


# 23rd INTERNATIONAL MULTIDISCIPLINARY SCIENTIFIC GEOCONFERENCE - SGEM 2023

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## FOREWORD

**The International Multidisciplinary Scientific GeoConference SGEM is focused on AGENDA 2030 Goals - GLOBAL WARMING, CLIMATE CHANGE, $CO_2$ REDUCTION, BIODIVERSITY, AND GREEN TECHNOLOGIES FOR A SUSTAINABLE FUTURE.**

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**In an ever-evolving world, amidst the vast expanse of the universe, Earth remains our singular home — a beacon of life, mystery, and endless curiosity.** It is with immense pride and anticipation that we present the **2023 Conference Proceedings in Earth and Planetary Science, a collective endeavor of minds passionate about our world and the celestial wonders beyond.**

The beauty of science, particularly in the realm of geosciences, lies in its ability to unite us. It transcends borders, politics, and cultural divides, binding us together in pursuit of knowledge, understanding, and enlightenment. This year, our focus aligns with the noble aspirations of Agenda 2030, reminding us that our planet's health and sustainability are integral to our shared future. Every paper within these pages stands as a testament to our collective commitment to that goal.

Moreover, this conference encapsulates the harmonious dance between the Earth as a place we live in and to care for, and the Planet, with its place in the Universe - different but intertwined, sharing the primary goal of interpreting, understanding and celebrating the Universe and our place in it. Like an artist who captures the majesty of a mountain range or the vastness of space, our researchers, too, paint with data, hypotheses, and discoveries, sketching a future where knowledge drives progress.

In a world where discord often grabs headlines, this gathering underscores that there exist realms untouched by strife, where camaraderie and cooperation reign supreme. This is the world of geosciences: a haven for curious minds, a frontier for explorers, a canvas for dreamers.

As you delve into these meticulously peer-reviewed articles, let them not only inform you but inspire you. Let them remind you of the magic that happens when diverse minds converge for a singular purpose. **And let them serve as a beacon of hope, signaling a future where science, with its inherent artistry, continues to light our way.**

**In anticipation of the better future and optimism,**
**Sincerely yours,**

**Prof. DSc. Oleksandr Trofymchuk**
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# SECTION

# ADVANCES IN BIOTECHNOLOGY

# AN IN VITRO STUDY: CITRUS LEMON ESSENTIAL OIL AS AN ALTERNATIVE TREATMENT AGAINST MICROBIAL INFECTIONS

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## ABSTRACT

A genuine and urgent need is to prevent and treat bacterial diseases primarily caused by germs that have developed antibiotic resistance. These factors led to several studies focusing on alternate or supplementary antimicrobial methods. With the expectation that, unlike antibiotics, they will be effective without causing resistance, researchers are becoming increasingly interested in antimicrobial substances made from natural resources, such as plant extracts, essential oils, and peptides. The present study investigated the antimicrobial behaviour of *Citrus lemon* essential oil against *S. pyogenes* (ATCC 19615), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14028), *H. influenzae* type B (ATCC 10211), *C. albicans* (ATCC 10231), and *C. parapsilopsis* (ATCC 22019). The microdilution assay demonstrated good antimicrobial efficacy of the essential oil with variable MIC values depending on the microbial species. The chromatographic analyses by GC-MS were performed because the antimicrobial activity is correlated with the chemical composition. The results revealed 24 compounds, with D-limonene (66.67%) and beta-pinene (12.39%) as the predominant compounds.

**Keywords: Citrus lemon, antimicrobial, Gram-positive, GC-MS**

## INTRODUCTION

Extracts and essential oils from plants have captured the attention of researchers more and more in recent years, demonstrating their effectiveness as therapeutic agents in parasitic, inflammatory and microbial diseases. The food flavouring and preservation industries use essential oils extensively because they are odorous and volatile elements of plant secondary metabolism.
The lemon (*Citrus lemon*) is a species of small evergreen tree in the *Rutaceae* flowering plant family. Its fruits have many uses in the food industry but exhibit antimicrobial activity due to their chemical composition and acidic pH ( pH 2-3).

## MATERIALS AND METHODS

Materials

The essential oil of *Citrus lemon* (CLEO) was purchased from doTERRA (Pleasant Grove, Utah, USA).

The reference microbial strains (ATCC) used in this study were obtained from the culture collection of the Microbiology Laboratory within the Interdisciplinary Research Platform of the University of Life Sciences "King Mihai I" From Timisoara.

The essential oil sample was tested on the following reference strains: *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* type B (ATCC 10211), *Candida albicans* (ATCC 10231) and *Candida parapsilopsis* (ATCC 22019).

Minimum Biofilm Inhibitory Concentration (MBIC) is the lowest concentration of the antimicrobial agent to inhibit microorganism growth. Our previous research has described the method as a microbial mass loss by measuring OD by spectrophotometry following ISO 20776-1:2019.

Gas Chromatography-Mass Spectrometry (GC/MS)

C-MS analysed BEO using Shimadzu QP 2010 Plus apparatus (Columbia, SC, USA) equipped with an AT WAX 30 m 0.32 mm 1 μm capillary column. The discharge rate of the carrier gas, helium, was 1 mL/min, and the temperatures of the injector and ion source were 250 °C and 220 °C, respectively. For compound separation, a temperature gradient was utilised with an initial oven temperature of 40 °C maintained for 1 min, followed by an increase to 210 °C at a rate of 5 °C/min and a subsequent 5-min hold at this temperature. The sample injection volume was 1 μL of a 2% BEO hexane solution, and a split ratio of 1:50 was utilised. The GC-MS analysis was executed in triplicate.

Microbiological method

A dilution of $10^{-3}$ of fresh strain was used to perform the test, an inoculum equivalent to a standard of 0.5 McFarland. The bacterial and fungal strains were revived by growing overnight in the Brain Heart Infusion (BHI) broth (Oxoid, CM1135) at 37 °C and subsequently switched to BHI Agar (Oxoid, CM1136) for 24 and 48 hours at 37 °C, respectively. The crops were then diluted to an optical density (OD) of 0.5 McFarland standard ($1.5 \times 10^8$CFU×mL) using BHI broth and evaluated with a McFarland densimeter (Grand-Bio, England). The dilutions were pipetted 100 μL into each well of the 96-well microdilution plate using a Calibra 852 digital multichannel pipette. The tested essential oil was added at 1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL, 10 mg/mL, 20 mg/mL, 40 mg/mL and 80 mg/mL. The plates were covered and left for 24 hours at 37 °C. After 24 hours, the DO was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Tripled tests were performed for all samples. Strain suspensions and BHI were used as positive controls.

The MIC was determined by measurement of OD using the spectrophotometric method, according to ISO 20776-1:2019. The MIC is the lowest sample concentration tested at which there is no discernible growth of microorganisms. BGR (bacterial growth rate) and BIR (bacterial inhibition rate) were calculated as indicators for interpreting the results using the following formulas:

$$BGR = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%)$$

$$BIR = 100 - BGR\ (\%)$$

## TABLES AND GRAPHS

Equations (1) and (2) were utilised in conjunction with the values displayed in Figures 1 and 2 to calculate the results as OD (optical density) values. The obtained and computed results are all shown graphically as follows. The microbiological investigation of the CLEO impact against Gram-positive bacteria identified different evolutionary patterns. Figure 1 shows the impact of CLEO on the growth rate of bacteria, namely Gram-positive bacteria.

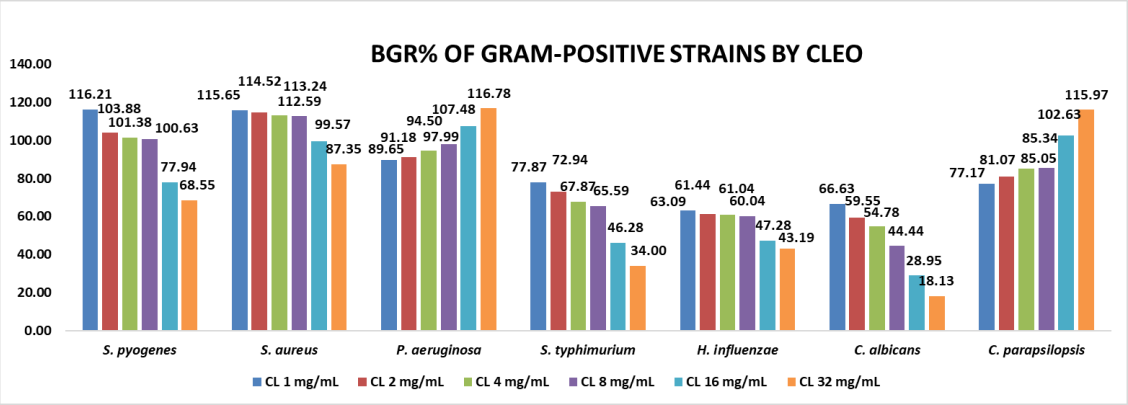



Fig. 1. BGR% of CLEO against Gram-positive strains tested

Figure 1 presents all the bacterial growth rates of the Gram-positive strain obtained after applying the concentrations of CLEO selected (1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL, 16 mg/mL and 32 mg/mL).

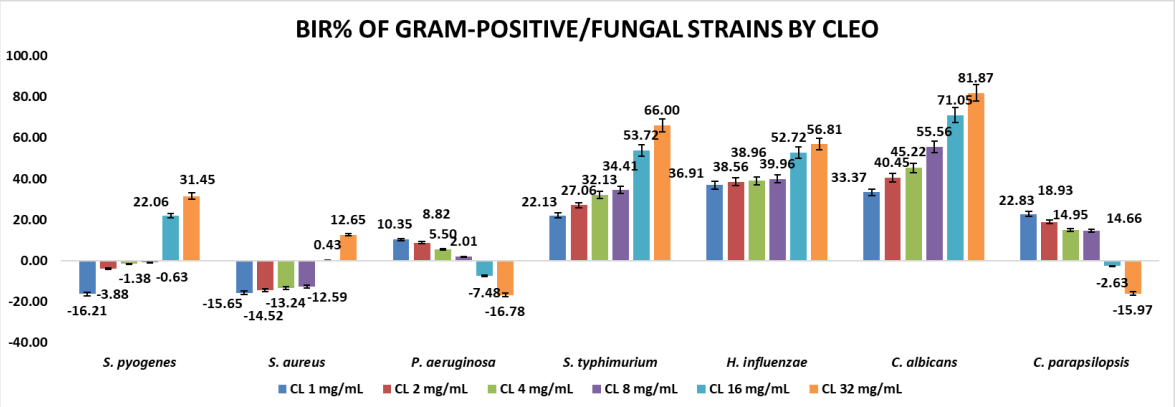



Fig. 2. BIR% of CLEO against Gram-positive strains tested

Figure 2 represents the bacterial inhibition rates (BIR%) values obtained by CLEO against the selected Gram-positive strains.

Table 1. MIC of CLEO on the selected ATCC strains

| | *S. pyogenes* | *S. aureus* | *P. aeruginosa* | *S. typhimurium* | *H. influenzae* | *C. albicans* | *C. parapsilopsis* |
|---|---|---|---|---|---|---|---|
| CL 1 mg/mL | | | | | | | |
| CL 2 mg/mL | | | | | | | |
| CL 4 mg/mL | | | | | | | |
| CL 8 mg/mL | | | | | | | |
| CL 16 mg/mL | | | | | | | |
| CL 32 mg/mL | | | | | | | |

The red colour highlights the samples where the MIC was determined, and the yellow highlights negative values but with a positive correlation, the values increasing alongside the increase in concentration. The grey colour represents the samples in which the MIC was found, but subsequent concentrations showed a potentiating effect, and therefore, the effect decreased together with the concentration.

## RESULT

The chromatographic analyses by GC-MS were performed because the antimicrobial activity is correlated with the chemical composition. The results revealed 24 compounds, with D-limonene (66.67%) and beta-pinene (12.39%) as the predominant compounds.
The Gram-positive bacteria tested showed different sensitivities to the selected concentrations of CLEO. *P. aeruginosa* and *C. parapsilopsis* proved to be the more resistant strains, with low BIR% values and a negative evolution trend. The inhibition activity is in negative correlation with the increase in concentration, so if the BIR% values at the smallest concentration tested were 10.35% for *P. aeruginosa* and 22.83% for *C. parapsilopsis* at 1 mg/mL, while at 32 mg/mL, the efficacy decreased to -16.78% and -15.97% respectively.
CLEO exhibited low antibacterial activity against *S. pyogenes* and *S. aureus*, with the first positive BIR% registered at 16 mg/mL in both strains. Still, the evolution recorded was positively correlated with the increase in concentration. On the other hand, CLEO proved good efficacy against *S. typhimurium*, *H. influenzae* and *C. albicans*, in which MIC was demonstrated from the first concentration tested, the BIR% values reaching maximum values ranging from 66% to 81.87%.

## DISCUSSION

According to studies, the antimicrobial activity of *Citrus lemon* essential oils is variable depending on the chemotype. Raspo et al. (2020), demonstrated that CLEO from Argentina and USA showed strong antimicrobial activity against *Leuconostoc mesenteroides*, *Escherichia coli* and *Lactobacillus plantarum*, with MIC ranging between 0.33 and 0.55 mg/mL and MBC between 0.42 and 0.95 mg/mL[1].
The antimicrobial activity was due to the high concentration of limonene, 69-72% respectively (Raspo et al., 2020). In their study, Ben Hsouna et. al (2017) confirmed that studied CLEO contained limonene (39.74%) as the dominant compound, followed by β-Pinene (25.44%) [2]. While limonene has been shown to have strong antifungal properties, although the mechanism of action of terpenes is not fully understood, it is thought to involve membrane disruption by the lipophilic compounds [4], [5].
These chemical compounds are important in preventing contamination from and growth of *L. monocytogenes* in minced beef meat [2]. Similar findings were presented by Asker

M. et al., who stated that *Citrus lemon* leaf oil was very strong regarding the antibacterial efficacy against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*B. cereus* and *S. aureus*) [3].
On the other hand, Li et al., 2020, demonstrated that CLEO had little inhibitory effect on *Lactobacillus* and *Bifidobacterium* had high efficacy against *Salmonella typhimurium*, *E. coli* O157: H7 and *E. coli* K88 [6].

## CONCLUSION

As a result of our research, we can say that the essential oil of *Citrus lemon* produced by doTERRA due to the synergistic activity of its constituents causes a strong inhibitory effect, especially on the bacterial ATCC strains of *S. typhimurium* and *H. influenzae*, as well as on the tested *C. albicans* strain. Since the chemical structures and their antibacterial capabilities are correlated, the antimicrobial activity of CLEO essential oils appears to be related to its terpenes-type primary components, which the GC analysis revealed to be D-limonene (66.67%) and beta-pinene (12.39%).

## ACKNOWLEDGEMENTS

We have performed this research with the support of the Interdisciplinary Research Platform belonging to the University of Life Sciences "King Michael I" from Timisoara, where the analysis was made.

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# ANALYSIS OF VEGETATION COVERAGE OF GRASSLANDS BASED ON NDVI VALUES. CASE STUDY: POIANA RUSCĂ MOUNTAINS

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## ABSTRACT

In any terrestrial area, but especially in the case of mountainous areas, the spatial distribution of vegetation is conditioned by a series of natural factors such as relief, climatic factors or soils. From another point of view, the distribution of vegetation is different, depending on the phenophases, implicitly on the observation period, during the growing season. In this context, the aim of the work was to analyze the vegetation coverage of the grasslands in the Poiana Rusca Mountains, by applying the Normalized Difference Vegetation Index (NDVI), in five different periods. Five satellite images from the months of March, May, July, August and October were used, on which NDVI was applied, later reclassified according to the obtained values. Quantitatively, the changes produced from one time interval to another were calculated, based on the matrix of transitions, and thus, the changes produced were highlighted. It was found that the highest degree of vegetation coverage of the grasslands was in July, the "peak" of the vegetation season. The application of NDVI for the analysis of vegetation distribution has several advantages: it complements measurements in the field, it is expressed in the form of thematic maps that can be integrated with other geospatial data, it allows the location of "problematic" or risky areas, in terms of vegetation cover..

**Keywords:** NDVI, satellite images, GIS, grasslands.

## INTRODUCTION

The Normalized Difference Vegetation Index (NDVI), introduced in the specialized literature by Rouse et al. (1973), became over time, through the wide range of applications in which it is used, one of the best-known remote sensing indices, applied to multispectral satellite images ([8], [9], [3], [2]). In this context, being an index that highlights different aspects of vegetation, it can be said that NDVI is applied, most intensively, in the analysis of the terrestrial biosphere ([11], [14]).

In the specialized literature, based on NDVI, numerous studies were designed, which aimed to: monitor the vegetation, evaluate the health of plants, especially in precision agriculture, analyze the spatio-temporal changes produced in the vegetation cover, mapping vegetation and so on.

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In the mountain area, the grasslands, the object of study of this paper, are elements of particular importance in terms of biodiversity ([4], [15]), are used in a traditional system [13], their vegetation being seasonal. On surfaces used as grasslands, for vegetation analysis, NDVI is applied, through different techniques and ways of interpretation.

In the previously outlined context, the purpose of the research presented in this study was to analyze the degree of vegetation coverage of the grasslands in the Poiana Ruscă Mountains, by applying the Normalized Difference Vegetation Index (NDVI), in five different periods (March, May, July, August and October) and to evaluate the changes produced in different stages of the growing season.

## MATERIALS AND METHODS

### Study area

The study area is located in the western part of Romania and includes the Poiana Ruscă Mountains (Figure 1), delimited according to the regionalization made by Badea and Posea (1984) [10].

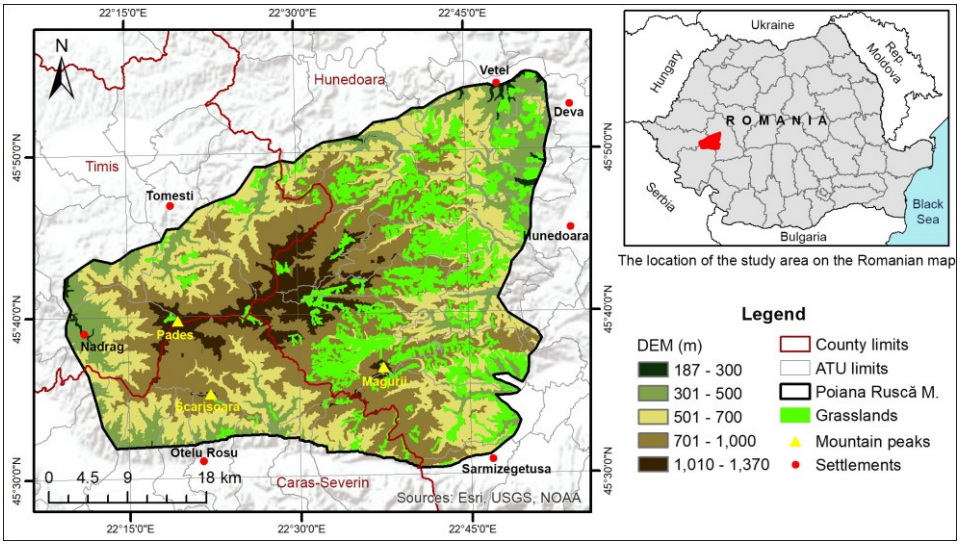



Figure 1 Location and description of the study area (processing after [5], [7], [10])

The analyzed territory, namely the Poiana Ruscă Mountains, is located at altitudes between 187 - 1370 m, with an average altitude of 706 m, which imposes certain characteristics of other environmental factors, such as climatic factors, pedological factors or vegetation. The meadows, identified on the surface of 21890 ha, are located, for the most part, in the eastern half of the Poiana Ruscă Mountains, in all altitudinal levels, with different participation and proportions (Figure 1).

### Research methodology

The methodology that was applied in this study is schematically described in Figure 2.

***1. Processing of satellite images*** - it was done in the SNAP software; Sentinel 2 satellite images from March, May, July, August and October were used [6]; considering the fact that the area of interest overlapped on two satellite scenes, in the first phase it

was necessary to mosaic them, for each considered time interval; in the next phase, the bands of the five resulting satellite scenes were brought to the same spatial resolution, namely 20 m; in the last phase of this stage, the area of interest was extracted from the satellite scenes;

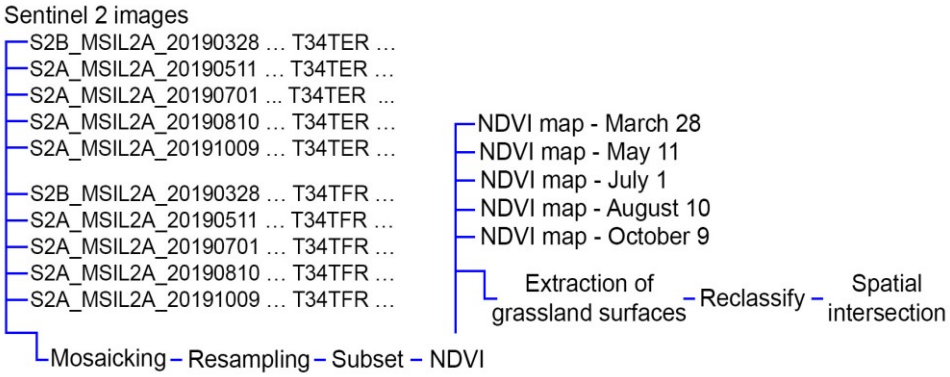



Figure 2 Work methodology

***2. Generation of NDVI maps*** was also carried out in the SNAP software, for each established moment in time; the formula for calculating this index involves the near-infrared band (B8) and the red band (B4), has the form NIR-RED / NIR+RED and is implemented in SNAP;

***3. Generation of the NDVI maps for the grassland surfaces*** was carried out in the ArcGIS software; from each NDVI map, grassland areas were extracted, later being reclassified into eight value classes: -1.00 – 0.00; 0.01 - 0.20; 0.21 - 0.30; 0.31 - 0.40; 0.41 - 0.50; 0.51 - 0.60; 0.61 - 0.70; 0.71 – 1.00;

***4. Change analysis*** - it was also carried out in the ArcGIS software, through the spatial intersection of two NDVI maps each, so over time intervals; four periods resulted: March – May; May – July; July - August; August – October. The changes in the NDVI values of the grasslands from one period to another were thus highlighted, which suggest changes in their vegetation cover; the results of the analysis of changes were expressed in the form of the matrix of transitions.

## RESULTS

In the Poiana Ruscă Mountains, the NDVI values were variable, both in time and in space (Figure 3). In March, the NDVI values ranged between -0.983 - 0.999, their average being 0.472. The lowest values were recorded in the case of lands not covered by vegetation, and the maximum values characterized the coniferous forests in the higher mountain areas. In May, the NDVI values registered an increase, they were between -1.00 – 0.999, but the average value was 0.798, which suggests a much higher degree of vegetation coverage. In July, NDVI recorded values between -0.138 – 0.999, the average values being 0.865. This temporal moment can be considered the "peak" of the growing season.

Towards the autumn period, the NDVI values register a decrease, so that, at the level of October, they were between -1.00 – 0.999, with the average values of 0.815. The high values of NDVI are explained by the presence, on extensive areas, of forest surfaces.

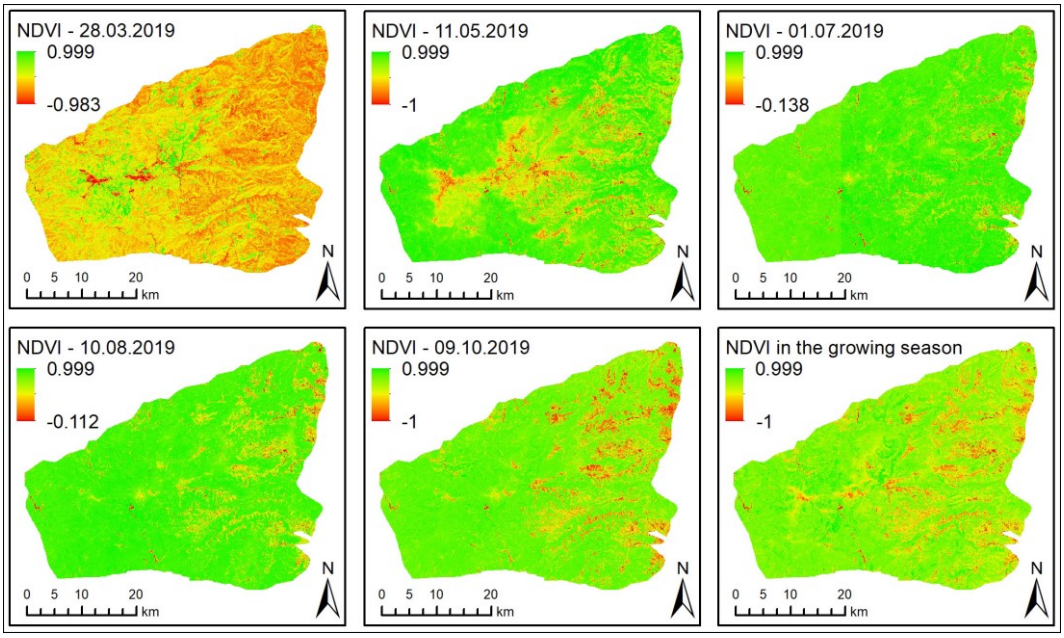



Figure 3 NDVI map in different time periods, in the Poiana Ruscă Mountains

In the case of areas used as grasslands, NDVI had a differentiated spatio-temporal distribution (Table 1).

Table 1 NDVI values on grassland surfaces

| Observation date | NDVI values | | |
|---|---|---|---|
| | Minimum | Maximum | Average |
| **28.03** | -0.4048 | 0.9233 | 0.3769 |
| **11.05** | -0.0032 | 0.9574 | 0.6958 |
| **01.07** | -0.0164 | 0.9995 | 0.8304 |
| **10.08** | -0.0395 | 0.9577 | 0.7854 |
| **09.10** | -0.0953 | 0.9990 | 0.6252 |

From the data presented in Table 1, it can be seen that the lowest average values were recorded in March, at that time the vegetation of the grasslands was still in the initial phase of development. The average of the values increases towards the summer season, when it reaches the maximum of 0.8304 and decreases towards the autumn period, with the change of environmental conditions (in particular, the decreasing temperatures).

The data summarized in table 1 provide an overview of the vegetation cover of the Poiana Ruscă Mountains, in the March - October interval, but in order to have a detailed situation, it was necessary to apply the analysis of changes, for each interval (stage of the season of vegetation) taken into account.

At the beginning of the analyzed interval, namely the month of March, 9936 ha of grasslands (50%) were defined by NDVI values between 0.31 - 0.40, and 6314 ha had NDVI values between 0.41 - 0.50. Towards the end of the interval, respectively 11.05, the transition of NDVI values, in particular, from classes C4 and C5 to classes C7 and C8 is observed, which means the increase in the degree of vegetation coverage (Table 2).

Table 2 Transitions matrix for NDVI values in the period 28.03 – 11.05

| 28.03 \ 11.05 | C 1 | C 2 | C 3 | C 4 | C 5 | C 6 | C 7 | C 8 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|
| C 1 | | 0 | 0 | 4 | 8 | 7 | 5 | 5 | 29 |
| C 2 | 0 | | 23 | 31 | 50 | 41 | 28 | 24 | 218 |
| C 3 | 0 | 16 | | 284 | 790 | 958 | 795 | 620 | 3529 |
| C 4 | 0 | 7 | 25 | | 600 | 1640 | 2679 | 4837 | 9936 |
| C 5 | 0 | 3 | 7 | 24 | | 302 | 862 | 5012 | 6314 |
| C 6 | 0 | 1 | 2 | 5 | 17 | | 114 | 988 | 1176 |
| C 7 | 0 | 0 | 0 | 1 | 3 | 9 | | 202 | 255 |
| C 8 | 0 | 0 | 0 | 0 | 1 | 2 | 9 | | 82 |
| Total (ha) | 0 | 47 | 124 | 498 | 1573 | 3008 | 4530 | 11758 | 21538 |
| Legend: C1: -1.00 – 0.00; C2: 0.01 - 0.20; C3: 0.21 - 0.30; C4: 0.31 - 0.40; C5: 0.41 - 0.50; C6: 0.51 - 0.60; C7: 0.61 - 0.70; C8: 0.71 – 1.00 | | | | | | | | | |

In the period 11.05 – 01.07, the NDVI values suggest an increase in the degree of vegetation cover, so that, at the end of the period, most of the grassland areas (96%) had NDVI values between 0.71 – 1.00 (Table 3).

Table 3 Transitions matrix for NDVI values in the period 11.05 – 01.07

| 11.05 \ 01.07 | C 1 | C 2 | C 3 | C 4 | C 5 | C 6 | C 7 | C 8 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|
| C 1 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C 2 | 0 | | 4 | 3 | 2 | 3 | 3 | 31 | 47 |
| C 3 | 0 | 0 | | 10 | 9 | 7 | 9 | 85 | 124 |
| C 4 | 0 | 0 | 1 | | 20 | 30 | 34 | 406 | 497 |
| C 5 | 0 | 0 | 0 | 3 | | 61 | 96 | 1390 | 1566 |
| C 6 | 0 | 0 | 0 | 1 | 8 | | 175 | 2766 | 2994 |
| C 7 | 0 | 0 | 0 | 1 | 4 | 22 | | 4343 | 4505 |
| C 8 | 0 | 0 | 0 | 1 | 10 | 49 | 157 | | 11805 |
| Total (ha) | 0 | 2 | 9 | 24 | 68 | 216 | 609 | 20608 | 21537 |
| Legend: C1: -1.00 – 0.00; C2: 0.01 - 0.20; C3: 0.21 - 0.30; C4: 0.31 - 0.40; C5: 0.41 - 0.50; C6: 0.51 - 0.60; C7: 0.61 - 0.70; C8: 0.71 – 1.00 | | | | | | | | | |

According to the data summarized in Table 3, the vegetation of the grasslands in the Poiana Ruscă Mountains, in July, has the maximum degree of coverage, and the high values can be explained by the high density of plants.

Table 4 Transitions matrix for NDVI values in the period 01.07 – 10.08

| 01.07 \ 10.08 | C 1 | C 2 | C 3 | C 4 | C 5 | C 6 | C 7 | C 8 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|
| C 1 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C 2 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| C 3 | 0 | 1 | | 1 | 0 | 0 | 0 | 1 | 9 |
| C 4 | 0 | 0 | 4 | | 4 | 1 | 1 | 3 | 24 |
| C 5 | 0 | 0 | 1 | 13 | | 10 | 4 | 11 | 68 |
| C 6 | 0 | 0 | 0 | 4 | 56 | | 26 | 55 | 216 |
| C 7 | 0 | 0 | 0 | 1 | 27 | 207 | | 170 | 609 |
| C 8 | 0 | 0 | 1 | 7 | 61 | 422 | 2686 | | 20759 |
| Total (ha) | 0 | 4 | 11 | 38 | 176 | 716 | 2921 | 17822 | 21688 |
| Legend: C1: -1.00 – 0.00; C2: 0.01 - 0.20; C3: 0.21 - 0.30; C4: 0.31 - 0.40; C5: 0.41 - 0.50; C6: 0.51 - 0.60; C7: 0.61 - 0.70; C8: 0.71 – 1.00 | | | | | | | | | |

In the interval 01.07 – 10.08, according to the matrix of transitions (Table 4), the degree of vegetation coverage of the grasslands is still high. However, the reduction of NDVI values is observed on certain grasslands; for example, from class C8, with NDVI values between 0.71 – 1.00, the initial surface of 20759 ha, was reduced to 17822 ha, the surface difference being included in value classes C7 and C6.

In the period 10.08 - 09.10, according to Table 5, the area of grasslands with NDVI values between 0.71 - 1.00 (C8) is significantly reduced, from 17673 ha to 7445 ha.

Table 5 Transitions matrix for NDVI values in the period 10.08 – 09.10

| 10.08 \ 09.10 | C 1 | C 2 | C 3 | C 4 | C 5 | C 6 | C 7 | C 8 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|
| C 1 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C 2 | 0 | | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| C 3 | 0 | 5 | | 1 | 0 | 0 | 0 | 0 | 11 |
| C 4 | 0 | 1 | 18 | | 4 | 1 | 0 | 1 | 38 |
| C 5 | 0 | 1 | 31 | 85 | | 14 | 4 | 2 | 176 |
| C 6 | 0 | 1 | 29 | 262 | 264 | | 40 | 21 | 717 |
| C 7 | 0 | 1 | 17 | 363 | 1460 | 772 | | 92 | 2930 |
| C 8 | 0 | 5 | 38 | 315 | 2457 | 4034 | 3495 | | 17673 |
| Total (ha) | 0 | 17 | 138 | 1039 | 4224 | 4921 | 3765 | 7445 | 21549 |
| Legend: C1: -1.00 – 0.00; C2: 0.01 - 0.20; C3: 0.21 - 0.30; C4: 0.31 - 0.40; C5: 0.41 - 0.50; C6: 0.51 - 0.60; C7: 0.61 - 0.70; C8: 0.71 – 1.00 | | | | | | | | | |

Towards the autumn period, the vegetation cover of the meadows, reflected by the NDVI values, is less, the grassland areas included in the C8 value class being moved to the classes with lower NDVI values.

## DISCUSSIONS

NDVI, used in numerous scientific researches, in different contexts, can also be used to evaluate the vegetation state of plants, during the growing season ([12], [1]). Starting from this idea, in the present study, NDVI was applied to Sentinel 2 images to evaluate the vegetation cover of grasslands, in different phases of the growing season.

The analysis of the vegetation coverage of the grasslands in the Poiana Ruscă Mountains was carried out between March and October (Figure 4), on four time intervals.

In March, the first stage of the vegetation season in the analyzed mountain grasslands, 16% of them recorded NDVI values between 0.21 - 0.30, a percentage of 46% of the grassland areas had NDVI values between 0.31 - 0.40, and 29%, between 0.41 – 0.50. At that time, against the background of restrictive climatic conditions, the degree of vegetation coverage of the grasslands was moderate. In May, an increase in NDVI values was observed, so that 14% of them had NDVI values between 0.51 - 0.60, a percentage of 21% fell between 0.61 - 0.70, and 55% of the grassland areas had NDVI values between 0.71 – 1.00. In the month of July, the maximum degree of vegetation cover was reached on the grassland surfaces, thus, 96% of them had NDVI values between 0.71 - 1.00. In August, a slight reduction in NDVI values was observed, but the vegetation cover remains at a very high level.

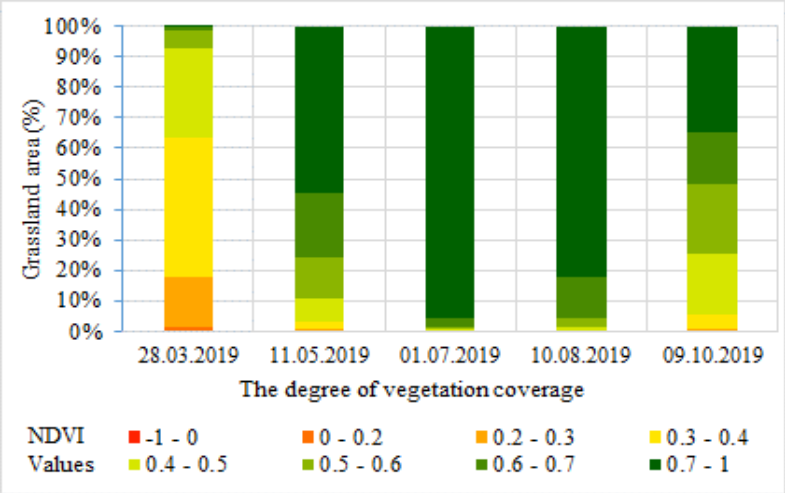



Figure 4 The variation of NDVI values in the interval 28.03 – 09.10

In the autumn period, the NDVI values decrease, which also means a reduction in the degree of vegetation coverage of the grasslands. However, it was found that 52% of the grassland areas had NDVI values above 0.61.

## CONCLUSION

The analysis of the degree of vegetation coverage of the grasslands in the Poiana Ruscă Mountains, carried out between March and October, by applying NDVI on Sentinel 2 satellite images, highlighted the following aspects: in March, the NDVI values were low, which reveals the fact that the grasslands had the lowest degree of vegetation coverage, against the background of restrictive climatic conditions; the NDVI values register a significant increase, until July, when the maximum vegetation coverage of the grasslands is reached, 96% of the surfaces having NDVI values between 0.71 – 1.00; towards the autumn period, the NDVI values decrease (48% of the grassland areas had NDVI values below 0.60) and also signal the reduction in the degree of vegetation coverage, due to the senescence of the plants in the grasslands.

The analysis of the temporal distribution of vegetation based on NDVI complements field measurements, and the data can be integrated with other geospatial data to locate "problematic" or at-risk areas in terms of vegetation cover.

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# ANALYZING THE GROWTH PATTERNS OF STERLET (*ACIPENSER RUTHENUS*) VS. HYBRID STURGEON (*A. RUTHENUS*, FEMALE X *A. GUELDENSTAEDTII*, MALE) FINGERLINGS REARED WITHIN A RECIRCULATING AQUACULTURE SYSTEM

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## ABSTRACT

Reusing and recirculating the water in which aquatic organisms are cultivated is a crucial goal in improving the sustainability of aquaculture. As a result, recirculating aquaculture systems (RAS) have become increasingly popular recently as a means of raising various fish species, including sturgeons. Sterlet (*Acipenser ruthenus*) and a sturgeon hybrid (a cross between male *A. gueldenstaedtii* and female *A. ruthenus*) were reared in a RAS, and their growth was monitored during the first 6 months of their life. The aim was to analyze the growth patterns of their main body traits while maintaining identical rearing conditions. The RAS used for our trials had 3 tanks with a volume of one cubic meter each. Three hundred sterlets and 300 sturgeon hybrids, each 2 months old, were placed in triplicate into the three tanks of the RAS, with each tank containing a combination of 200 fish (100 sterlets and 100 hybrids, respectively). Thirty sterlets and 30 hybrids were randomly measured twice per month to determine key body traits, including total length, standard length, maximum body depth, and body weight. At 2 months old, the disparities in primary body traits between sterlets and hybrid sturgeon were minimal and statistically insignificant ($p>0.05$). However, by the time they reached 6 months of age, these differences had increased highly significant ($p\leq0.001$). The hybrid sturgeons significantly outperformed the sterlet in the same RAS rearing conditions, being much heavier and longer at the age of 6 months.

**Keywords:** growth dynamic, RAS, sterlet, sturgeon hybrid

## INTRODUCTION

Reusing and recirculating water in the cultivation of aquatic organisms is a fundamental objective for enhancing the sustainability of aquaculture practices. Recirculating aquaculture systems (RAS) have seen increased utilization in fish farming across many European countries, allowing for the controlled and sustainable cultivation of multiple high-value species [5, 7]. This approach represents a crucial step toward more environmentally friendly and efficient sturgeon farming operations. Sturgeons typically exhibit slow growth and late maturation. Nevertheless, sturgeons grow faster and reach

maturity in captivity much earlier than in the wild [15]. This has sparked significant interest in cultivating these fish under controlled conditions, not only for caviar production but also for meat. Although sturgeon aquaculture was initially focused primarily on caviar production, recent decades have witnessed a positive trend in demand for sturgeon meat [2, 4].

Recirculating aquaculture systems can provide excellent control over the physico-chemical parameters of water to accelerate the growth rate of sturgeons and facilitate sexual maturity [13]. The choice of sturgeon species or hybrids also influences the duration of the production cycle in sturgeon farming. The sterlet (*Acipenser ruthenus*) stands out as the earliest maturing species among farmed sturgeon, attaining sexual maturity in captivity at approximately 3 years old [1, 14]. Hence, it garners interest and is prevalent in many sturgeon farms in Romania, well-suited for cultivation in RAS [13].

Sturgeons are prone to interspecific hybridization, even between species differing in ploidy levels, such as sterlet (2n) and Russian sturgeon (*A. gueldenstaedtii*) (4n). These hybrids could exhibit advantages of hybrid vigor or positive heterosis, but they are sterile [3, 9]. Nonetheless, for meat production in farms with RAS designed for sterlet farming, these sturgeon hybrids are usually found. Quantifying the influence of hybrids in the technological flow of the farm could be very useful.

The aim of the study was to analyze the growth patterns of the main body traits in sterlet and hybrid sturgeon while maintaining identical rearing conditions for them in RAS.

## MATERIALS AND METHODS

*Experimental design*. For the experimental setup, an indoor RAS was employed, comprising three individual tanks, each with a one-cubic-meter capacity. Within this controlled environment, 300 juvenile sterlets and 300 sturgeon hybrids were introduced, all of which were at the age of 2 months. To ensure robust statistical analysis, these fish were distributed in triplicate distribution model, with each tank accommodating a mix of 200 fish -comprising 100 sterlets and 100 hybrids.

*The fish.* The juveniles of sterlet and sturgeon hybrid used in this study were obtained through artificial reproduction methods [7, 11], utilizing broodfish exclusively raised indoors in a recirculating aquaculture system. In the initial month of their lives, the fry sturgeons were reared indoors in elongated fiberglass tanks. Subsequently, until they reached the age of two months, the fish were transferred to indoor one-cubic-meter square tanks, all integrated into a RAS. During this period, the fish gradually transitioned from natural feed to dry pellets, becoming accustomed to the new feeding diet.

*Rearing conditions*. During the experimental period, the three experimental tanks connected to a RAS maintained consistent water quality, with water from each tank continuously mixed into the treatment unit. The water exchange rate was set at 2 times per hour for each rearing tank. To ensure optimal conditions, half a cubic meter of water from each tank was replaced daily with fresh water sourced from a well, previously heated to match the temperature of the RAS.

The main physical-chemical parameters of the water were daily monitored. The values of these parameters were: ammonium <0.2 mg/l, nitrites < 0.7 mg/l, nitrates <130 mg/l, dissolved oxygen 4.5-6.5 mg/l, temperature 21.3±2°C.

*Fish feeding*. The fish were nourished with dry pellets manufactured by Alltech-Coppens, ranging from 1.2-1.5 mm (ADVANCE) to 2 mm (STAR ALEVIN) and 3 mm

(SUPREME-10), tailored to the respective sizes of the fish. The daily feed quantity was determined as a percentage of the total fish biomass recorded during bi-weekly measurements. For fish aged 2-3 months, the feed was adjusted to 5% of the fish biomass per day. This percentage gradually decreased to 4% for 4-month-old fish and 3% for those aged 5-6 months. Feeding was conducted around the clock, employing semi-automatic FIAP (Germany) belt feeders, one feeder per tank.

*Growth analysis*. Thirty sterlets and 30 sturgeon hybrids were randomly measured twice per month to determine key body traits, including total length (TL), standard length (SL), maximum body depth (MBD), and body weight (BW) [7, 8]. The fish were anesthetized using clove oil (0.05 ml/500 ml water) before measurements to prevent their movements and minimize stress during handling [6].

The measurement data served for calculating rearing parameters:

Specific growth rate; $SGR_{BW}$ (% $d^{-1}$) = [(ln $BW_f$ - ln $BW_i$) / $\Delta T$] x 100

$SGR_{TL}$ (% $d^{-1}$) = [(ln $TL_f$ - ln $TL_i$) / $\Delta T$] x 100

Daily growth rate; DGR (g) = ($BW_f$ –$BW_i$) / $\Delta T$

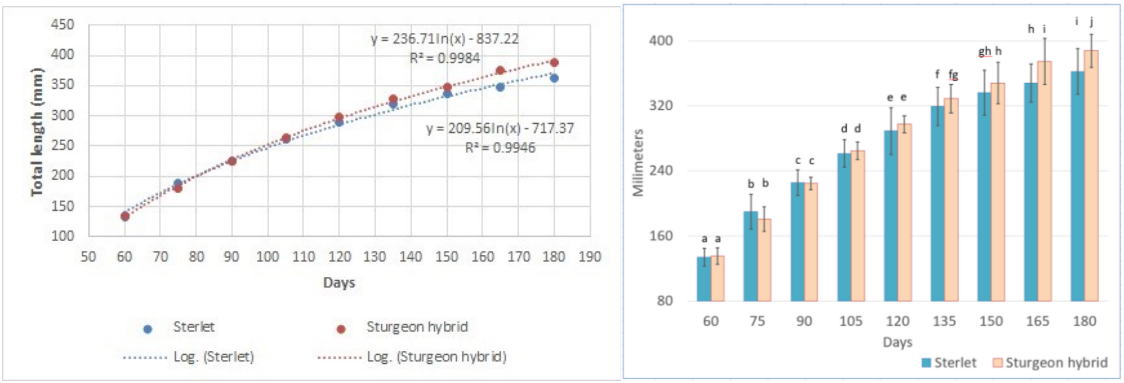
Fulton's condition factor; K = (BW (g) x 100) / $TL^3$ (cm)

where: BW - body weight, TL - total length, f - final, i - initial, $\Delta T$ - the duration of the experiment.

*Data analysis and statistics*. The data were analyzed using STATISTICA10 software. The significance of differences was assessed using the Duncan test. The statistically processed data are presented in the paper as Mean ± SD. Microsoft Excel was utilized for creating graphs.

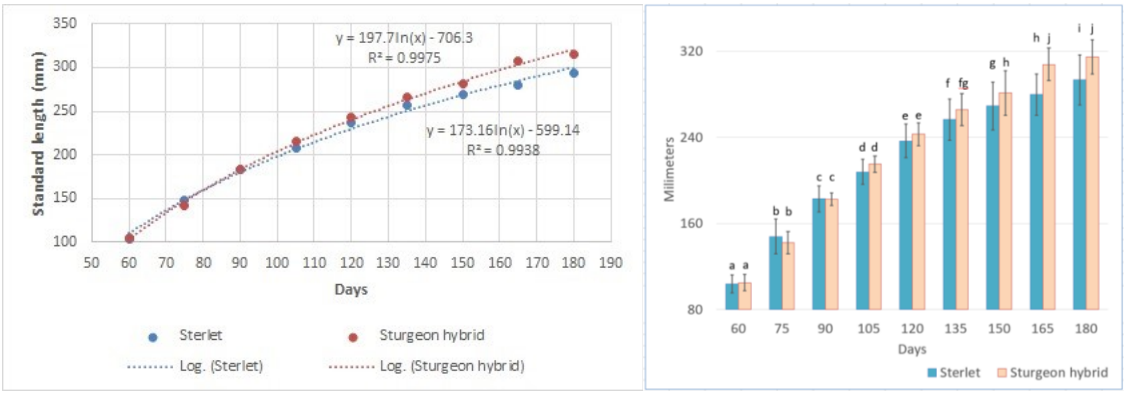
## RESULTS

During the study period (from 60 to 180 days post-hatching), the total length (TL) of the body exhibited a highly accelerated growth in both the sterlet and the hybrid sturgeon. The TL increased in the sterlet from 133.65±10.97 mm to 362.07±28.02 mm and in the sturgeon hybrid from 135.35±10.28 mm to 387.43±20.35 mm, with the increase being significant (*P≤0.05*) at 15-day intervals, during which measurements were taken (Figure 1, a and b). Therefore, the hybrid sturgeons were longer than sterlet with about 6,54%. Over the entire study period, the total length increased 2.71 times in the sterlet and 2.87 times in the hybrid.




**a.** **b.**

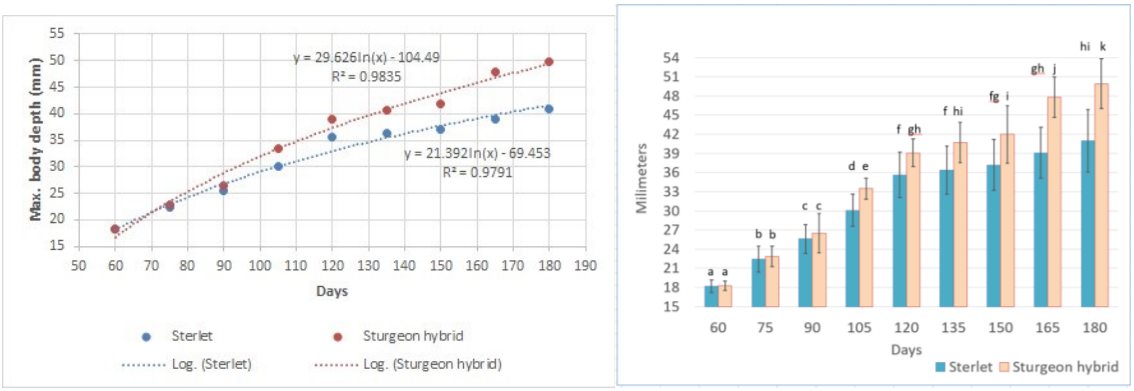
**Figure 1.** Growth patterns of the total length, mathematically adjusted (a) and significance of the differences (b), between sterlet and hybrid sturgeon. Different letters indicate significant differences (*P≤0.05*)




**a.** **b.**

**Figure 2.** Growth patterns of the standard length, mathematically adjusted (a) and significance of the differences (b), between sterlet and hybrid sturgeon. Different letters indicate significant differences (*P≤0.05*)

At the end of the study, the hybrid sturgeons were significantly longer (*P≤0.05*) than the sterlet, and the same findings applied to the standard length (SL) as well (Figure 2, a and b). The SL increased in the sterlet from 103.90±8.32 mm to 293.53±23.22 mm and in the sturgeon hybrid from 105.97±7.61 mm to 314.97±15.80 mm, with the increase being significant (*P≤0.05*) at 15-day intervals.

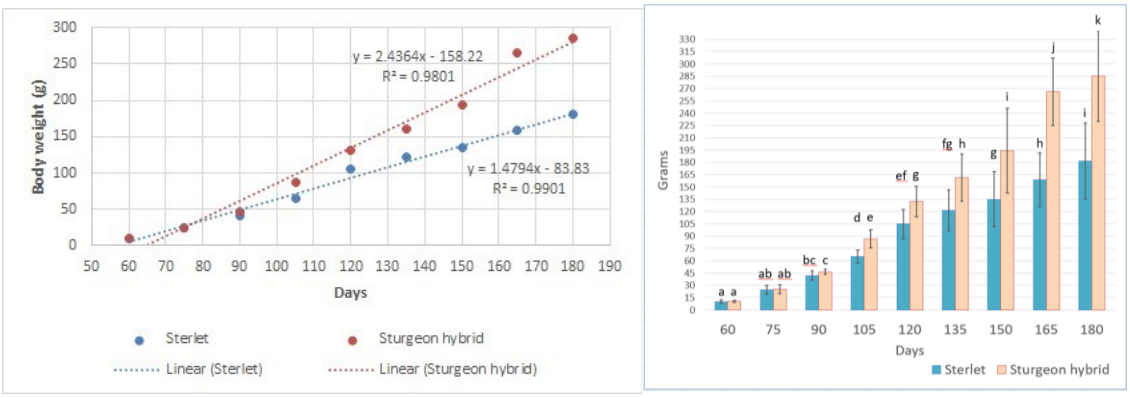



**a.** **b.**

**Figure 3.** Growth patterns of the maximum body depth, mathematically adjusted (a) and significance of the differences (b), between sterlet and hybrid sturgeon. Different letters indicate significant differences (*P≤0.05*)

The maximum body depth (MBD) of the sterlet increased by a factor of 2.25 over the study period, from 18.20±0.98 mm to 40.97±4.88 mm (figure 3). In the hybrid sturgeon, the MBD increased significantly, rising from 18.28±0.76 mm to 49.93±3.89 mm, indicating a growth of 2.73 times.

The MBD trend in the sterlet over the four months displayed a distinctive pattern, characterized by a prominently steep curve in the first half. In comparison, the hybrid sturgeon exhibited a relatively consistent growth trend in MBD over the same period.




**a.** **b.**

**Figure 4.** Growth patterns of the body weight, mathematically adjusted (a) and significance of the differences (b) between sterlet and hybrid sturgeon. Different letters indicate significant differences (*P≤0.05*)

The body weight (BW) demonstrated substantial growth in the sterlet, increasing from 10.11±2.12 g to 181.80±46.61 g. However, the growth was notably more significant in the hybrid sturgeon, escalating from 10.39±1.48 g to 285.20±55.16 g in the same period and under the same rearing conditions (figure 4). While the sterlet increased its body weight by approximately 18 times, the hybrid sturgeon achieved a growth of 27.45 times. These data indicate that, under the same conditions in a RAS, hybrid sturgeons reached a higher body weight than the sterlet at 6 months, surpassing it by about 36%.
At the end of the experimental period, all other rearing parameters indicated superior growth in the hybrid sturgeon compared to the sterlet in the RAS under identical conditions (table 1).

**Table 1.** Rearing parameters of the sterlet and hybrid sturgeon calculated at the end of experimental period

| Parameter | Sterlet | Sturgeon hybrid |
|---|---|---|
| Initial body weight (g) | 10.11 ± 2.12 | 10.39 ± 1.48 |
| Final body weight (g) | $181.80^{a}$ ± 46.61 | $285.20^{b}$ ± 55.16 |
| Daily growth rate DGR (g /day) | $1.43^{a}$ ± 0.39 | $2.29^{b}$ ± 0.46 |
| Specific growth rate $SGR_{BW}$ (% day-1) | $2.40^{a}$ ± 0.34 | $2.75^{b}$ ± 0.19 |
| $SGR_{TL}$ (% day-1) | 0.83 ± 0.11 | 0.88 ± 0.09 |
| Initial condition factor $K_i$ | 0.42 ± 0.04 | 0.42 ± 0.06 |
| Final condition factor $K_f$ | $0.38^{a}$ ± 0.06 | $0.49^{b}$ ± 0.05 |

Absence of superscript letters indicates no significant differences between sterlet and sturgeon hybrid (*P>0.05*)
Mean values with different uppercase letters indicate significant differences (*P≤0.05*) between sterlet and hybrid sturgeon for the same parameter.

## DISCUSSIONS

In RAS-designed farms for sterlet production, sturgeon hybrids are occasionally present. Assessing their impact on the farm's technological flow would be valuable. Establishing and evaluating growth curves for farmed sturgeons are critical for the technological management of the farm, enabling quick interventions when necessary and providing an overview of the technological flow. While data indicating the growth of the sterlet in the first 6 months of age have been published, comparisons among different sturgeon

species and hybrids were made [10], information regarding fish reared in RAS or other systems under similar conditions is sparse. Different rearing conditions undeniably influence growth patterns, leading to changes in morphological characters and rearing parameters [8]. For instance, Lenhardt et al. [8] calculated a higher value for the Fulton condition factor (K=0.42) in wild sterlet compared to cultured ones (0.30). Our results show a higher K for cultured sterlets (K=0.38) and even higher for hybrid sturgeon (K=0.49). Prokeš et al. [10] demonstrated that the growth intensity of sterlet was moderately lower than that of the Siberian sturgeon but higher than that of the stellate sturgeon reared in similar conditions. Our results generally lead to the conclusion that sturgeon hybrids exhibit superior growth compared to sterlet when reared in the same conditions in RAS, with hybrids being significantly longer and heavier, and with all rearing parameters superior to sterlet. These findings align with published information, highlighting interspecific hybridization as a potential tool for improving productivity in aquaculture [12].

## CONCLUSIONS

At 6 months old, the total length of the hybrid sturgeons exceeded that of the sterlet by approximately 6.54%. During the period from 60 to 180 days post-hatching, the total length increased by 2.71 times in the sterlet and 2.87 times in the hybrid, reaching 362.07±28.02 mm in the sterlet and 387.43±20.35 mm in the hybrid.

The SL significantly increased in both the sterlet (from 103.90±8.32 mm to 293.53±23.22 mm) and the hybrid sturgeon (from 105.97±7.61 mm to 314.97±15.80 mm), mirroring the pattern observed in total length.

The maximum body depth trend in the sterlet over the four months displayed a distinctive pattern, marked by a prominently steep curve in the first half. In contrast, the hybrid sturgeon showed a relatively steady growth trend in body depth over the same period. This trait increased significantly in the hybrid sturgeon, rising from 18.28±0.76 mm to 49.93±3.89 mm, indicating a growth of 2.73 times, significantly higher than in the sterlet (2.25 times).

The growth of body weight in the hybrid sturgeon was significantly more pronounced than in the sterlet, occurring during the same period and under identical rearing conditions. The hybrid sturgeon's weight increased from 10.39±1.48 g to 285.20±55.16 g, compared to the sterlet, which reached only 181.80±46.61 g.

Our data indicate that, under the same conditions in a RAS, hybrid sturgeons could reach a higher body weight than the sterlet at 6 months, surpassing it by about 36%. Between the age of 2 and 6 months, the body weight of the sterlet increased approximately 18-fold, while the hybrid sturgeon experienced a growth of 27.45 times.

All other rearing parameters indicated superior growth in the hybrid sturgeon compared to the sterlet in the RAS. The differences were significant for daily growth rate, specific growth rate and final condition factor.

## ACKNOWLEDGEMENTS

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# APPROACHING THE *PHYTHOPHTORA INFESTANS* PATHOGEN IN POTATO CULTURE BY BIOLOGICAL MEANS

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## ABSTRACT

In the current context, in which the European Union, through various projects, requires the reduction of the consumption of pesticides it is necessary to investigate some different products to control the *Phytophthora infestans* potato blight, biological ones, that have the role of improving the structure and health of the soil, avoiding contamination of it but also of groundwater with synthetic active substances. Potato production is diminished by the presence of weeds, pathogens and pests. One of the most important phytopathogenic agents is *Phytophthora infestans* (Mont.) deBary, which produces the disease called mange, an important disease that occurs in all areas where potatoes are grown causing economic losses up to 50%. In general, disease management of this pathogen by biological means is a difficult task, especially when the level of disease pressure is high, together with favorable environmental conditions. The study was performed, in Belint area, Timis county, under the climatic conditions of the year 2022. The test product used in study protocol were Fitocid, Fitohelp, Mycohelp, Viridin and Taegro in various rate of application and an untreated check was also included. The assessments were performed at 3, 7, 14 DAT, assessing the effectiveness of the products in respect of the frequency and intensity of the attack. All five bioproducts reduced the severity of the pathogen compared to the untreated control, the antagonism shown by the Fitocid and Fitohelp products against the fungus Phytophthora infestans was maximum seven days after application.

**Keywords:** *Phytophtora infestans,* biopreparation, control, severity, efficacy

## INTRODUCTION

Potato is an important crop worldwide, being grown in 149 countries [1]. The production of this crop is depreciated and diminished by a series of pathogens such as *Phytophthora infestans* (Mont. de Bary) (Oomycota), *Rhizoctonia solani* (Basidiomycota), *Streptomyces scabies* (Actinobacteria), of which the fungus *P. infestans* causes the highest damage, being considered the most important biotic factor threatening global production [2]. Downy mildew is the disease caused by the

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phytopathogen *P. infestans* and causes annual losses of approximately 5.2 billion euros globally [3]. This pathogen infects several species of the *Solanaceae* family [5]. For the outbreak of an epidemic, it is enough that in the planting material for 100 ha, the frequency of the affected tubers is 0.002% [5]. Downy mildew is an acute disease, in 7-10 days it can completely destroy the potato plant [3] being present during vegetation, harvesting, transport and storage [4,6]. Managing the pathogen P. *infestans* is a significant problem [7,8], and the most effective approach involves the use of chemical fungicides. However, the use of these chemicals products has negative repercussions on the environment and human health [9]. A major issue related to these fungicides is the evolution of resistance in *P. infestans* populations [10]. Thus, fungicides are not a sustainable and continuous solution for disease control. The use of biological control has been established as a non-chemical alternative in the fight against the fungus *P. infestans*. Over the past three decades, several bacterial agents have been evaluated, including *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, and *Pseudomonas fluorescens* [2, 3, 11]. There are also antagonistic fungal agents that can act as possible biocontrol agents against *P. infestans*, including *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen, *Pythium oligandrum* Dreschler and Trichoderma species [11, 12]. Although biocontrol agents have been shown to be highly effective in laboratory studies, they are limited by their inconsistent and unpredictable performance under field conditions. This inconsistency can be attributed to continuous changes in biotic factors, such as the presence and competition with native microbiota, and abiotic conditions, such as temperature, humidity, ultraviolet radiation, etc., in the environment. These conditions influence the physical form and performance of biocontrol agents [1, 13]. The purpose of this study was to control the fungus *P.infestans*, from the potato agroecosystem, by applying biopreparations.

## MATERIALS AND METHODS

The biological control of the pathogen *P. infestans* was carried out under natural conditions, in the field. The trial was located near Belinț - Timiș county, in Western Romania, in the year 2022 (figure 1). The variety used in the experiment was the Marabel seed potato, a semi-early variety.

The trial was placed as completely randomised blocks following of EPPO and Good Agricultural Practices guidlines. Each plot is subdivided into 3 repetitions, with six variants having dimensions of 10 x 3.0 m (30m2), in total we have 18 microplots. The experimental variants were differentiated by the applied biological product. All 5 biological treatments were analyzed for their potential to reduce the *P. infestans* fungus. The experimental variants (Figure 2) were treated as follows:T0 – untreated control, T1 Fitocid 0,300 l (*Bacillus subtilis* $1{,}0x10^{9}$ CFU/$cm^3$); T2 - Fitohelp 0,450 l (*Bacillus subtilis* $4{,}0x10^{9}$ CFU/$cm^3$); T3 – Mycohelp 0,600 kg (mixture of *Bacillus subtillis, Azotobacter*, *Enterobacter, Enterococcus* and fungi *Trichoderma lignorum, Trichoderma viride,* $1{,}0 \times 10^{9}$ CFU/g); T4 - Viridin 3 l (Trichodermin); T5 -Taegro 0,370 kg (*Bacillus amyloliquefaciens* strain FZB24 - $1x10^{13}$ UFC/kg).

In each variant, the biopreparations were applied in three different growth stages: BBCH 22-23 (shoot formation); BBCH 32-33 (20% plants close the row) and BBCH 35-36 (50% plants close the row) (figure 3).

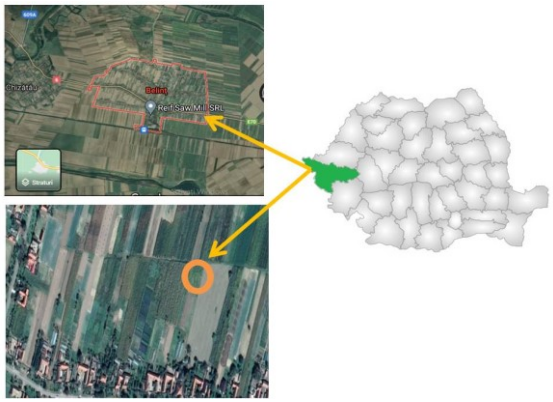


Figure 1 Trial location (image processed by Stef based on information https://en.m.wikipedia.org/wiki/File :Romania_judete.svg/https;www.google.com/maps/place/Biled)



Figure 2 The trial setup where the research was carried out (Photo Cărăbeț A., 2022)

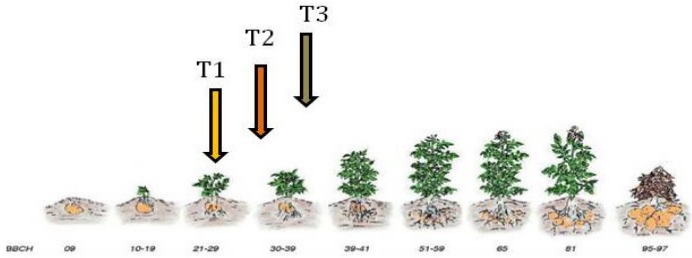



Figure 3. BBCH stages where biological products were applied (image processed by Ștef based on the information on the site https://www.pesticid.ro/fungicid-nando-500-sc)

To establish the severity of the attack and the effectiveness of the biological products, four assessments were performed (figure 4), so that after 10 DAA each assessment was made with only one exception registered at the third application when it was also carried out at 20 DAA.

Attack severity was calculated using equation:

$$I = \frac{\sum(i \times f)}{n}$$ where:

$I$ - expressed in percentage represents the attack intensity of the fungus;

$i$ – the proportion in which the plant is attacked;

$f$ – the number of samples observed (analyzed);

$n$ – the total number of plants attacked;

Their effectiveness was established considering the attack intensity. At the four evaluations, 10 plants/plot were examined, noting the intensity of the attack, after which the Abbott formula was applied.

The efficacy of biological products was calculated with the Abbott formula:

$$\text{Abbott \%} = \left(1 - \frac{n \text{ in } T}{n \text{ in } C}\right) x\ 100$$

n – intensity of *Phytophthora infestans*; T- treated variant; C- control (untreated)



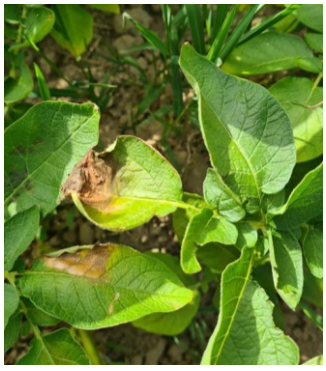


Figure 4 Evaluation of potato plants regarding the severity of the pathogen *P. infestans* (Photo Cărăbeț A., 2022)

## RESULTS

The impact of foliar application of biological products based on *Bacillus subtilis*, *Bacillus amyloliquefaciens, Trichoderma* sp. and the mixture of the bacteria *Bacillus subtilis, Azotobacter, Enterobacter, Enterococcus* and the fungi *Trichoderma lignorum, Trichoderma viride*, used to induce systemic resistance and as antagonistic agents used to reduce the severity of the disease produced by *P. infestans* in potatoes under field conditions is shown in Figures 5 and 6.

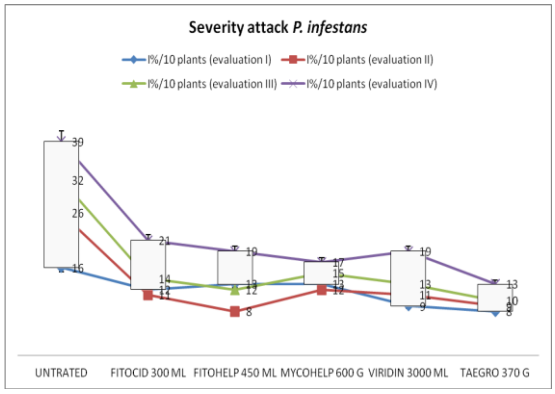



Figure 5. The severity of the attack of the pathogen *Phytophthora infestans* on potato plants at 4th assessments

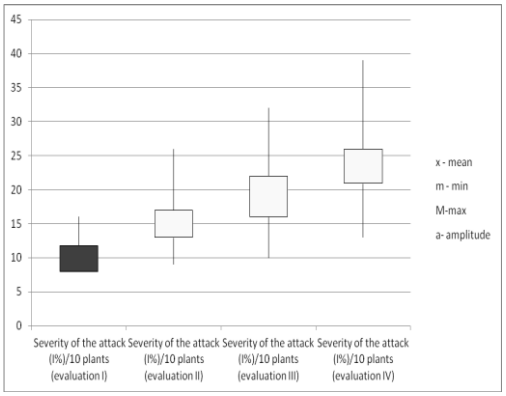
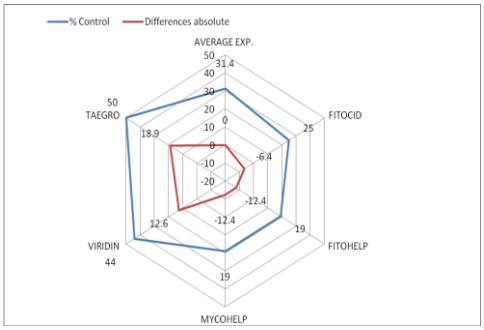



Figure 6. Amplitude of *Phytophthora infestans* attack severity in the experimental field

The results show that all treatments had a significant impact in reducing the severity of the disease. The most effective treatment was proved to by Taegro based on *Bacillus amyloliquefaciens*. The severity shown by the pathogen *P. infestans* on untreated potato plants varied between 16% (first assessment) and 39% (at the fourth assessment) (figures 5). The climatic conditions, from the year 2022, recorded in the experimental site, did not stop the evolution of the pathogen, so that at the second assessment (10 days after the second application of biopreparations) a virulence of 9-26% (figure 6). The maximum amplitude (26) of the severity of the disease was recorded at the last evaluation (Figure 6). Meanwhile, the Fitocid and Fitohelp treatments (based on Bacillus subtillis) controlled the pathogen at highest, 10 days after the application of the second treatment (assessment II), reducing the severity of the disease from 26% (control variant) to 11 % and 8% respectively.

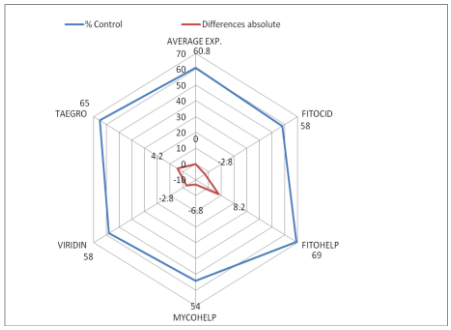
Control percentages varied between 19-50%, 10 days after the first application of biofungicides. Comparing these percentages with the average of experience revealed that the biofungicides based on *Trichoderma* sp. and *Bacillus amyloliquefaciens* controlled the pathogen *P. infestans* in a proportion of 44% and 50% respectively, the results being very significant (Figure 7). The efficacy of the bacterial and fungal antagonism was confirmed, considering the reduction of the pathogen *P. infestans* up to 69%, in the variants treated with Fitohelp (at the 2nd assessment). This was followed by treatment with Taegro, based on *Bacillus amyloliquefaciens*, which controlled the pathogen by 65%. After 10 days from the application of the second treatment, it was observed that the pathogen *Phytophthora infestans* was controlled by an average of 60.8%. On the other hand, the biofungicides Fitocid and Viridin failed to control the fungus, not exceeding the average percentage of the experience, and the results did not reveal significant differences.

The average percentage of control of the pathogen *Phytophthora infestans*, at the third assessment, decreased by 0.8% compared to the previous assessment (Figure 9). Inhibition of the fungus *Phytophthora infestans* development produced by parasitism of the fungus *Trichoderma* sp. (found in the composition of the Viridin product) did not show significance compared to the average of the experience, the same situation was recorded in the variants treated with Fitohelp (*Bacillus subtilis*).



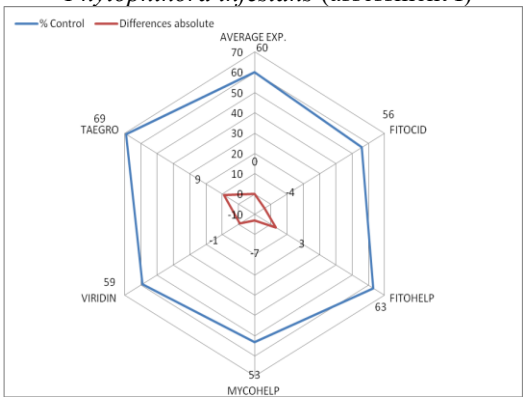

DL 5% = 5.76; DL1% =8,2; DL 0.1% = 11.87

Figure 7. The efficacy of biofungicides against *Phytophthora infestans* (assessment I)



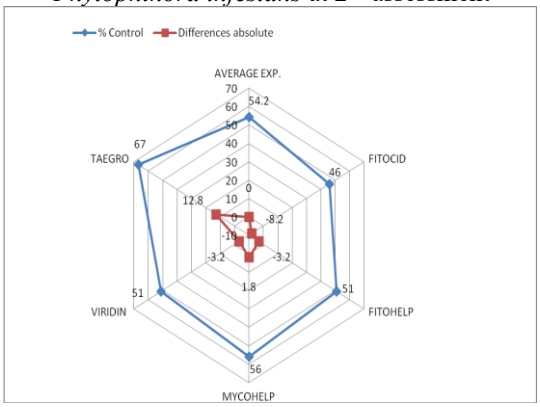

DL 5% = 3.95; DL1% = 5,62; DL 0.1% = 8.14

Figure 8. The efficacy of biofungicides against *Phytophthora infestans* at 2nd assessment




DL 5% = 3.31; DL1% = 4,71; DL 0.1% = 6.82

Figure 9. The efficacy of biofungicides against *Phytophthora infestans* (3rd assessment)




DL 5% = 4.24; DL1% = 6.03; DL 0.1% = 8.74

Figure 10. The efficacy of biofungicides against *Phytophthora infestans* (4th assessment)

The formulation based on *Bacillus subtilis* (Fitocid) reduced the severity of the disease by 56%, 4% less than the mean of experience, the difference being significantly negative. The product based on bacteria and fungi (Mycohelp) no longer effectively

protected the potato plants against the mycotic pathogen, the outcome being very significantly negative, compared to the experience average. The potato plants of the variants treated with Taegro, which contains *Bacillus amyloliquefaciens*, were protected in proportion to 69%, the absolute difference (9%) being very significant compared to the average of the experience (60%) (figure 9).

The biofungicides used in the experiment had a disease control between 46-67% (figure 10), and the results were significantly better than in the case of the untreated control. At the 4th assessment (20 days after the last application of biofungicides), their ranking related to the experience average was: Taegro, Mycohelp, Viridin, Fitohelp and Fitocid. Differences in *Phytophthora infestans* control ranged from (-8.2%) to (12.81%) compared to the mean of experience (Figure 10). Products based on *Bacillus subtilis* (Fitocid and Fitohelp) recorded negative differences of (-8.2) and (-3.2) respectively compared to the experience average, the results being significantly negative or insignificant. Taegro's product ranked first in potato blight control, with a percentage of 67% (figure 11).

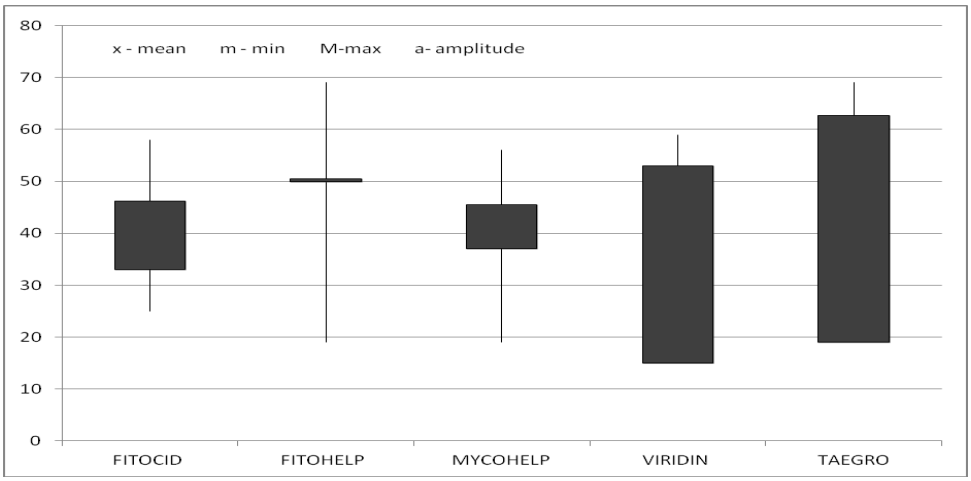



Figure 11. The amplitude of biofungicides efficacy against the pathogen *P. infestans*

## DISCUSSION

The potato is an important crop both economically and socially, playing an important role in the current diet in many parts of the world. However, in addition to the need to ensure the protection of this culture against pathogens and pests, there is also a need to protect the environment, implicitly the soil and water. For this reason, a safer approach is necessary, something that can be achieved by introducing the use of biofungicides in this pathosystem downy mildew (*Phytophthora infestans*)- *Solanum tuberosum.* There is relatively few information on biological control as a potentially successful alternative for managing the devastating potato downy mildew epidemic. Two factors make the biological control of the phytopathogenic agent P. *infestans* difficult, namely the rapid establishment of the infection and the acute evolution of the disease [13].In these conditions, proposed by the study plan, the products based on bacteria and fungi, as well as mixtures between these organisms (*Bacillus subtilis*, *Bacillus amyloliquefaciens, Trichoderma* sp. and the mixture of the bacteria *Bacillus subtilis, Azotobacter, Enterobacter, Enterococcus* and the fungi *Trichoderma lignorum, Trichoderma viride*,), have brought to attention very significant results, which

encourage the introduction of these biofungicides on the widest possible scale in potato culture technology. In in vitro tests carried out by several researchers [14, 15, 16, 17] the antagonistic species *T. harzianum* and *B. subtilis* significantly reduced the growth of the pathogen that produces potato blight by various percentages 75.1% - 88%, compared to the control. The observations in the present study highlighted that the highest severity of the disease was achieved in the control plots, and the lowest was in the variants where the bio fungicides were applied. Neither antagonistic bacteria nor antagonistic fungi were able to provide greater than 70% protection of potato plants. The success of the biological control of the pathogen *P. infestans* depends on the method of application and the repetition of treatments. Bacteria with antagonistic activity against *P. infestans* are mainly found in the genera *Pseudomonas* and *Bacillus* [14, 15].

## CONCLUSION

The biological products based on *B. subtilis* (Fitocid and Fitohelp) controlled 46.25% (mean of all four assessments) and 50.5% (mean of all four assessments) respectively of the P. infestans fungus, the maximum effectiveness was reached after a II –th application (2$^{nd}$ assessment). The bacteria *Bacillus amyloliquefaciens*, active ingredient of Taegro product, exerted the highest effectiveness against the pathogen that produces potato downy mildew throughout the experiment. The product that has in its composition a mixture of bacteria and fungi, Mycohelp, protected the potato plants at the lowest, the average control of the pathogen *P. infestans* being 45.5%. The fungus Trichoderma sp., in Viridin product, exerted an average control of 53%, the maximum effectiveness being recorded at the 3$^{rd}$ assessment (59%).

The results of the present study show that the efficacy of the biopreparations increased when the treatment(s) was/are repeated. The highest values regarding the reduction of the pathogen P. infestans in the potato crop were recorded at the 2$^{nd}$ and 3$^{rd}$ assessments. At 20 DAA from the 3$^{rd}$ application (assessment IV), the efficacy of the biofungicides decreased. Following the results obtained, we encourage the introduction and intercalation of these biofungicides in the potato culture technology, thereby protecting the environment as well as the final consumer.

## ACKNOWLEDGEMENTS

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# ASSESSMENT OF CHEMICAL AND NUTRITIONAL QUALITY OF RAPESEED MEAL INTENDED FOR MONOGASTRIC LIVESTOCK FEEDING

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## ABSTRACT

Rapeseed meal is a valuable source of protein for animals feeding. It can be used as an alternative to soybean meal in the mixtures of combined feeds intended for farm animals. Thus, the purpose of this study was to evaluate the chemical and nutritional composition of the rapeseed meal intended for use in the feeding of monogastric livestock. 70 samples of rapeseed meal arrived at the Laboratory of Chemical Analyses for Animal Nutrition, were analysed. The following values were obtained for the main nutritional compounds: crude protein (CP) – 37.95%, with variations between 35.26 and 41.67%; ether extract (EE) – 1.84%, with values between 1.55 – 2.06%; crude fiber (CF) – 11.93%, with variations between 9.85 – 14.93%; and nitrogen-free extracts (NfE) – 41.07%, varying significantly between 35.75 – 45.82%. In order to evaluate the nutritional value of rapeseed meal, the digestible forms of nutrients and the value of metabolizable energy (ME) for pigs and poultry were calculated, using predictive formulas. The average ME value for pigs was 1278.57 kcal/kg, varying between 1145.63 - 1398.27 kcal/kg, and for poultry the ME value was 935.06 kcal/kg, being between 837.90 - 1022.55 kcal/kg. These data underline the importance of the chemical and nutritional evaluation of feed ingredients before they are included in the diets of farm animals in order to have the certainty of correctly calculated rations and to ensure the amounts of nutrients in accordance with their feeding norms, in order to maximize animal production.

**Keywords:** rapeseed meal, nutritional value, pigs, poultry, animal nutrition

## INTRODUCTION

Rapeseed meal is a by-product of the rapeseed oil extraction process and represents about 60% of whole rapeseed. Rapeseed oil is mainly used for human consumption and rapeseed meal is commonly used as a source of protein in animal feed [15]. Rapeseed meal has a high crude protein content and has well-balanced essential amino acids, thus being very close to the values for soy protein [10, 11]. As rapeseed

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meal is a cost-effective source of protein in animal feed, it can be an alternative ingredient to soybean meal, considering that according to US Department of Agriculture/Foreign Agricultural Service (USDA/ FAS, 2018/19) reports, rapeseed meal is the second most produced protein meal in the world after soybean meal [13]. Considering that Romania leads the export of rapeseed and rapeseed meal to the European Union, it is justified to include this feed raw material in animal feed, increasing the economic efficiency of animal production, in pigs and poultry [6].

Although rapeseed meal contains anti-nutritional factors and toxic substances, in recent years, improved rapeseed crops with low glucosinolate content have been developed extensively, increasing its use in the animal feed market. Various technologies have also been developed to improve the digestibility of nutrients in rapeseed meal, including fermentation and husk removal [1, 12].

Due to its valuable protein composition and high and balanced content of essential amino acids, rapeseed meal is considered a good protein resource in diets for monogastric animals. Recent research has shown that a small amount of rapeseed meal could be used in pig diets without detrimental effects on growth performance and meat quality [3-5]. Choi et al., 2015, reported that the addition of rapeseed meal to finishing pig diets increased feed conversion ratio, improved meat color, and enhanced meat taste and flavor [3].

In this context, the aim of this study was to evaluate the chemical composition and nutritional value of rapeseed meal intended for use in monogastric animal diets for optimal balancing and rationalization of diets for maximum utilization by farm animals.

## MATERIALS AND METHODS

This study involved the analysis of 70 samples of rapeseed meal. The rapeseeds samples were collected from the Western Plain of Timis County, Romania, in the 2022 harvest. The samples were processed in the Animal Nutrition Laboratory of the Faculty of Veterinary Medicine in Timisoara. In order to evaluate the crude chemical composition, the following parameters were examined: moisture (M%), dry matter (DM%), ash, crude protein (CP%), ether extract (EE%), crude fiber (CF%) and nitrogen-free extract (NfE%).

Standardised methods of feed chemical analysis (Official Methods of Analysis), adapted by the manufacturer of the laboratory equipment, were used in the processing of the samples, according to the application notes provided. The equipment used was FOSS brand. Thus, for the analysis of crude protein by the Kjeldahl method, the FOSS analyser with components such as the Kjeltec 8400 Analyzer Unit and the Kjeltec Sampler 8420, together with the FOSS Tecator Digestor Auto and the FOSS Tecator Scrubber were used. Crude fat determination was performed with the FOSS Soxtec 2055. Crude fiber analysis was carried out with the FOSS Fibertec 2010 analyser, supplemented by the FOSS Cold Extraction Unit 1021. Sample calcination took place in the Nabertherm B150 calcination furnace. A Binder oven was used for the determination of dry matter and moisture, applying the formula: 100 - DM% = M%.

To estimate the nutritional value, the percentages of digestible nutrients were calculated using the determined crude chemical composition values and digestibility coefficients for poultry and pigs, respectively 68.6 and 50.3 [after Burlacu, 1983, cited by Hotea et al., 2020] [7], applying the formula:

$$D.N.\% = \frac{C.C. \times D.C.}{100}$$

- D.N. = digestible nutrients;
- C.C. = crude composition;
- D.C. = digestible coefficient.

The assessment of nutritional value for monogastric animals focuses mainly on metabolisable energy. Thus, the calculation of metabolisable energy (ME kcal/kg) was performed using the ATWATER formula based on 4/9/4 system [14]:

$$M.E.(kcal/kg) = [(4 \times D.P.) + (9 \times D.E.E.) + (4 \times D.F.) + (4 \times D.NfE.)]\, x\, 10$$

- D.P. = digestible protein,
- D.E.E = digestible ether extract,
- D.F. = digestible fiber,
- D.NfE. = digestible nitrogen-free extract

The data were subjected to statistical processing using Excel's data analysis function. In this process, statistical correlations and descriptive statistics parameters were calculated. The analysis also included the identification of positive or negative correlations between the data obtained. The results obtained from this statistical processing were represented in tables and charts, thus providing a visual insight into the relationships and trends identified in the data collected.

## RESULTS

Following the crude chemical analysis of the 70 samples, the values obtained varied, and Table 1 shows the mean values for each parameter analyzed, as well as their lower and upper limits.

**Table 1**

Average values of chemical compounds in rapeseed meal

| % | Mean | Minimum | Maximum | SD* | Confidence Level (95.0%) |
|---|---|---|---|---|---|
| **M** | 10.76 | 9.09 | 12.76 | 0.961925 | 0.229362982 |
| **DM** | 89.23 | 87.24 | 90.91 | 0.961925 | 0.229362982 |
| **Ash** | 7.20 | 5.63 | 8.15 | 0.664357 | 0.158410332 |
| **CP** | 37.95 | 35.26 | 41.67 | 1.693091 | 0.403703327 |
| **CF** | 11.93 | 9.85 | 14.93 | 1.330091 | 0.317148993 |
| **EE** | 1.84 | 1.55 | 2.06 | 0.115486 | 0.027536731 |
| **NfE** | 41.07 | 35.75 | 45.82 | 2.451103 | 0.584444894 |

*SD – Standard Deviation

The chemical analyses show statistically correlated mean values for moisture and dry matter. The higher the water content, the lower the dry matter value, i.e. the content of elements with nutritional value.

For mineral substances the variations ranged from 5.63 to 8.15%, with an average value of 7.20%. Protein, fiber and nitrogen-free extract content showed significant variations, as follows: crude protein content showed an average value of 37.95%, with variations ranging from 35.26% to 41.67%; fiber content varied between

9.85 and 14.93%, and for nitrogen-free extract limits of 35.75 and 45.82% were obtained. The fat content of the samples analysed showed tighter limits, ranging from 1.55 to 2.06%, with an average value of 1.84% (Table 1).

In the statistical analysis of the correlations between the parameters analysed, the inter-influence of the chemical components of the rapeseed meal samples was observed (Fig. 1).

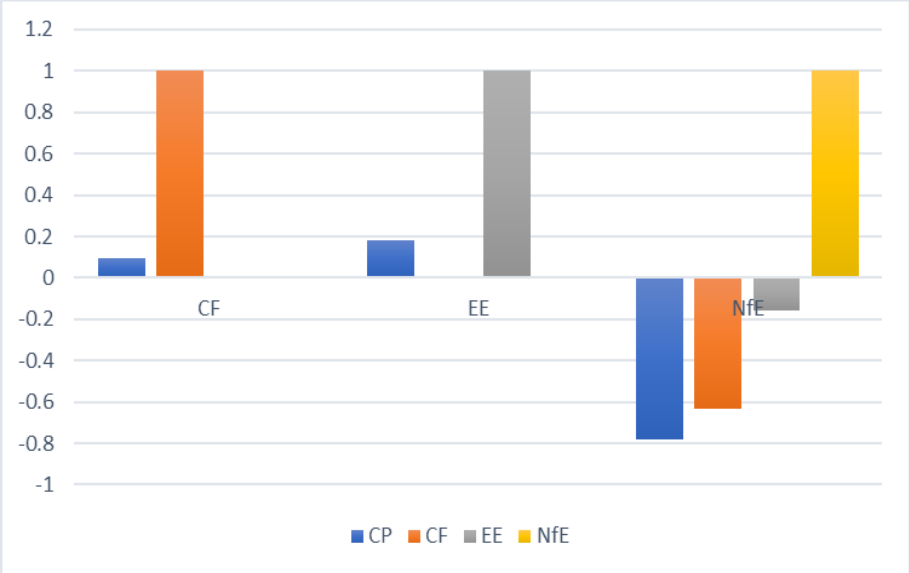



**Figure 1.** Interdependence of chemical components in rapeseed meal

From Figure 1 it can be seen how crude protein is directly influenced by the fiber content of rapeseed meal. The higher the fiber value in the samples, the lower the crude protein value. On the other hand, the fat content of the meal positively influenced the protein content. Samples with a higher fat content also had a higher protein content. In contrast, nitrogen-free extract negatively influenced the other nutritional parameters. The higher the nitrogen-free extract, the lower the protein, fat and fiber content.

Following the calculation of the digestible forms of the nutritional parameters, applying the digestibility coefficients established by Burlacu et al., 1983, cited by Hotea et al., 2020, slightly different values were obtained for the two species (figure 2).

Figure 2 shows differences in digestibility of the nutritional parameters taken in the study. For each element a better digestibility is observed in poultry, the differences being proportional to the values of the raw chemical components obtained in the analysis. Thus, in both species, the highest digestibility is shown by nitrogen-free extract, closely followed by protein. Lower digestibility was found for fiber in rapeseed meal. As the fat content of rapeseed meal is lower, the digestible form of rapeseed meal has the lowest values.

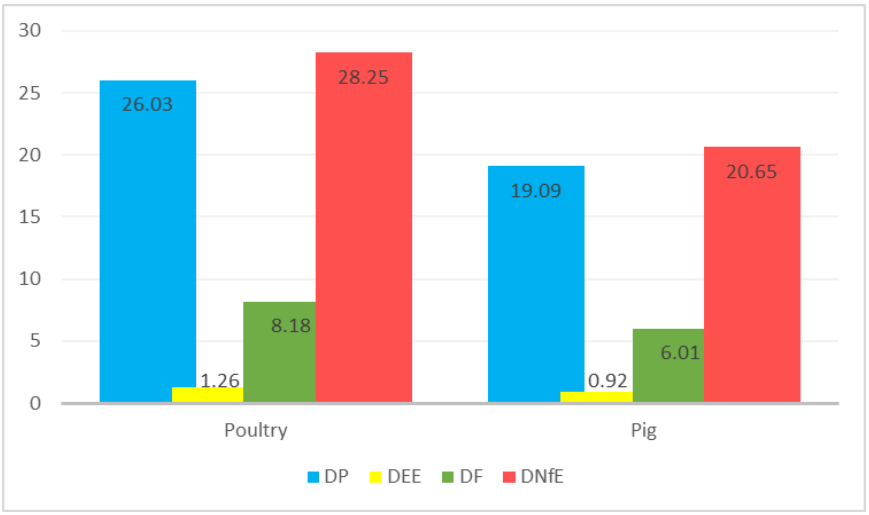



**Figure 2.** Digestible values of chemical components of rapeseed meal in poultry and pigs

Table 2 shows the energy values of rapeseed meal samples for poultry and pigs calculated with the ATWATER - 4/9/4 system:

**Table 2**

Metabolizable energy value of rapeseed meal for poultry and pigs

| *kcal/kg* | Mean | Minimum | Maximum | SD* | Confidence Level (95.0%) |
|---|---|---|---|---|---|
| ***ME poultry*** | 1278.57 | 1145.63 | 1398.27 | 61.4223 | 14.6456 |
| ***ME pig*** | 935.06 | 837.89 | 1022.55 | 44.8936 | 10.7045 |

*SD – Standard Deviation

## DISCUSSIONS

The aim of this study was to calculate the nutritional energy value of the rapeseed meal samples taken in the study. From the analysis of the data a higher nutritional value for poultry can be observed, constituting a valuable source of nutrients (Table 2).

Corroborating the results obtained from this study, the increased protein content of rapeseed meal is evident, with an average value of 37.95%, but also with a significant energy content of 1278.57kcal/kg ME for poultry and 935kcal/kg ME for pigs. These data underline the nutritional value of this feedstuff which can be successfully used in the feeding of monogastric animals as an alternative source for other protein feeds.

Rapeseed meal is the industrial by-product left over from the extraction of oil from rapeseed. Following an efficient extraction of the oil, the lipid content of rapeseed meal is lower and, therefore, the caloric value of this product is lower, but the protein value is maintained, with important values of almost 40%. In this sense, rapeseed meal can be used as an alternative protein source for other protein feed. For example, rapeseed meal can be used as a protein alternative in pig and poultry rations instead of the more expensive soybean meal, or sunflower meal which has a higher content of fibre that is more difficult for monogastric animals to digest. Thus, expert studies in the

field of animal nutrition support these claims with their research. The IBNA Institute (National Research and Development Institute for Animal Biology and Nutrition, Balotești, Romania), following a series of studies, recommends the following levels of inclusion of rapeseed meal in poultry feed: hens: 15% (replacing 50% of soybean meal; at the same time reducing feed costs by 5-7%, depending on the purchase price of rapeseed meal); broilers: Starter - 5%; Grower - 8%; Finisher - 11% [8].

The efficiency of digestion is an intimate interaction between genetics, the nature of diets and the physiological state of the animal (age, health, etc.). It has long been demonstrated that diet composition and protein source have an impact on animal performance and nutrient utilisation. Soybean meal is currently the most effective vegetable protein incorporated in the diet of chickens, but there is growing interest in developing alternative feeds. Among these, rapeseed meal has been recognized as a highly concentrated protein source with a well-balanced amino acid profile [5].

Cheng et al, 2022, published a review article highlighting the chemical composition of rapeseed meal compared to soybean meal and outlining its use in monogastric feed. As a protein feed raw material, the crude protein content in rapeseed meal is typically between 35% and 40%. The protein content of rapeseed meal is influenced by rapeseed type, growing medium, fiber content and so on [1, 5]. The crude fat concentration can vary in the dry matter of rapeseed meal from 1.42% to 14.55%, with an average of 7.78% [1]. Rapeseed type, impurity content, processing technology, planting, growing conditions in different production areas, climate and other atmospheric factors can all affect the crude fat content of rapeseed meal [2]. Carbohydrates represent about one third of this meal and are mainly composed of monosaccharides, sucrose, oligosaccharides, starch and non-starch polysaccharides. The content and structure of non-starch polysaccharides in rapeseed meal varies depending on the type of rapeseed [2].

soybean meal, the use of rapeseed meal in poultry and pig feed is limited due to the highly digestible carbohydrates. Some studies have shown that genetic selection of rapeseed types and processing technologies influence crude fibre content. Also, the use of hulled seeds in obtaining rapeseed meal, makes it contain less crude fibre, being more easily digestible [9]. Recent research has shown that a small amount of this meal can be fed to pigs without detrimental effects on growth performance and meat quality. Do et al., 2017 demonstrated that a diet containing 8% rapeseed meal does not harm growth performance in weaned pigs [4]. Also, Choi et al., 2015, demonstrated that the addition of 10% rapeseed meal in the diet does not negatively influence the production performance of finishing pigs [3].

Thus, this study highlights the nutritional value of rapeseed meal through the analysed crude chemical composition, with emphasis on the high protein content, as well as by determining the caloric value of metabolizable energy for monogastric species, poultry and pigs, supporting the use of this feed source in animal nutrition. Knowing the nutritional value of a feed material, before its inclusion in animal diets, makes it possible to apply rational, precision feeding that makes animal production more efficient and increases its use in an economically profitable way. As rapeseed meal is a cheaper feed raw material than soybean meal, by using it, livestock farmers can benefit from higher quality feed at a better price-quality ratio. The good nutritional value and competitive price of rapeseed meal makes it attractive to the feed industry, both as a feed material for the formulation of commercial compound feeds and for local animal feed producers, who can use it in feed mixtures used in monogastric diets.

## CONCLUSIONS

By performing chemical analyses on rapeseed meal, the variation of chemical compounds and their interactions was presented, with special attention paid to the crude protein, crude fiber and nitrogen-free extract content.

Using digestibility coefficients specific for pigs and poultry, in combination with the equations for calculating metabolizable energy according to the ATWATER - 4/9/4 system, the nutritional value of the feed tested for monogastric animals was highlighted.

The direct impact of chemical compounds on the energy value, evident in this study, underlines the importance of knowing the methods to estimate the nutritional value of rapeseed meal in order to integrate it efficiently into animal diets.

The use of mathematical formulations in assessing the nutritional value of feed simplifies the process of formulating animal diets, helping to reduce the economic losses associated with inefficient feed use and, in turn, increasing animal production.

Based on the nutritional values revealed by this study, rapeseed meal can be recommended for use in poultry and pig diets as an economically profitable alternative protein source.

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# BEHAVIOUR OF ANIMAL-ORIGIN GELATINS TO ARTIFICIAL AGEING IN THE PROCESS OF RESTORING GILDED WOOD

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## ABSTRACT

Animal-origin glues are adhesives made by boiling animal tissues for a long time, and from the chemical point of view they are gelatinous and albuminoid protein substances bearing different names. The most frequently used are gelatins. They are extracted from raw animal hide residues, from cartilages, tendons, and from the gelatinous matter extracted from bones, on which the name and quality of gelatin depends. The gelatin extracted from unhaired hide waste/pelt waste resulting from unhairing bovine hide from tanneries was used in this article to study the behaviour to accelerated ageing of two types of gelatins used for gilding some wood samples with gold leaf. In this paper we aimed to find the damaging effects of heat and relative humidity on the gelatines (extracted from bovine hide and rabbit skin) based adhesives used for wood gilding. Therefore, a gold leaf was applied to a fir wood (pine tree) conditioned for ten years using commercial gelatin extracted from rabbit skin as well as gelatin extracted from bovine hide by-products. The gilding technique followed the traditional recipe in which adhesive, multiple layers of plaster, bolus were employed in order to fix the gold leaf. The gilded wood prepared as such was artificially aged in a special Binder-type aging chamber at 60°C for 7 days, followed by conditioning in an incubator at 70% R.H., at 25°C for 2 days, in nine cycles. The physical–chemical changes of gelatin-based adhesives resulting from these conditions, as well as stratigraphic and microstructural characteristics of gilding layers were analyzed by applying optical microscopy, colorimetric measurements and infrared spectroscopy with attenuated total reflection (FTIR-ATR). The obtained results showed that both gelatins have very good adhesive and structural properties and could be successfully used in the field of conservation and restoration.

**Keywords:** gelatin, gilded wood, conservation, restoration

## INTRODUCTION

Museums, castles, and churches house a substantial collection of wooden gilded items, including frames, furniture, religious icons, and statues. To ensure their optimal preservation, it is crucial to comprehend both their composition and the processes of

deterioration. This knowledge guides the establishment of appropriate environmental conditions and restoration procedures. The traditional gilding method involves the application of a gold leaf onto a wooden surface that has undergone prior treatment with multiple layers. In general, the initial layer comprised animal glue, followed by a preparatory coating in the second layer, which was a blend of glue with gypsum or calcite. The third layer, known as bolus, was composed of animal glue, kaolin, and iron oxide [1, 2]. Animal glues, among the earliest adhesives employed by humans, have demonstrated their durability over time. As a result, they are extensively utilized in the restoration of artworks. Animal glues are derived from collagen, which is the primary component found in skin, bones, and connective tissue. They are produced through the prolonged boiling of animal tissues, and from a chemical perspective, they are gelatinous protein compounds known by various names. The most frequently used are gelatins. They are extracted from raw animal hide residues, from cartilages, tendons, and from the gelatinous matter extracted from bones, on which the name and quality of gelatin depends. Residues from the processing of leather in tanneries, known as hide waste, were utilized in the production of gelatin [3, 4, 5, 6].

In this work we aimed to study the damaging effects of heat and relative humidity on commercial gelatin extracted from rabbit skin as well as gelatin extracted from bovine hide by-products for the purpose of their use in gilded wooden historical objects restoration. The long-term stability of gelatins was assessed by subjecting the gilded wood samples to artificial aging within an environment characterized by severe hydrothermal conditions. The research employed optical microscopy, colorimetric measurements, and infrared spectroscopy with attenuated total reflection (FTIR-ATR) to analyze the stratigraphic and microstructural features of the gilding layers, as well as the physical and chemical alterations in the gelatin-based adhesives.

## MATERIALS AND METHODS

Gelatin extracted from decalcified bovine hide by-products was prepared at the National Research and Development Institute for Textile and Leather, Division Leather and Footwear Research Institute in Bucharest with the purpose of providing the restoration field with a material with a much better extraction yield and a much lower price compared to rabbit skin gelatin, commercial product. The gelatin extraction was carried out at a solid/liquid ratio of 1:3, at acidic pH lower than the isoelectric point, adjusted with formic acid, under continuous stirring for 5 hours at 70oC. After hot filtration, settling-cooling at a temperature of max. 10oC for 14-16 hours, the gelatin fraction with a homogeneous appearance was collected, which was later dried at 60oC in exhaust mode and chopped as flakes. Analytical data indicate that the gelatins extracted under these conditions has an average molecular mass of over 40 kDa and a strength of 700 grams, determined by the Bloom test under standard conditions, which also indicates a very strong cohesion.

Commercial gelatin extracted from rabbit hide was purchased from “Divolo” company. Both gelatins were individually employed in the gilding process of a pine wood stick that had been conditioned for ten years. The gilding process adhered to the traditional recipe, which involved using gelatine, multiple layers of plaster, and bolus to affix the gold leaf.



*Images from the gilding process*



*Images of gilded wood samples:*
*Up- with bovine hide glue; Down- with rabbit skin glue*

The gilded wood prepared as such was artificially aged in a special Binder-type aging chamber at 60°C for 7 days, followed by conditioning in an incubator at 70% R.H., at 25°C for 2 days. Table 1 shows the symbols of new and artificially aged gilded wood samples and the aging conditions.

**Table 1**. List of the new and artificially aged samples and their ageing conditions.

| **Sample** | **Ageing conditions** | | **Sample** | **Ageing conditions** | |
|---|---|---|---|---|---|
| gilded wood samples for which gelatin extracted from rabbit hide was used | | | gilded wood samples for which gelatin extracted from bovine hide by-products was used | | |
| P0_CI | untreated | | P0_GBP-C | untreated | |
| P1_CI | 168 h at 60°C | 48 h at 70% R.H. and 25°C | P1_GBP-C | 168 h at 60°C | 48 h at 70% R.H. and 25°C |
| P2_CI | 336 h at 60°C | | P2_GBP-C | 336 h at 60°C | |
| P3_CI | 504 h at 60°C | | P3_GBP-C | 504 h at 60°C | |
| P4_CI | 672 h at 60°C | | P4_GBP-C | 672 h at 60°C | |
| P5_CI | 840 h at 60°C | | P5_GBP-C | 840 h at 60°C | |
| P6_CI | 1008 h at 60°C | | P6_GBP-C | 1008 h at 60°C | |
| P7_CI | 1176 h at 60°C | | P7_GBP-C | 1176 h at 60°C | |
| P8_CI | 1344 h at 60°C | | P8_GBP-C | 1344 h at 60°C | |
| P9_CI | 1512 h at 60°C | | P9_GBP-C | 1512 h at 60°C | |

Stereomicroscopic examinations of the gilded wooden wands' surface were conducted using a fixed optical stereomicroscope, specifically the StereoZoom Leica S8 APO, at 20X and 160X magnification.

FTIR ATR measurements were conducted using an ALPHA FTIR instrument from Bruker Optics, Germany, which was equipped with a Platinum ATR module. Spectra were obtained by averaging 32 scans within the range of 4000 to 400 $cm^{-1}$, with a spectral resolution of 4 $cm^{-1}$. The Opus 7.0 software from Bruker Optics was utilized for acquiring and processing the spectra, while the graphical representation was generated using Origin 7.5 software.

The colorimetric analysis was conducted utilizing a DataColor Check II Plus spectrophotometer, which features diffused illumination and an 8º viewing angle. It utilized a pulsed xenon light source with a spectral range of 360-700 nm and had effective bandwidth and wavelength bandwidth of 10 nm and 2 nm, respectively. For calibration, black and white standards were employed. The analyses were conducted with measurement times of less than 2.5 seconds, covering a measuring range between 0-200% reflectance, and employing an aperture spot size of 6.5 mm. Each surface was examined three times to establish an average and to detect any discrepancies within the gilded wooden wand surfaces. Variations in individual color components (Δa and Δb), in brightness (ΔL), as well as the overall color deviation (ΔE), were computed based on the CIEL*a*b* (1994) color space theory [7, 8, 9] using the Microsoft Excel program.

## RESULTS

In Figure 1, stereomicroscopic images (20X/160X) of specifically chosen artificially aged gilded wood samples are presented. It was noticed that, as a result of the ageing process, cracks of relatively large sizes were identified, of 102 μm and 360 μm, in the case of using rabbit skin gelatin (samples P8_CI and P9_CI), while, in the case of bovine hide gelatin, cracks of smaller sizes are noticed, of 233 μm and 61 μm (samples P8_GBP-C and P9_GBP-C).

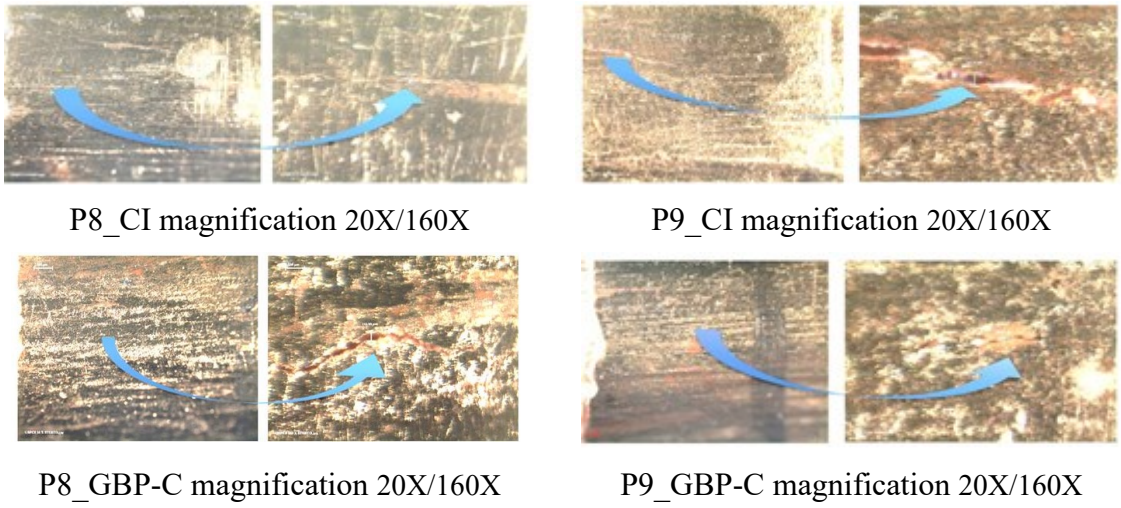



**Figure 1.** Stereomicroscopic images (20X/160X) of new and some artificially aged gilded wood samples.

Colorimetry helped quantify the change in colour after artificial ageing. This assessment technique is necessary to evaluate the yellow and golden colour of gilded wood samples subjected to ageing because the colour space is particularly narrow, in terms of the wavelength from the visible spectrum of these colours [10]. The results of the colorimetric measurements as well as the consequent computation of brightness (*ΔL*), colour component (*Δa* and *Δb*), and overall colour variations (*ΔE*) are summarised in Table 2.

**Tabel 2.** Overall colour variation (ΔE) and changes in colour coordinates (Δa and Δb) and brightness (ΔL) for the new and artificially aged gilded wood samples for which gelatin extracted from rabbit skin and bovine hide was used.

| **Sample** | **ΔL** | **Δa** | **Δb** | **ΔE** | **Sample** | **ΔL** | **Δa** | **Δb** | **ΔE** |
|---|---|---|---|---|---|---|---|---|---|
| P0_CI | untreated | | | | P0_GBP-C | untreated | | | |
| P1_CI | 6.90 | 0.31 | 8.90 | 11.27 | P1_GBP-C | 3.13 | -0.29 | 1.67 | 3.56 |
| P2_CI | 5.84 | 0.30 | 5.27 | 7.87 | P2_GBP-C | 1.76 | -0.07 | -0.94 | 2.00 |
| P3_CI | 6.86 | -0.08 | 7.09 | 9.87 | P3_GBP-C | 3.04 | 0.41 | 2.19 | 3.77 |
| P4_CI | 8.86 | -0.92 | 5.05 | 10.24 | P4_GBP-C | 4.69 | 0.66 | 3.42 | 5.84 |
| P5_CI | 1.45 | 0.72 | 4.70 | 4.97 | P5_GBP-C | 4.33 | -0.05 | 5.73 | 7.18 |
| P6_CI | 7.10 | -0.08 | 6.17 | 9.41 | P6_GBP-C | 6.50 | 0.43 | 8.88 | 11.02 |
| P7_CI | 0.37 | 0.89 | 2.39 | 2.58 | P7_GBP-C | 3.05 | -0.46 | 1.95 | 3.56 |
| P8_CI | 1.15 | 1.15 | 3.29 | 3.67 | P8_GBP-C | 5.55 | -0.20 | 4.52 | 7.16 |
| **P9_CI** | **1.95** | **0.40** | **2.01** | **2.83** | **P9_GBP-C** | **3.08** | **0.22** | **3.62** | **4.76** |

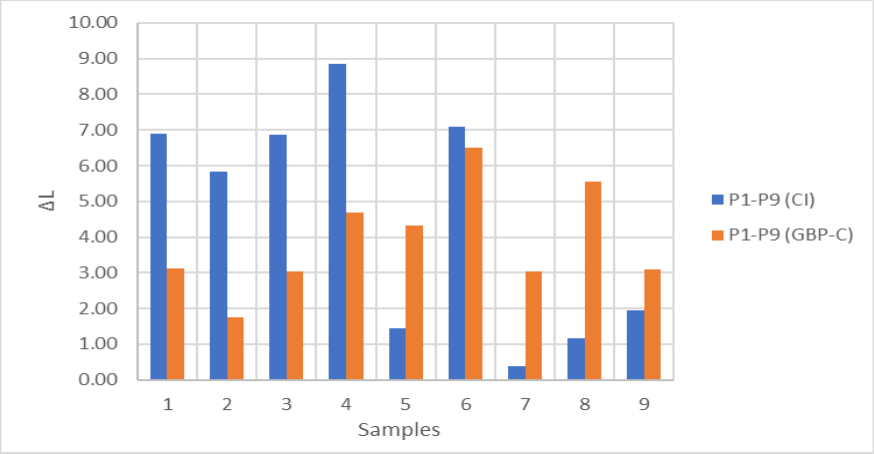



**Figure 2.** Variation of brightness for both types of samples (CI and GBP-C), relative to P0_CI for samples with rabbit skin glue and P0_GBP-C for samples with bovine hide glue.

The FTIR-ATR spectra of gelatins extracted from rabbit skin (CI) and bovine hide by-products (GBP-C) are illustrated in Figure 3. The prominent absorption bands, associated with vibrational transitions in the peptide chain, were identified in both gelatins: amide A ($A_A$) at 3275 $cm^{-1}$, linked to stretching vibrations of the amide N–H bonds; amide B ($A_B$) at 3065 $cm^{-1}$, attributed to the Fermi resonance overtone of the amide II vibration; amide I ($A_I$) at 1630 $cm^{-1}$, primarily arising from the C=O stretching vibration; amide II ($A_{II}$) at 1545 $cm^{-1}$, associated with NH in-plane bending and CN stretching vibration; and amide III ($A_{III}$) at 1232 $cm^{-1}$, corresponding to NH bending, CN stretching vibration, with minor contributions from both CO in-plane bending and CC stretching vibration [11-13]. The most used spectral regions to the protein secondary structural components are the amide I (1700−1600 $cm^{-1}$) and amide II bands (1600−1500 $cm^{-1}$) [14-18].

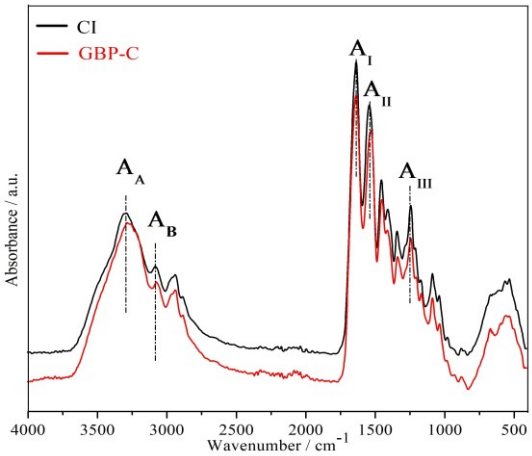



**Figure 3.** FTIR-ATR spectra of gelatins extracted from rabbit skin (CI) and bovine (GBP-C) hide. The main gelatin absorbtion bands ($A_A$, $A_B$, $A_I$, $A_{II}$ and $A_{III}$) are highlighted.

The FTIR analysis of both new and artificially aged gilded wood samples (Figure 4) confirmed the presence of both calcium carbonate (at 1788, 1415, and 875 $cm^{-1}$) and

kaolinite (at 3695, 3605, 1103, 1022, 995, and 900 $cm^{-1}$), which were utilized in the gilding process of the wooden stick. Due to the overlap between the absorption band of calcium carbonate (starting from 1415 $cm^{-1}$) and that of amide II, only the amide I band was taken into consideration for evaluating gelatin deterioration resulting from artificial aging.

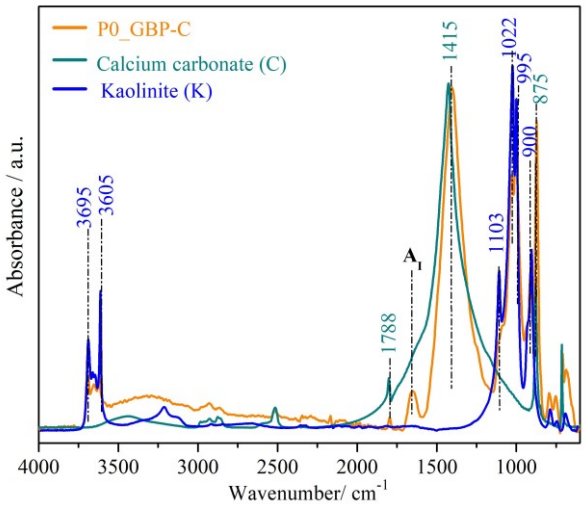



**Figure 4.** FTIR-ATR spectrum of the untreated sample P0_GBP_C as compared with those attributed to calcium carbonate (1788, 1415 and 875 $cm^{-1}$) and kaolinite (3695, 3605, 1103, 1022, 995 and 900 $cm^{-1}$).

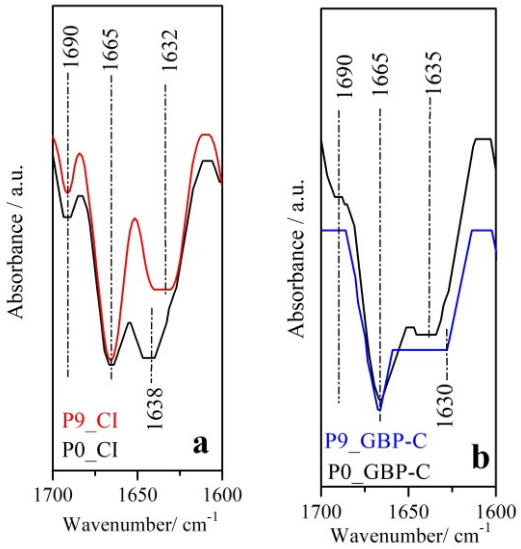



**Figure 5.** (a) The second derivative FTIR-ATR spectra in the $A_I$ region (1700–1600 $cm^{-1}$) of the untreated sample P0_CI and the artificially aged one P9_CI and (b) The second derivative FTIR-ATR spectra in the amide I region (1700–1600 $cm^{-1}$) of the untreated sample P0_GBP-C and the artificially aged one P9_GBP-C. The components of $A_I$ are highlighted.

## DISCUSSION

From a colorimetric point of view, the data in table 2 are very important for determining the color change, as a result of the accelerated aging process. Samples P8_CI, P9_CI, P8_GBP-C, P9_GBP-C can be considered as the most relevant in defining the characteristics of gelatins, because they participated in all accelerated aging cycles. Thus, it can be seen from figure 2, where the ΔL parameter was highlighted, i.e., that of the brightness variation, that samples P8_GBP-C and P9_GBP-C had a better behavior compared to samples P8_CI and P9_CI, because the surface of the samples in which gelatin extracted from rabbit skin was used shows cracks that reduce the brightness parameter. This phenomenon correlates with the morphological changes of the surface of the samples, observed by stereomicroscopy and shown in figure 1.

According to FTIR-ATR spectra, the second derivative spectra of both new and artificially aged gilded wood samples in the spectral range of amide I facilitated the identification of different secondary structures within the gelatin. Specifically, α-helices were identified at approximately 1665 $cm^{-1}$, β-sheets at around 1690 $cm^{-1}$, and random coils at about 1638-1630 $cm^{-1}$. [14-18]. It is noteworthy to emphasize that subtle alterations in the molecular structure of both gelatins, employed in the gilding process,

were observed only after the eighth aging cycle. Figure 5 shows the second derivative FTIR-ATR spectra in the $A_I$ region (1700–1600 $cm^{-1}$) of the untreated samples (P0_CI and P0_GBP-C) and the artificially aged ones (P9_CI and P0_GBP-C). It was observed that the amide I component at 1635-1638 $cm^{-1}$ (associated with random coil structures) shifted towards shorter wavenumbers (1630-1632 $cm^{-1}$), whereas the amide I component at 1665 $cm^{-1}$ (related to α-helices) showed no alterations. Regarding the amide I component corresponding to β-sheets (~ 1690 $cm^{-1}$), it remained unaltered in the case of the gelatin extracted from rabbit skin after nine cycles of artificial aging, whereas for the gelatin extracted from bovine by-products, it completely disappeared.

## CONCLUSION

This study was carried out in order to recover a waste, i.e., the scraps of unhaired hide (pieces of hide from the neck area, knees or belly, of variable sizes that cannot be processed into finished leather due to their irregular topography) from the leather industry in order to make glue or gelatin necessary for the restoration field. The extraction of gelatin was carried out through an original process, and the behavior of bovine hide gelatin was compared with that of rabbit skin gelatin, a commercial product. Two types of gilding experiments were carried out in parallel: one with the use of rabbit skin gelatin, the other with bovine hide gelatin, according to a classic procedure for restoring gilded wood, in which a 24-karat gold leaf was glued onto a wooden support prepared with several layers of mixture (bolux, bovine hide/rabbit skin gelatin, plaster). The samples were subjected to accelerated aging, up to 9 cycles of varying the temperature from 25°C to 60°C, for 168 hours/cycle and humidity from 10 to 70% RU, for 48 hours/cycle. The methods of evaluating the accelerated aging behavior of the samples were stereomicroscopy, color determination by the CieLa*b* method and ATR-IR, which highlighted a superior behavior of bovine hide gelatin compared to rabbit skin gelatin for the restoration of gilded wood.

The bovine hide gelatin ensured a very good cohesiveness and adhesion of the materials used for gilding, compared to the rabbit skin gelatin which showed cracks in the gold leaf following the variation of temperature and humidity parameters.

## ACKNOWLEDGEMENTS

This work was supported by the grants of the Romanian National Authority for Scientific Research and Innovation CCCDI - UEFISCDI, project CAPTAN, PN-III-P3-3.5-EUK-2019-0196 / contract no. 253/2021 and by contract no. 4PFE/2021, PNCDI III - Program 1 – Development of the national RD system, Subprogram 1.2-Institutional Performance-RDI excellence funding projects.

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# BIOACTIVE COMPOUNDS FROM HORSERADISH FOR BIOTECHNOLOGICAL APPLICATIONS

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## ABSTRACT

The horseradish (*Armoracia rusticana*) is a root vegetable that belongs to the botanical family *Brassicaceae* and due to the high content of bioactive compounds, it has of great interest in many biotechnological fields. The high content of polyphenols, phenols, flavonoids, vitamin C and detoxification enzymes makes horseradish to have a great antioxidant potential and positive effects on the health, being used from ancient times as medicinal plant. The abundance of minerals, vitamins and dietary fibre make horseradish worth to be considered as a natural functional food with a high energy value. In addition to all these biologically active compounds with specific properties for the pharmaceutical and food industry, peroxidase from horseradish (HRP) is known as a green biocatalyst for degradation of wastewater containing phenol. For economical efficient biocatalysts processes, and for a better stability of HRP in the harsh conditions of phenol degradation, immobilized enzymes are frequently used. All the immobilization methods have proven to be effective for HRP, starting to binding methods (physical adsorption, covalent binding), crosslinking, encapsulation and entrapment in porous biomaterials. The immobilized enzymatic preparations showed high specificity and selectivity and very good efficiency in removing the hazardous substants. HRP in immobilized form was also used to develop smart biosensors for detection of pollutants from wastewaters resulted from industrial processes.

**Keywords**: horseradish, bioactive compounds, peroxidases-green biocatalyst, pharmaceuticals, functional-food

## INTRODUCTION

Among the vegetables of the *Brassicaceae* family, horseradish (*Armoracia rusticana*) is of great interest, being an exceptionally rich source of biologically active compounds that could be used for biotechnological purposes. Horseradish is a perennial plant, cultivated especially in Europe and North America. Horseradish is an extremely resistant plant, which can be productive for at least 10-15 years. Both, the root and the leaves of horseradish contain significant amounts of bioactive compounds for agro-industrial and pharmaceutical purposes [1]. The special properties of horseradish have made it to be used since ancient times both as a medicinal plant and as a spice. The efficient use of horseradish plants (roots, leaves and even seeds) requires extraction of bioactive compounds by environmentally friendly methods, using solvents other than conventional ones and optimization of the processes in order to obtain compounds with high bioactivity and selectivity and low toxicity [2].

## MATERIALS AND METHODS

The work was carried out as a result of studying bibliographic references, of which 82% are from the last 5 years.

## RESULTS AND DISCUSSION

### Biological active compounds in horseradish

The biologically active compounds found in the greatest amount in horseradish are glucosinolates (GSL). Until now up to 200 glucosinolates are identified [3]. GSL are sulfur-containing biochemical compounds synthetized as secondary metabolites in plants such as *Brassicaceae* family, including horseradish. Several authors have found sinigrin as the major GSL in horseradish. The research published by Popovic [4] noticed that among all analysed GSLs the three glucosinolates that are found in the highest proportion in horseradish roots are gluconasturtiin (64.9%), sinigrin (33.91%) and glucobrassicin (1.19%). In the same study in the horseradish leaves sinigrin was found in a percentage greater than 99.9%. In fresh, unprocessed horseradish, GSLs are hydrolyzed by myrosinase to several products like isothiocyanates, nitriles, thiocyanates, indole-3-carbinols and oxazolidine-2-thiones [4].

Horseradish plants contain also (1) flavonoid glycosides, mostly based on kaempferol and quercetin structures, (2) organic acids - ferulic acid, gallic acid, 4-hydroxybenzoic acid, malic acid, caffeic acid, syringa acid, chlorogenic acid, sinapinic acid, citric acid, and γ-aminobutyric acid and fatty acids - eicosadienic acid, 13*Z* or 13*E*-hexadecenic acid-, 3-hydroxypalmitinic acid, 9,10-dihydroxyhexadecanic acid, (9*Z*)-17-hydroxy-9-octadecanic acid-, 14-hydroxystearinic acid, myristinic acid, pinolenic acid, 10,11-dihydroxystearinic acid, 3-*p*-cumaroyl-quinic acid, 5-*p*-cumaroyl-quinic acid, (3) vanillin, (4) aminoalcohol - sphingosin, (5) tannin - glucogallin, (6) phosphoglycerides, (7) phosphatidylinositole, (8) amino acids - arginine, lysine, and proline [5].

Knez (2022) [6] published a very interesting review about the composition of root vegetables carrot, radish, potato, yam, ginseng, celery, parsley, horseradish (Figure 1).

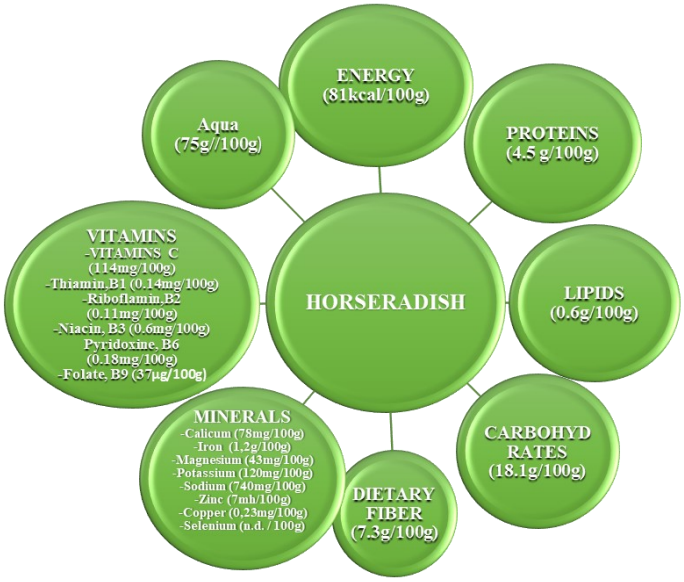



Figure 1. Composition of horseradish roots [6]

Horseradish, among the other root vegetables, has the smallest content of water and the highest energy value. All these root vegetables have a high content of fibre, horseradish being the richest.

Compared to other root vegetables, horseradish is the richest in other biochemical compounds: proteins, lipids, carbohydrates, minerals, vitamins. The richness in vitamin C is 2.5 times higher than in parsley root, and 14 times higher than in celery root.

In addition to the bioactive compounds known in horseradish plants, Negro (2022) [5] have carried out a study of the phytochemical composition of horseradish extract and new compounds that have not been described before have found.

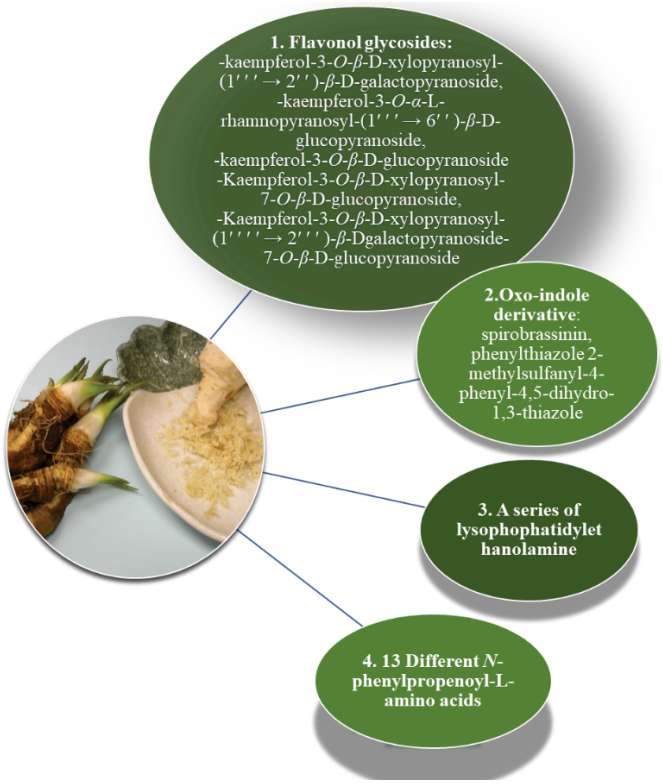
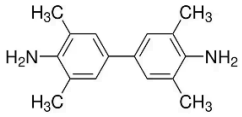



Figure 2. New compounds identified in fermented and non-fermented horseradish root extracts [5]

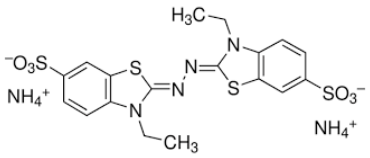
Another very representative bioactive compound in horseradish is the enzyme peroxidase. Horseradish peroxidase (HRP) (EC 1.11.1.7) is an enzyme from the class of oxidoreductases, with wide applicability in industrial biocatalysis, pollution and environmental applications, but also in clinical diagnosis.

HRP is a heme-enzyme with many isoforms and molecular weight of 44 KDa. Its structure includes a polypeptide chain with four disulfide bridges, hemi-$Ca^{2+}$ group and carbohydrate, being a glycoprotein. The heme group is essential for a proper biocatalytic activity, being redox cofactor. The reaction catalysed by HRP is oxidation of organic compounds involving the hydrogen peroxide. Horseradish peroxidase has an accessible active site and many compounds can bind to the reaction site. The intermediary complex formed between HRP and $H_2O_2$ oxidizes in the next step of the reaction mechanism a different chromogenic hydrogen donor. Commonly chromogenic hydrogen donors used as HRP substrates [7] are 3,3',5,5'-tetramethylbenzidine (TMB), 2,2' -azino-di-[3-

ethylbenzthiazoline-6-sulfonic acid] (ABTS), chemiluminescent substrates - luminol and isoluminol, fluorogenic substrates - tyramine, homovanillic acid, and 4–hydroxyphenyl acetic acid etc (Figure 3). HRP is inhibited by various organic compounds (azides, dichromate, cyanides, L–cystine, ethylenethiourea, hydroxylamine, sulfides, *p*–aminobenzoic acid), or transition metal cations ($Cd^{+2}$, $Cu^{+2}$, $Co^{+2}$, $Fe^{+3}$, $Ni^{+2}$, $Mn^{+2}$, $Pb^{+2}$). The optimum pH of HRP is at a near-neutral pH (6.0-6.5) [7].

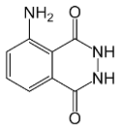


3,3',5,5'-tetramethylbenzidine (TMB)

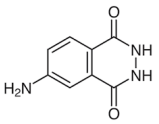


2,2' -azino-di-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS)

**Chemiluminescent substrates**

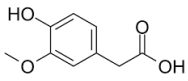


luminol

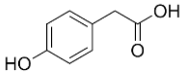


isoluminol

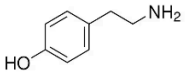
**Fluorogenic substrates**



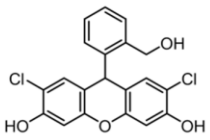
homovanillic acid



4–hydroxyphenyl acetic acid



tyramine



2,7-dichloro-9-(2-(hydroxymethyl) phenyl)-9H-xanthene-3,6-diol

Figure 3. Some of the most used HRP substrates [7]

Even HRP is a robust enzyme, it has a good stability in the pH range 5 to 9, the industrial application requires high catalytic activity and high operational, thermal stability and long-time preservation.

Arnodo (2023) [8], in a very well documented review, noticed that the activity, stability and the performance of HRP appeared to be higher in deep eutectic solvents (DESs) based on ChCl. The all DESs investigated have shown to highly stabilize the HRP structure.

DESs are not expensive and biocompatible solvents [9]. Biocatalysis in DESs has been exploited for HRP in the reaction of free radical polymerization of acrylamide in ChCl/U 1:2 and ChCl/Gly 1:2. The tested catalytic system was HRP/$H_2O_2$/2,4-pentanodione, temperature $50^0$C. In all the tested catalytic systems the polymerization reaction yield was higher than 90%.

Non-conventional solvents, such as DESs, used in biocatalytic transformations develop more sustainable processes by increasing the efficiency and performance of enzymes. The redox transformation catalysed by HRP in DESs were carried out higher conversion rate compared to biotransformation in aqueous solvents [8].

The immobilization of enzymes in/on insoluble supports is an important tool to increase enzyme stability and to reuse the enzymes in industrial processes. By immobilizing the enzymes, the biocatalytic activity upon reuse is maintained and the catalytic and operational stability in different reaction media is improved. The production costs of biotechnological products are greatly reduced by reusing the enzymes. The supports used for enzymes immobilization are both organic and inorganic. The supports can be made of single biomaterials or of a mixture of them that lead to hybrid matrices. The type of support is chosen according to the technological process in which the enzymatic biocatalyst is used. For biotechnological applications the hybrid matrices are more suitable.

The mixture Ca alginate-starch was tested as hybrid support for immobilization of HRP. The immobilized biocatalyst was successfully applied in the field of pollutant bioremediation. The biodegradation of phenol red dye was evaluated in a batch system with free and immobilized HRP. The immobilized HRP exhibited a higher biodegradation capacity compared to the free enzyme, the quantity of the phenol red dye degraded by the immobilized HRP was 10 times higher in the same timeframe. Reusability of Ca alginate-starch immobilized HRP in the biodegradation reaction of phenol red dye was studied in 15 reuse cycles. The biodegradation capacity decreased constantly, but after 6 reuse cycles Ca alginate-starch-HRP still showed approximately 40% of its initial capacity. After 15 cycles of reuse the morphology of porous beads containing the enzymes remained unchanged. [10]

Bilal (2019) [11] immobilized HRP in a hybrid agarose-chitosan hydrogel, the cross-linker used was N-hydroxysuccinimide. The immobilization efficiency of HRP in the hydrogel was up to 90%. The immobilized HRP have shown wide-working pH and temperature stability, and good reusability and resistance of biomaterial containing enzyme in the oxidation of RB-19 dye, without the loss of enzymes.

HRP was entrapped in silica nanospheres synthetized by Triton X-100 (TX-100) and tetraethoxysilane (TEOS). The results of the study revealed that the catalytic activity of the entrapped HRP could be retained, the enzymatic activity of the immobilized preparation toward the substrate TMB was good. The entrapped HRP performed good electrocatalytic reduction toward $H_2O_2$. The native structure of the enzyme into the nanotailored support was investigated by spectroscopic methods. It turned out that the structure of the protein enzyme molecules was retained. The bioelectrode obtained by HRP entrapped in silica nanospheres could effectively achieve direct electron transfer. The electrochemical response of HRP confined in silica nanospheres was good. That could be due the biocompatibility of the silica nanospheres, having good effect on preserving the structure and activity of enzyme. On the other hand, in the morphology of support there are mesoporous tunnels that can accelerate the electron transfer between heme-HRP entrapped in nanopores and the electrode [12]

Yang (2023) [13] synthesized a hollow CuO/Cu2O octahedron adsorbent for HRP immobilization. The surface of the adsorbent was grafted with a mixture of 3-aminophenyl boric acid (APBA), polyoxometalate (PW9) and silanization reagents. The

toxicity of the products after the degradation of phenol was studied on aquatic plants growth in different waters: clean water, biotreated wastewater with immobilized HRP and phenol-containing wastewater. The plants have grown normally in the solution after the phenolic wastewater treatment, proving that adsorbed HRP efficiently reduced the toxicity of phenol.

An efficient immobilization method of horseradish peroxidase enzyme was done on transition metal carbides, as a result of progress in biotechnological applications of 2D nano-biomaterials. HRP was immobilized by adsorbtion on $Ti_3C_2$ and $Ti_3C_2OH$ by noncovalent method. This method has the advantage that preserve the conformational structure of the enzyme through immobilization process, which was demonstrated by studies of the secondary structure of HRP adsorbed on MXenes surface. Also, the higher stability of immobilized HRP at higher temperature and acidic conditions was demonstrated [14].

In the scientific literature of the last years several nano-biomaterials are mentioned to be used for HRP immobilization used in biocatalytic degradation of pollutants from the environment. Bilal (2020) [15] in a review noticed them as being carbon nanoonions (CNOs), nanodots (NDs), graphene oxide (GO), and reduced graphene oxide (rGO), MNPs and Fe3O4-nanodiamond (ND), nanofibers (NFs), nanogel (NG), metal-organic frameworks (MOFs) and ordered mesoporous silica (OMS). The HRP immobilized using nanostructured carriers are multifunctional applications.

**Application of biological active compounds from horseradish plants**

The compounds with biological activity in horseradish have effects on the functions of living organisms (humans, insects or microbes), but also on the properties of food. That is why horseradish extract is used to obtain pharmaceutical products with health promoting effects, antimicrobial effects, insecticidal effects, improving and preserving conventional food, obtaining biofunctional food, improving food packaging materials (Figure 4) [5].

The intensity of the taste and flavour of horseradish roots made them a very used spice in the food industry to give specific piquant aroma to many kinds of dishes, no matter if they are vegetables or meat, cooked or pickled. The horseradish spices also have a protective effect on food, protecting it from spoilage. The specific aroma of horseradish is given by the constituent volatile compounds - isothiocyanates. These sulphur compounds are released under the action of myrosinase after grinding horseradish root [16], [1].

Since ancient times, horseradish has been used as a medicinal plant, being used to treat many ailments. Over the years all these properties of horseradish used in the treatment of diseases have been intensively studied and their positive effect has been scientifically proven. Moreover, other pharmacological activities were highlighted. Nowadays commercial products can be found in the market for infections treatment [16], [1], [5].

The antimicrobial activity against pathogenic bacteria, antifungal and insecticidal activity of horseradish bioactive compounds must also be mentioned. The reduced toxicity of the volatile biocomponents of horseradish makes it interesting for the agro-industrial field, being an alternative to synthetic insecticides.

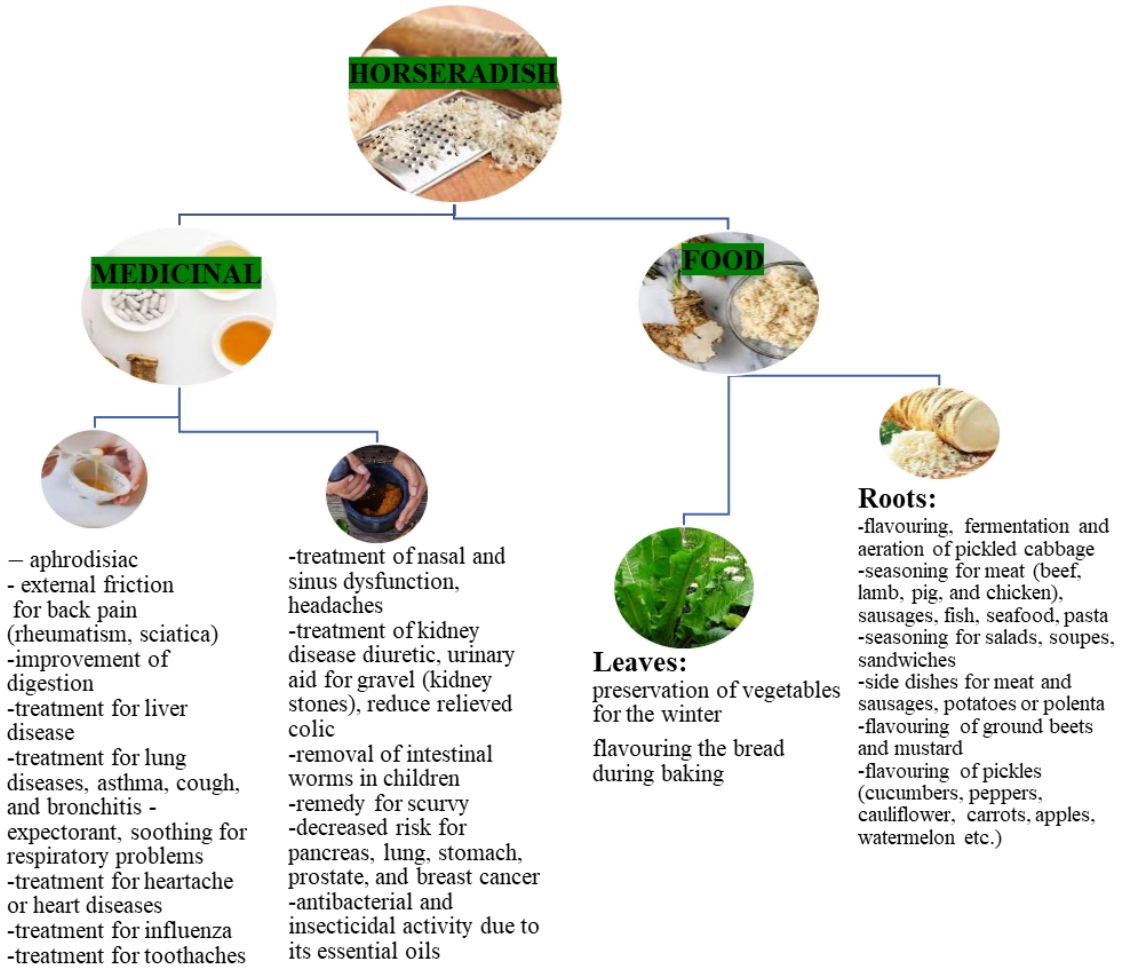



Figure 4. Biological activity of horseradish compounds [1]

The immobilized HRP, for most of the methods or carriers tested, have shown improved biocatalytic activity, for different applications. The fields where the immobilized enzyme can perform successfully are biotechnology, including detection and degradation of pollutants from the environment, biosensing, but also other industrial biocatalytic processes [15].

It is known that industrialization brings with it an important pollution of the environment and all of these pollutants have negative effects on humans and other living organisms as a result of contact with water, air and soil, as parts of the contaminated environment. There is a high demand for performant monitoring systems. The smart biosensors based on immobilized HRP are characterized by selectivity, sensitivity, and reproducibility and thus have an important role in the detection of specific pollutants [17].

Horseradish peroxidase is used in very specific analytical methods like immunochemistry, immunoblotting and immunohistochemistry. In this techniques HRP is used as a label for Immunoglobulins. The connection between antibodies and HRP is made by cross-linking [7].

## CONCLUSION

The horseradish is a true reservoir of bioactive compounds with beneficial effects on the health. The degradation products of the glucosinolates have shown biological activities, including anti-inflammatory, anticarcinogenic, antioxidant, antimicrobial, gastroprotective effects. Isothiocyanates are the main volatile compounds of horseradish which ensures the flavour and aroma of this vegetable. Horseradish bioactive compounds change the conventional food and transform it in biofunctional food with high nutritional value, having also food preservation activities.

Horseradish peroxidase is a very efficient biocatalysts used more and more in green technologies based on its high activity, selectivity and specificity. The immobilized HRP perform in the harsh conditions of the industrial processes, having a better operational and in time stability, reusability without losing the biocatalytic activity. HRP was successfully immobilized using different immobilization methods, in several biomaterials (organic, inorganic and hybrid porous hydrogels, nanomaterials, microcapsules etc.).

The future research will address different sustainable procedures for the extraction of biologically active compounds from horseradish, in order to obtain compounds with the best possible biological activity. Based on its qualities, the applications of HRP can be studied in many other fields as well, for different purposes, with immobilized enzyme on/in numerous previously untested organic-inorganic hybrid supports.

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# CHARACTERISATION OF GENETIC DIVERSITY OF SOME BREAD WHEAT GROWN IN SEMI-ARID ENVIRONMENT IN ALGERIA USING SSR MARKER

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## ABSTRACT

The assessment of genetic diversity is a key step in evaluating the adaptation of populations to new environmental conditions, and thus in the selection of new cultivars. The present investigation aimed to assess genetic variability of bread wheat (*Triticum aestivum* L.) genotypes grown in Algeria. The 17 hexaploid genotypes of bread wheat were assessed using 16 molecular microsatellites of SSR (Simple Sequence Repeat). Among the 16 microsatellite markers tested, only 11 markers were the most polymorphic and reproducible. The Polymorphism Information Content (PIC) values per locus varied from 0.14 to 0.70 with an average of 0.48 and 0.49. Genetic similarity between genotypes varied from 0.27 and 0.92 with an average of 0.60. The highest genetic distance value of 0.92 has been scored between Kauz/Pastor/Fiscal and Wbll1*2/Brambling. The lowest value of 0.27 scored between Cham6 and Pastor/Wbll1. Genetic similarity calculated from molecular data and used to produce a dendrogram. The genotypes involved were divided into two clear groups according to their origin and pedigree. The first cluster included wheat genotypes Ain Abid (local genotypes), Attila/2 Pastor, and 5119 (introduced genotypes). The second group revealed high polymorphism and subdivided into four sub-groups. The high diversity revealed among the wheat accessions grown in Algeria is likely to be used in genetic improvement programmes.

**Keywords:** *Triticum aestivum*, PIC, Microsatellites, Genetic diversity

## INTRODUCTION

The cereals and derivatives sector is one of the mainstays of the agri-food industry in Algeria. This importance is due, in particular, to the predominant role played by cereals and cereal derivatives in human nutrition, especially semolina (couscous and other cereals).Cereals and their by-products play in the human diet, particularly semolina (couscous and pasta) and flour (bread), as well as in the food industry. Bread wheat (*Triticum aestivum* L.) has a total production of more than 600 million tons per year; it is one of the richest crops in calories and protein (Oury and Godin 2007; Grassini et al. 2013)[29][14].

A growing world population requires a steady increase in wheat production. However, wheat production in Algeria is characterised by highly unstable fluctuations. Algerian wheat production remains relatively low and unstable from one year to the next, mainly due to highly variable and often unfavourable climatic conditions: irregular rainfall, disease, drought, etc. (Allahverdiyev 2015; Toklu et al. 2015; Ozlem and Begum 2018)[3][30][39]. This situation leads to significant variations in production and yield. The lack of water and the poor distribution of rainfall over the year largely explain the significant variations in cereal production (Boudour at al. 2011; Kara and Brinis 2012)[8][25].

Algeria is one of the world's leading wheat importers (with 65% of the African market), due to the limited resources available to the national industry to meet the growing consumption needs of the population (Maggie 2000; Kara and Brinis 2012)[18][25]. Bread wheat and durum wheat are the main staple foods for consumers in this country, with local cereal production accounting for just over 30% of the country's needs. Wheat is grown in Algeria over an average area of 1.9 million hectares, but yields are low at 18kg/ha for durum wheat and 17kg/ha for common wheat. (CIC 2010)[10].

In semi-arid regions such as Algeria, variations in bread wheat yields largely linked to the effects of water and heat stress (Rampino 2006; Flagella et al. 2010; Farzad et al. 2013; Kara et al. 2014; Yao et al. 2014)[32][13][12][40]. This situation requires a variety of improvement strategies. It is essential to identify genetic variations if genetic resources managed and used optimally (Chao 2007)[9]. Various markers suitable for genetic diversity studies identified and developed a few years ago. The emergence of new PCR-based molecular markers, such as SSRs and SNPs, has made it possible to characterise collections of genetic material that were previously impossible to collect, and to launch a productive wheat-breeding programme. (Drikvand et al. 2013; Reza et al. 2015; Allen et al. 2017)[11][4][33]. Microsatellites have a high potential use for the genetic analysis because of their high degree of polymorphism and they are codominantly inherited (Huang et al. 2002[15]; Ali et al. 2008[2]; Reza et al. 2015)[33]. Therefore, the present study aims to estimate the coefficient level of genetic variation among seventeen of local and introduced genotypes of bread wheat using microsatellites SSR markers.

## MATERIALS AND METHODS

### Plant materials collection.

This research carried out on the ITGC experimental farm. (Technical Institute of High Culture) in Constantine /Algeria. In this investigation, three local varieties and fourteen introduced genotypes were represented in Table 1.

**Table 1.** Origin and pedigree of the studied genotypes

| N° | Genotypes | Pedigrees | Origin |
|---|---|---|---|
| **V1** | **Ain abid** | ITGC/ Algeria | Algeria |
| **V2** | **Arz** | ITGC/ Algeria | Algeria |
| **V3** | **Hidhab** | ITGC/ Algeria | Algeria |
| **V4** | **Hamam 1** | ICW92-0455-1AP-1AP-2AP-3AP-0AP | ICARDA/Syria |
| **V5** | **5119** | | SERASENE /France |
| **V6** | **Milan/S87230/babax** | CMSS97MO-3687T6040Y-03M-020Y-030M-015Y-38M | CIMMYT/ Mexico |

| | | | |
|---|---|---|---|
| **V7** | **Angi-4** | ICW92-0326-12AP-1AP-2AP-3AP-0AP | ICARDA/Syria |
| **V8** | **Cham 6** | CM39992-8M-7Y-OM-0AP | CIMMYT/ Mexico |
| **V9** | **Attila** | CM85-836-50Y-0M-OY-3M-0Y | CIMMYT/ Mexico |
| **V10** | **Attila/2 Pastor** | CGSS97Y00042M-099T0PB-058Y-099M-099Y-099B | CIMMYT/ Mexico |
| **V11** | **Pastor/Wbll1** | CMSA00Y00586-0POY 040M 040SY-030M-17ZT | CIMMYT (Mexico) |
| **V12** | **Babax/Lr42//Babax*2/3vivitsi** | CGSS01B00046T-099Y-099M-099M-099Y-21Y-0B | CIMMYT (Mexico) |
| **V13** | **Wbll1*2/Brambling** | CGSS01B00062T-099Y 099M-099M-099Y-099M-77Y-0B | CIMMYT (Mexico) |
| **V14** | **Thelin//2*Attila*2/Pastor** | CGSS02Y00089T-099B-099M-099Y-099M-4Y-0B | CIMMYT (Mexico) |
| **V15** | **Kauz/Pastor/Fiscal** | CMSS02M00325S-030M-15Y-0M-0Y | CIMMYT (Mexico) |
| **V16** | **Prl/2*Pastor** | CGSS97Y00034M099T0PB-027Y-099M-099Y-099M | CIMMYT (Mexico) |
| **V17** | **Babax/Lr42//Babax*2/3/Kukuna** | CGSS01B00048T-099Y-099M-099M-099Y-099M-31Y-0B | CIMMYT (Mexico) |

The genotypes were sown and grown in the ITGC experimental field in a complete randomized block model with two replicates per genotype,

**DNA isolation and amplification.** The extraction of total DNA was performed from frozen and freshly harvested young leaves using a modified CTAB method described by Ben Naceur (1998)[5]. Using this protocol, a consistent yield of good quality DNA fragments achieved with a minimum of 20 µg DNA/g leaves. The DNA was purified by RNase (10µg/ml) treatment and phenolic extraction. The concentration of purified DNA estimated at 0.8% agarose gel. DNA dissolved and preserved in TE buffer.

The amplification reaction carried out in total reaction mixture of 25µl containing 50-100ng of genomic DNA, 0.2 mM dNTPs, 0.25 µM of each primer and 1U of Taq DNA polymerase (GoTaq, Promega) and its one-time concentrated buffer containing 1.5 mM $MgCl_2$.

The PCR amplification was performed with the following steps: one cycle of 94°C for 3 minutes, 35 cycles of 2 min denaturing step at 94°C, 1 min annealing temperatures between Ta between 52-64°C (Table 2) depending on the primers, 2 min extension at 72°C and followed by a final extension for 1 min at 72°C. Electrophoresis of DNA chain polymerization amplification performed on 2% agarose gel electrophoresis in 1xTris- Borate-EDTA buffer (pH 8.3). To better discern some fine bands, SSR products were also separated using 40% non-denaturing polyacrylamide gel electrophoresis in 1x TBE buffer. DNA Ladder (Promega) at 100bp used as a molecular size standard.

**Table 2.**Description of tested SSR primers (http:www.wheat.pw.usda.gov/ggpages/SSR/WMC)

| Primer | Sequences | Motifs | Chromosome location | Alleles size (pb) |
|---|---|---|---|---|
| ***WMC 14F*** | ACCCGTCACCGGTTTATGGATG | (CT) (CA) | 7D | 239 |
| ***WMC 14R*** | TCCACTTCAAGATGGAGGGCAG | | | |
| ***WMC 15F*** | AGTCCGATTCGGACTCCTCAG | (CT) (CA) | 4A | 295 |
| ***WMC 15R*** | GGACTAACCGAGGGTAGTTG | | | |
| ***WMC 16F*** | ACCGCCTGCATTCTCATCTAA | (CT) | 4B | 165 |
| ***WMC 16R*** | GTGGCGCCATGGTAGAGATTG | | | |
| ***WMC 17F*** | ACCTGCAAGAAATTAGGAAC | (CA) | 7A-7B | 182 |
| ***WMC 17R*** | CTAGTGTTTCAAATATGTCGA | | | |
| ***WMC 18F*** | CTGGGGCTTGGATCACGTCATT | (CA) (CT) | 2D | 237 |
| ***WMC 18R*** | AGCCATGGACATGGTGTCCTTC | | | |
| ***WMC 19F*** | CTGACATGCGGCATTCACTTCC | (CA) | 1A | 153 |
| ***WMC 19R*** | AGGCTTAGAACACACCGACACG | | | |
| ***WMC 20F*** | TTAAAAACACGCGGATCTTCTC | (CA) | 1A | 119 |
| ***WMC 20R*** | GTACTCACATATTTCTCGGTCT | | | |
| ***WMC 21F*** | CGCTGCCGTGTAACTCAAAATC | (GA) 37 | | 136 |
| ***WMC 21R*** | AGTTAATTGGGCGCTCCAAGAA | | - | |
| ***WMC 22F*** | ATCATTGGTTTCCTCTTCACTT | (GT) 24 | | 169 |
| ***WMC 22R*** | GTGGACTATTTAACATCTTCAT | | - | |
| ***WMC 23F*** | ATTCGCTCATACGATAGGGTTG | CT) 22 (CT) 18 | | 314 |
| ***WMC 23R*** | AGAGGCTGGTGTAGTTGGTTTG | | - | |
| ***WMC 24F*** | GTGAGCAATTTTGATTATACTG | (GT) 28 | 1A | 136-155 |
| ***WMC 24R*** | TACCCTGATGCTGTAATATGTG | | | |
| ***WMC 25F*** | TCTGGCCAGGATCAATATTACT | (GT) 26 | 2B | 166 |
| ***WMC 25R*** | TAAGATACATAGATCCAACACC | | | |
| ***WMC 27F*** | AATAGAAACAGGTCACCATCCG | (GT) 25 | 2B-5B | 352-398 |
| ***WMC 27R*** | TAGAGCTGGAGTAGGGCCAAAG | | | |
| ***WMC 48F*** | GAGGGTTCTGAAATGTTTTGCC | (GA) 9 | 4B | 139-190 |
| ***WMC 48R*** | ACGTGCTAGGGAGGTATCTTGC | | | |
| ***WMC 50F*** | CTGCCGTCAGGCCAGGCTCACA | (GT) 10 (GT) 16 | 3A | 219-236 |
| ***WMC 50R*** | CAACCAGCTAGCTGCCGCCGAA | | | |
| ***WMC 283F*** | CGTTGGCTGGGTTATATCATCT | (CA) 19 (CA) 8 | 4A | |
| ***WMC 283R*** | GACCCGCGTGTAAGTGATAGGA | | | |

**Microsatellites Markers Analysis.**

The reaction of DNA amplification by PCR was performed with 16 microsatellite primer pairs: *WMC 14, WMC15, WMC16, WMC17, WMC18, WMC19, WMC20, WMC21, WMC22, WMC23, WMC24, WMC25, WMC27, WMC48, WMC50 and WMC283* reported in Table 2.

The SSR profiles was transformed into a binary matrix where the presence of the generated band at a precise level is scored as 1 and its absence is scored as 0. The similarity matrix was generated with NTSYS software pc-2.02j (NTSYS-Numerical Taxonomy and Multivariate Analysis (Rohlf 1989)[34]. A dendrogram was produced using the UPGMA method (Unweighted Pair-Group Method using Arithmetic Averages) cluster analysis.

The PIC (Polymorphism Information Content), according to NEI and LI (1979)[28], is calculated by the following formula:

$$\mathbf{PIC} = \mathbf{1} - \sum_{i=1}^{k} \boldsymbol{Pi^2}$$

where *k* is the total number of alleles detected for a locus of a marker *Pi* is the proportion of the population carrying the *i* th alleles, scored for each locus.

## RESULTS AND DISCUSSION

In this study, eleven microsatellite markers for 11 loci were used to characterize and evaluate the genetic diversity of seventeen wheat genotypes (Table 3).

**Table 3.** PIC values, alleles number and annealing temperature generated by polymorphic primers

| Primer | PIC | Ta (°C) | Nb Alleles | Primer | PIC | Ta (°C) | Nb Alleles |
|---|---|---|---|---|---|---|---|
| ***WMC 14*** | 0.67 | 58 | 5 | ***WMC 25*** | 0.62 | 52 | 3 |
| ***WMC 15*** | 0.39 | 55 | 2 | ***WMC 27*** | 0.67 | 55 | 4 |
| ***WMC 17*** | 0.70 | 54 | 5 | ***WMC 48*** | 0.48 | 64 | 2 |
| ***WMC 20*** | 0.49 | 54 | 2 | ***WMC 50*** | 0.70 | 60 | 5 |
| ***WMC 21*** | 0.14 | 55 | 2 | ***WMC 283*** | 0.59 | 60.4 | 3 |
| ***WMC 24*** | 0.52 | 52 | 2 | | | | |

Amplified microsatellite loci were analyzed for polymorphism using polyacrylamide gel electrophoresis and the result revealed that all the primer pairs detected polymorphisms among the wheat genotypes analyzed. A total of 34 alleles were detected. The maximum number of alleles were observed at *WMC 14* primer. According to Nei's (1972)[28], the similarity coefficient between cultivars was ranged from 0.27 and 0.92 with an average of 0.60 (Table 4).

**Table 4**. Similarity matrix for bread wheat varieties based on their microsatellite markers

| | V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 | V9 | V10 | V11 | V12 | V13 | V14 | V15 | V16 | V17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 1,00 | | | | | | | | | | | | | | | | |
| V2 | 0,61 | 1,00 | | | | | | | | | | | | | | | |
| V3 | 0,56 | 0,45 | 1,00 | | | | | | | | | | | | | | |
| V4 | 0,56 | 0,64 | 0,67 | 1,00 | | | | | | | | | | | | | |
| V5 | 0,64 | 0,45 | 0,50 | 0,67 | 1,00 | | | | | | | | | | | | |
| V6 | 0,50 | 0,38 | 0,70 | 0,52 | 0,61 | 1,00 | | | | | | | | | | | |
| V7 | 0,58 | 0,48 | 0,70 | 0,61 | 0,70 | 0,91 | 1,00 | | | | | | | | | | |
| V8 | 0,35 | 0,40 | 0,36 | 0,45 | 0,55 | 0,48 | 0,57 | 1,00 | | | | | | | | | |
| V9 | 0,38 | 0,43 | 0,48 | 0,64 | 0,56 | 0,67 | 0,75 | 0,70 | 1,00 | | | | | | | | |
| V10 | 0,72 | 0,55 | 0,50 | 0,33 | 0,67 | 0,61 | 0,70 | 0,55 | 0,48 | 1,00 | | | | | | | |
| V11 | 0,48 | 0,73 | 0,50 | 0,67 | 0,50 | 0,43 | 0,52 | **0,27** | 0,56 | 0,42 | 1,00 | | | | | | |
| V12 | 0,50 | 0,76 | 0,52 | 0,70 | 0,52 | 0,55 | 0,55 | 0,29 | 0,50 | 0,43 | 0,87 | 1,00 | | | | | |
| V13 | 0,48 | 0,55 | 0,75 | 0,83 | 0,50 | 0,61 | 0,70 | 0,55 | 0,64 | 0,42 | 0,67 | 0,70 | 1,00 | | | | |
| V14 | 0,56 | 0,45 | 0,83 | 0,83 | 0,67 | 0,70 | 0,78 | 0,45 | 0,64 | 0,50 | 0,50 | 0,52 | 0,83 | 1,00 | | | |
| V15 | 0,56 | 0,64 | 0,75 | 0,83 | 0,50 | 0,61 | 0,70 | 0,64 | 0,64 | 0,50 | 0,58 | 0,61 | **0,92** | 0,83 | 1,00 | | |
| V16 | 0,48 | 0,73 | 0,67 | 0,75 | 0,50 | 0,70 | 0,78 | 0,64 | 0,72 | 0,58 | 0,67 | 0,70 | 0,83 | 0,75 | 0,92 | 1,00 | |
| V17 | 0,35 | 0,50 | 0,55 | 0,64 | 0,55 | 0,48 | 0,57 | 0,80 | 0,61 | 0,55 | 0,45 | 0,48 | 0,73 | 0,64 | 0,82 | 0,82 | 1,00 |

Most of the genotypes showed a high level of genetic similarity. The lowest genetic distance value of 0.27 has been scored between Cham6 and Pastor/Wbll1. Results indicating that they are genetically similar to each other. The highest genetic distance value of 0.92 has been scored between Kauz/Pastor/Fiscal and Wbll1*2/Brambling.

A dendrogram was drawn using the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) cluster analysis among 17 genotypes of bread wheat (figure 2).

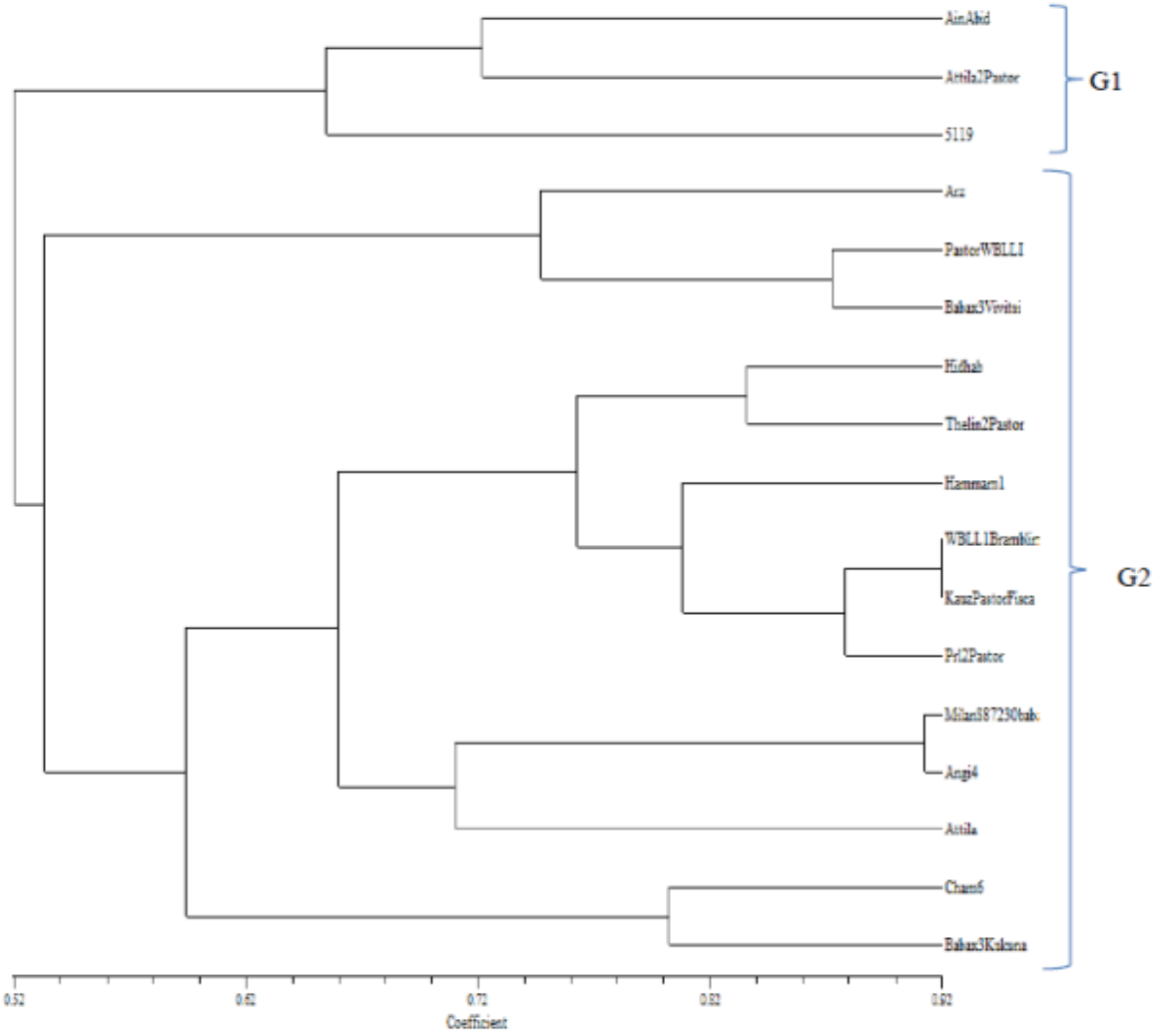



**Figure 2.** Dendrogram based on data of 11 microsatellite primers from 17 bread wheat genotypes constructed by UPGMA

Of a total of 16 SSR primers tested, only 11 produced polymporphic bands (*WMC 14, WMC15, WMC17, WMC20, WMC21, WMC24, WMC25, WMC27, WMC48, WMC50, WMC283*) (table 3). No amplified products obtained with WMC 16, WMC18, WMC 19, WMC 22 and WMC 23 primers. The Polymorphism Information Content (PIC) varied from 0.14 (WMC 21) to 0.70 (WMC 50 and WMC 17) with an average of 0.48 and 0.49 (table 3). This indicates that the markers are highly informative. These results confirmed in earlier studies on Moroccan wheat where the PIC values were between 0.29 and 0.79 (Zarkti et al. 2010)[41]. In another study, the PIC mean value (0.30) for SSRs is lower than the mean value of 0.48 and 0.49 observed in the present study in Egyptian hexaploid

bread wheat (Salem et al. 2014)[34]. In another recent study, Bellatreche et al (2019)[6] found that in the local bread wheat resources of the oases of Algeria, the information content on polymorphism (PIC) shifted from 0 to 0.55. However, these results are much higher in wheat Tunisian genotypes (PIC) from 0.33 to 0.94 respectively for WMC 25 and WMC 50 primers (Babay et al. 2015)[5] varied from 0.25 to 0.89 with an average of 0.69 in Cameroonian bread wheat (Tékeu et al. 2017)[38] and ranged from 0.7-0.89 with an average of 0.82 in Iranian wheat (Salehi 2018)[35]. The PIC value was used to estimate the level of gene variation in a plant. If the PIC value is > 0.5, the locus is a highly informative marker, whereas if the PIC value is < 0.25, the locus is considered to be low in diversity (Nagy et al. 2012; Ramadugu et al. 2015)[27][31]. The allele numbers per locus are 2 for WMC 15, WMC 20, WMC 21, WMC 24, WMC 27 and WMC 48 primers and 5 for WMC 14, WMC 17 and WMC 50 primers, with an average number of 3.2 alleles per locus. Salem et al (2008) and Islam et al (2012) have reported similar results in Egyptian and Bangladesh bread wheat accessions. Khaled et al (2015) found a high number of alleles per markers in Egyptian wheats, varied from 3 to 11 with a mean of 5.59. For Omani hexaploid bread wheat, Al Khanjari et al (2007)[1] found an average number of 8.70 alleles/locus. Jain et al (2004)[17] and Kondić-Špika et al (2016)[23] found a higher number of alleles per locus. The numbers were varied from 3 to 22, with an average of 7.8 alleles per locus. Leisova et al (2007)[24] showed that European wheat cultivars have an average of 12 alleles per locus. The same variation in alleles was also detected at other loci (Malik et al. 2013; Babay et al. 2015)[26] [5]. In contrast, Huang et al (2002)[15] used 26 microsatellites to study 998 bread wheat accessions from 68 countries and found an average of 18.1 alleles. Genetic diversity increases proportionally with the number of alleles at a given locus. (Khaled et al. 2015)[22].

Amplified microsatellite loci revealed that all the primer pairs detected polymorphisms among the wheat genotypes analyzed. It has been detected a total of 34 alleles. The maximum number of alleles observed at *WMC 14* (fig.1*). WMC 17* and *WMC 50* and their size ranged from 182 to 239 bp (Kara et al. 2017) [21].

The higher genetic distance indicates that wheat genotypes were genetically diverse. It is an indication of their genetic dissimilarity (Kondić-Špika et al. 2016)[23]. The consensus tree showed that it divided the wheat genotypes into two main clusters, the first included wheat varieties Ain Abid, Attila/2 Pastor and 5119. Results confirmed by Kara et al. (2016)[20.]. The second main cluster revealed high polymorphism and was divided into four sub-clusters (fig.2).The dendrogram demonstrate the ability of microsatellites to detect large amount of genetic diversity in genotypes with expected narrow genetic pool (fig. 2).

The cophenetic coefficient was r = 0.74, indicating that there is good fit between dendrogram clusters and the similarity matrix.

## CONCLUSION

In the present study, an alternative approach adopted by analyzing a small number of wheat genotypes of diverse origins using a larger number of SSRs to ensure better genome coverage. The SSR markers were able to discriminate between bread wheat cultivars. SSR markers successfully discriminated bread wheat cultivars. The majority of the primers used in this study showed high polymorphism. These results clearly demonstrate the reliability, usefulness and effectiveness of SSR microsatellites in analyzing genomic diversity (Salem et al. 2008)[36]. Microsatellite markers, because of their high level of

polymorphism and ease of manipulation, appreciated for various applications in wheat breeding and used to assess the genetic diversity of hexaploid wheat. As a result, they can be useful for screening, genetic diversity assessment and molecular mapping studies of bread wheat.

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# CHEMICAL AND RHEOLOGICAL CHARACTERIZATION OF HOMEMADE SOY MILK

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## ABSTRACT

The objective of this work was to evaluate the physical, chemical and rhelogical characteristics of vegetable milk of soybean (soymilk homemade prepared, and some types of soymilk purchased from Romanian supermarkets).

Soymilk is an alternative to dairy products and it has long been a traditional drink in China, Japan and other parts of Asia. The soybean (Glycine max) is the most important bean in the world, providing a wide range of vegetable proteins. Soybean is an important source of many bioactive compounds, which are important for the health benefits conferred.

Soymilk is a colloidal solution obtained in the form of water extract from swollen and ground soybeans. Soymilk is rich in protein, calcium and has no saturated fat. It is low in calories, being the perfect alternative for people with lactose intolerance. This kind of drink is especially used for vegetarian people, people with lactose intolerance, and those who hold religious fasting.

The soymilk has been prepared from analyzed grains and then some chemical and physical characteristics of milk have been assessed. We investigated moisture and total dry content substance (TDC), total mineral content- ash content, macronutrients content (fat, protein and carbohydrates content) for soybeans and soymilk samples. For soymilk, we established total solid content (brix grade), pH, sensory evaluation, and some rheological characteristics.

**Keywords:** soymilk, rheological characterization, moisture, fat, protein content

## INTRODUCTION

Soymilk is an alternative to dairy products and it has long been a traditional drink in China, Japan and other parts of Asia [1, 2]. The soybean (Glycine max) is the most important bean in the world, providing a wide range of vegetable proteins. Soybean is an important source of many bioactive compounds, which are important for the health benefits conferred [3, 4]. Soymilk is one of the most popular and traditional products

and it contains eight essential amino acids [5, 6]. It is a stable emulsion of oil, water and protein. [4, 7].

The objective of this work was to evaluate the rheological, physical, chemical and sensorial characteristics of vegetable milk of soybean (soymilk homemade prepared, and some types of soymilk purchased from Romanian supermarkets).

In the present study, we evaluated physicochemical characteristics of soymilk beverages: pH, total content of soluble solids – TSS, sensorial and rheological characteristics; and we investigated total mineral content (ash), moisture (water content) and total dry substances content – TDC, macronutrients content (fat, carbohydrates and protein content) on soybean used for homemade soymilk and for soymilk samples. The investigated homemade soymilk has been prepared by boiling.

## MATERIALS AND METHODS

In order to conduct the experimental research, homemade soymilk was prepared by boiling the mixture. Fresh spring water was used for preparing the soymilk.

We have employed the Biovita-10 multifunctional device for making plant milk. The ratio between the spring water quantity and the soy beans quantity was of 1:15.

Before introducing them in the device, the soy beans had been soaking in water for 12 hours. Subsequently, the soaking liquid was drained and the soy beans were introduced in the device, with water. The device contains an incorporated blender, which – during the process – has also grinded the beans very finely. Finaly, after getting cold, the resulting boiled mixture (of soy and water) was filtrated through a very fine sieve (the diameter of the sieve being smaller than 0.5 mm), thus obtaining both the soymilk and the mass of solid vegetable substance separated through draining.

The experimental analysis has been conducted both on the raw material employed for the milk production and on the resulting products (the remaining solid mass and the plant milk).

It was evaluated the two samples (A, B). The samples A is soymilk samples obtained by boiling by us and B and C are two kind of soymilk purche from supermaket.

Based on the analysed samples, the assessment of the following aspects has been made: the, pH, the total content of soluble solids – TSS (the Brix degre), total dry matter content – TDC, humidity, ashes and rheological behaviour.

### *Rheological measurements*

For the rheological measurements conducted on soymilk, we have employed a Brookfield LVDV III Vetra, equipped with a temperature control device. For the assessment, we have employed a cylindrical geometry (DIN-85) and a double-wall cylindrical cell, where the water that ensures the thermostating can be recirculated during the measurements.

Inside the cylindrical cell, the soymilk samples obtained by boiling (sample noted A) and respectively soymilk purche from supermaket (sample noted B) have been introduced (5-7ml).

Temperature control (±0,1ºC) was ensured by a water bath. The computerized control of the rheometer was achieved by employing the BEVIS programme.

All the assessed values have been collected with the Rhocalc 32 software and have been exported as Excel files. Rheogrammes have been obtained for both the soymilk samples.

***Sensory evaluation***

For sensory evaluation we have selected twenty panelists to evaluate aroma, colour, taste, smell, consistence and appearance. This group consisted of 8 men and 12 women who were undergraduate and graduate students. The samples (30 mL) were served cold (10 ± 2 °C) in 50 mL plastic cups codified with three-digit random numbers (each sample being three times given for tasting, without the panelists' awareness regarding this aspect). Mineral water at room temperature and saltwater biscuits were served to allow the assessors to cleanse their palates between samples. Each consumer evaluated the three samples (A, B) in a single session. The samples A is soymilk samples obtained by boiling by us and B is soymilk purche from supermaket.

***Physical and chemical analyses***

*The determination of the dry matter and water content* was made by using a Memmert oven (beans, milk). The water content actually represents the weight loss achieved by heating at the temperature indicated by the analysis method until reaching the constant mass.

*The ash content* (*total mineral content*) was evaluated by incineration at 500±15ºC.

*The determination of the total soluble solids* TSS (Brix degree**)** in the milk samples has been achieved by employing the KRÜSS (Germany) optometric equipment. The determination of pH has been obtained with the help of a pH-meter.

***Macronutrients composition***

Both the soybeans and the soymilk were examined through standard procedures, for their nutritional chemical composition (lipids, proteins, ash and carbohydrates). The crude protein content (Nx6.25) found in the samples was estimated by the macro-Kjeldahl method. The crude fats were determined by extraction with petroleum ether from a known weight of dried and grinded grains, using a Soxhlet extractor. For soymilk, we applied the same evaluation methods as for milk. We used the MilkoScan S54B device. The amount of carbohydrates was calculated by differences, according to the following equation:

$$Carbohydrates\ (\%) = 100 - [\ Proteins(\%) + Lipids\ (\%) + Moisture(\%) + ash(\%)]$$

The energy values of samples formulas was calculated according to the following equation:

$$Total\ Energy\ (kcal/\ 100g) = 4x[Proteins(\%) + Carbohydrates\ (\%)] + 9x[Lipids\ (\%)].$$

***Statistical analysis***

All determinations were performed in triplicate, calculating their arithmetic mean of three separate determinations. The data were statistically analyzed using the program Microsoft Excel.

## RESULTS

The results of our study are presented in the following figures:

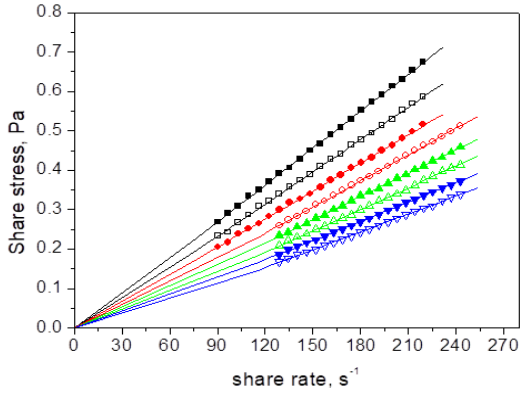



**Figure 1.** Rheograms for homemade soymilk obtained by boiling (■ – **5ºC;** □ – **10ºC;** ● – **15ºC;** ○ – **20ºC;** ▲**- 25ºC;** Δ – **30ºC;** ▼– **35ºC** ▽ – **40ºC;** continous lines – calculated rheograms

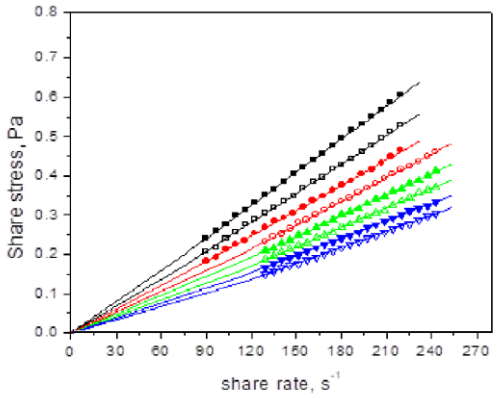



**Figure 2.** Rheograms for supermarket soymilk (■ – *5ºC;* □ – *10ºC;* ● – *15ºC;* ○ – *20ºC;* ▲**-** *25ºC;* *Δ – 30ºC;* ▼– *35ºC* ▽ – *40ºC;* continuous lines – calculated rheograms)

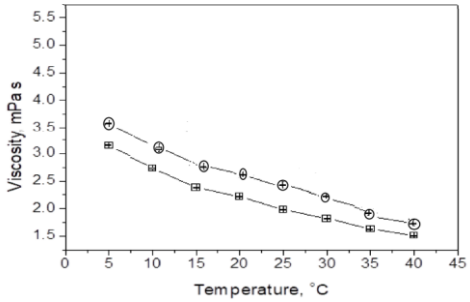



**Figure 3.** The influence of temperature on viscosity in soymilk (□ – supermarket soymilk; ○ – home made soymilk obtained by boiling)

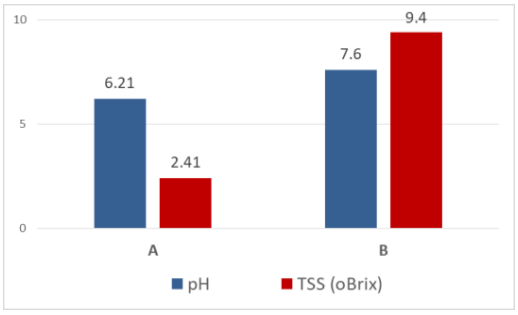



**Figure 4.** Total soluble solids (Brix°), pH in soymilk sample

Note: The samples **A** is soymilk samples obtained by boiling by us
The samples **B** is soymilk purche from supermaket.

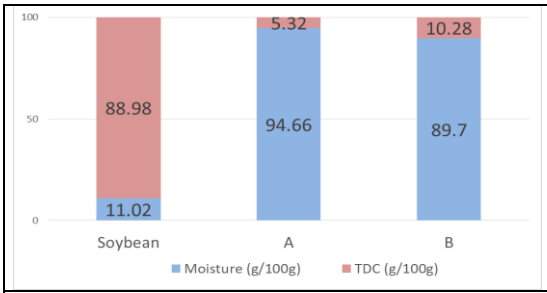



**Figure 5.** Moisture and TDC total dry weight contents of sample

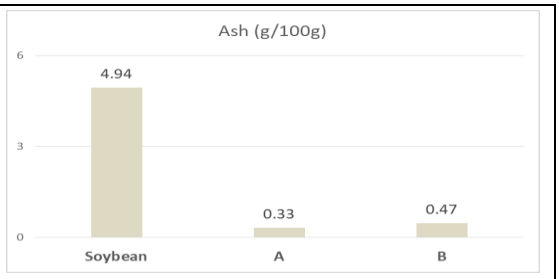



**Figure 6.** Total mineral content (ash) of sample

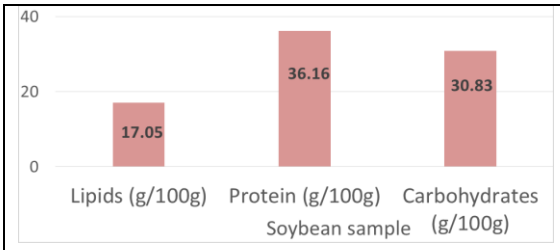



**Figure 7.** Macronutrients content in soybean samples

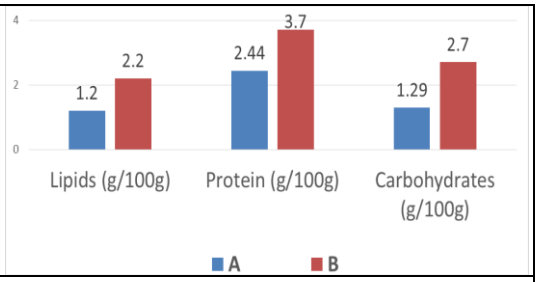



**Figure 8.** Macronutrients content in soymilk samples

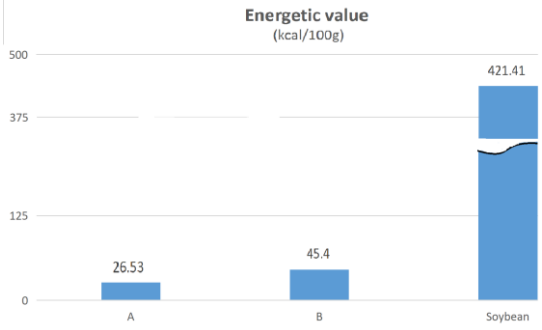



**Figure 9.** Energy value of soymilk samples

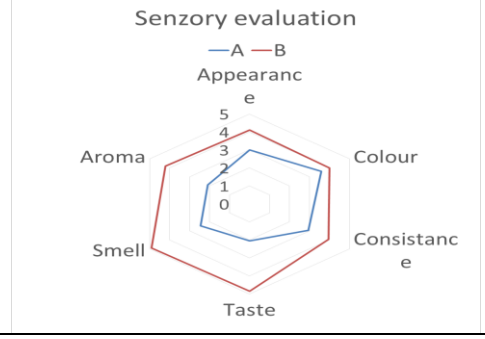



**Figure 10.** Sensorial aspects of soymilk samples

## DISCUSSION

### *Rheological analyses*

Flowing is a continuous deformation of fluid, which takes place when the resultant of forces acting on the fluid is not zero.

The ideal fluid (Pascal's fluid) is not viscous, thus being an ideal fluid which cannot be encountered among real fluids.

Real fluids are those that resist deformation and flowing, due to the friction forces between the layers. The intensity of these forces is expressed in the dynamic viscosity of fluids, thus leading to the conclusion that real fluids have viscosity.

Newtonian fluids are the fluids where the viscosity depends only on the fluid's state parameters and not on the parameters related to the forces acting on the fluid (the frictional force and the frictional speed).

The rheological behaviour of alimentary fluids depends on their composition and on the temperature. Their flow behaviour is also influenced by the shear speed/shear velocity, the shear duration and the pre-shearing time/the history previous to the shear. Rheograms express the dependence of the shear tension on the shear speed [8].

Soymilk has been characterized from a rheological point of view at temperatures ranging between 5 – 40 $^0$C, the interval for temperature rise being of 5 $^0$C.

Experimmntal rheograms for homemade soymilk (obtained by boiling) and for the one found in supermarkets are graphically shown in figures 1 and 2 as linear dependences $\sigma = f(\gamma)$, ($\sigma$- shear stress/tension, $\gamma$-shear speed).

Soymilk - both the homemade one obtained by boiling and the one purchased from the supermarket - behaves as a Newtonian fluid.

The influence of temperature on viscosity, shown in Figure 3, displays a linear decrease in viscosity as temperature rises. Consequently, from a rheological point of view soymilk behaves as a Newtonian fluid.

***Sensorial, physical and chemical characterization***

From the data displayed in Figure 4 we can note that the value of total soluble solids content (TSS content – brix degree) was prominent in B sample and the lower value measured for A sample (homemade soymilk). The brix values are higher for supermarket soymilk as compared to homemade soymilk.

As regards the pH, one may notice that the values range within the 6.2 - 7.7 frame. The highest pH value belongs to B sample, while the lowest is recorded for the milk prepared by us.

The results of the basic nutritional and physico-chemical characterization of soymilk samples and soybean are shown in figures 5 – 8.

The data listed in Figure 8 show that the lowest macronutrient content is found in A sample. The differences between these A and B samples can be explained by the fact that various soybeans quantities were used in proportion with the water added for the milk preparation. Likewise, for the improvement of milk sensory qualities other products are also introduced along with the soybeans, with specific macronutrient content.

The energetic value of the studied soymilk ranges between 26 and 45 (kcal/100g). The lowest energetic value is found in homemade soymilk. Homemade soymilk shows the lowest values for all parameters.

The results of *sensorial* assay are presented in Figure 8. Where, B is commercial product. One of the objectives of our research was to relate the physical and chemical characteristics of soymilk beverages to its sensory acceptance.

The sensorial properties were evaluated by scoring scale (1 – 5) (1-low, 5-excellent). From a sensorial point of view, the soymilk sample from supermarkets was well appreciated.

As expected, the least appreciated was the milk prepared by us, since it does not contain any addition meant to improve its sensorial qualities. The lowest score was registered on the taste level (2.05) and the highest score was recorded by the B sample, for the taste,

(4.85). On the aroma, the lowest level score was 2.1 for milk prepared by us and the highest score was 4.2 recorded by the B sample The milk prepared by us can improve its sensorial qualities by adding some components (such as salt, inverted sugar, etc.).

TSS values for soymilk purchased from Romanian supermarkets have average 9.4 °Brix. These data are in the range from 8.1 to 10.10 °Brix, reported by Terharg et al. (2013) [3] and Micula et al. (2016) [1]. Callou et al. (2010) [9] reported higher protein contents for soy beverages commercialized in the Brazilian market. Our data is lower than reported by Villegas, Carbonell and Costell (2011) for Spanish vanilla-flavored soy beverages (10.5 to 18.3 °Brix) [10].

This result confirms that the presence of aroma and flavour, darker colour, and different viscosity are important attributes in the acceptance of soymilk plain beverages. Villegas,

Carbonell and Costell (2011) [9,11]. have suggested that it is possible to increase the acceptability of vanilla-flavoured soy beverages by increasing their sweetness, viscosity, and intensity of vanilla flavour.

## CONCLUSION

Soybean is a product with a rich macronutrients content. Soybeans represent a significant source of protein, having beneficial properties for health. The moisture content in soybeans is relatively low.

Unlike milk of animal origin, vegetable milk contains neither cholesterol nor lactose, being an alternative food used by people who are lactose intolerant or have allergies to gluten and casein. It is also accepted by vegans, vegetarians and fasting people.

Homemade soymilk reveals the lowest value for all parameters. TSS values for soymilk ranged between 2.4 and 9.5 $^{o}$Brix. Homemade soymilk displayed the lowest value. The macronutrients content in soymilk samples range from 1.2 (lipids in sample A) to 3.7 g/100g (proteins in sample B).

Soymilk - both the homemade one obtained by boiling and the one purchased from the supermarket- behaves as a Newtonian fluid.

Non-linear dependences suggest a Newtonian character of the fluid (the soymilk obtained by boiling).

The presence of aroma and flavour, darker colour, and different viscosity degrees are important attributes in the acceptance of soymilk beverages.

The preparation recipe of homemade soymilk must be improved regarding all the physico-chemical and sensorial characteristics.

Physicochemical analyses of soy milk make it possible to know the recommended daily intake in the diet.

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# COCONUT MILK-BASED CHEESE ALTERNATIVES - ANTIOXIDANT, NUTRITIONAL AND SENSORY PROPERTIES

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## ABSTRACT

Vegan alternatives to cheeses are in growing demand in European markets as more and more people are either lactose intolerant or switching to a plant-based diet. When the consumer chooses to buy a vegan cheese, his expectations are that the vegan product has the nutritional and organoleptic characteristics as similar as possible to those of conventional cheeses. This work aimed to obtain two variants of vegan alternative to cheese, using coconut milk as the raw material: one with the addition of white pepper and garlic powder (C1) and the second with the addition of turmeric and paprika (C2). Also, the finished products obtained were analyzed regarding the total content of polyphenols (Folin-Ciocalteu assay), antioxidant activity (DPPH free radical method), proximate composition, energy value and sensory properties (five points hedonic scale assay). The C2 product variant with the addition of turmeric and paprika stood out for the highest content of total polyphenols (5,17±0,14 mg gallic acid/g) and the best DPPH free radical inhibition activity (55,86±0,34 %). Concerning the proximate composition and energy value, the differences between the two products were small, and at the sensory analysis the C1 variant obtained good scores (over 4) and C2 very good scores (over 4.5) in all sensory characteristics.

**Keywords:** coconut milk, vegan cheese alternatives, polyphenols, antioxidant activity,

## INTRODUCTION

Cheese is a food product very loved by many consumers, being used as such or in various preparations. Dairy cheeses are obtained from the milk of various animals (especially cow, goat, sheep, buffalo). In recent years, due to the increase in the number of people with a vegan diet, as well as to the fact that some consumers have allergies to dairy products or lactose intolerance, the market demand for plant-based alternatives for

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cheeses and dairy products has grown considerably [1,2]. A series of plant-based alternatives to cheeses have appeared on the market, using as raw materials different nuts, legumes, seeds, cereals, to which different oils, salt, spices, yeast, agar-agar or tapioca flour are added, and the assortment ranges are in continuing development at present [3,4]. If for producers obtaining vegan alternatives to cheeses with sensory properties as close as possible to dairy ones, from a nutritional point of view, plant-based cheese alternatives are perceived to be healthier than the original dairy versions, because they do not contain lactose and cholesterol [2]. Coconut grows in a palm tree: *Cocos nucifera*, part of the *Palmaceae* family. This nut is made up of three elements: pulp, water and shell. Coconut milk is made from the pulp of the nut, which is grated and then pressed or squeezed, with or without water addition. The composition and quality of coconut milk - which is practically an oil-in-water emulsion - is influenced by the quality of the raw material but also by the processing conditions [5]. The main components of coconut milk are lipids, carbohydrates and proteins, as well as some minor components including phenolic compounds [6]. In cow's milk, the amount of fat is approximately equal to that of protein, while in coconut milk, the amount of oil is ten times greater than the protein. The total lipid content of coconut milk varies between 5.88% and 21.83%, depending on whether water is added or not during the production process [7]. The analysis of antioxidant activity by different methods proved that coconut milk has stronger antioxidant properties than cow's milk [8].

The purpose of this work was primarily to obtain two variety of coconut milk-based alternatives to cheese: one with garlic powder and white pepper and second with turmeric powder and paprika, and then to analyze the finished products in terms of total polyphenols content, antioxidant activity, proximate composition, energy value and sensory characteristics.

## MATERIALS AND METHODS

*Coconut milk-based cheese alternatives preparation*

Two cheese alternatives coconut milk-based were obtained: C1 with the addition of garlic powder and white pepper and C2 with the addition of turmeric and paprika, using the raw and auxiliary materials in the proportions shown in Table 1.

**Table 1** Recipes used to obtain the coconut milk-based cheese alternatives

| **Raw and auxiliary materials (%)** | **C1** | **C2** |
|---|---|---|
| **Coconut milk** | 64 | 64 |
| **Agar-agar powder** | 3 | 3 |
| **Lemon juice** | 3 | 3 |
| **Inactive dry yeast flakes** | 8 | 8 |
| **Water** | 19 | 19 |
| **White pepper** | 0.8 | - |
| **Garlic powder** | 0.8 | - |
| **Paprika** | - | 0.8 |
| **Turmeric** | - | 0.8 |
| **Pink Himalayan salt** | 1.4 | 1.4 |

To prepare the two types of vegan cheese alternative, all the necessary ingredients were weighed in a bowl, after which they were homogenized in a blender and boiled for 6 minutes (with continuous stirring) until the composition was thickens. The warm composition was poured into glass molds previously greased with a little oil and left to cool. The cooled products were refrigerated for 3 hours at 4°C. After cooling, the products were removed from the molds, packed and storing in the refrigerator.



a) b)

**Figure 1. – Coconut-based cheese alternatives: a) C1 - with white pepper and garlic powder; b) C2 – with turmeric and paprika**

*Assessment of total polyphenols content*

The total polyphenols content (TPC) was determined from the two types of vegan alternatives to cheese C1 and C2, as well as from the coconut milk, white pepper, paprika, turmeric, lemon juice, garlic powder, using Folin-Ciocalteu assay and the same work methodology as that presented by Dumbrava *et al*., [9].

*Assessment of antioxidant activity*

To evaluate the antioxidant activity of both finished products and of raw materials, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (RSA) was determined [10]. For this purpose, 2 g of each finished product or raw material were weighed and then subjected to extraction with 20 ml of 70% (v:v) ethanol for 2 hours, at 22°C, under continuous stirring. The alcoholic extracts were filtered through Whatman No 1 filter paper, then 1 ml of each filtrate was pipetted into a test tube over which 2.5 ml of 0.1 mM DPPH solution in 70% (v:v) ethanol was added. The content of each test tube was well homogenized and left to rest for 30 minutes at 22°C, in the dark, after which the absorbance was read at 517 nm against 70% ethanol as a reference.

The DPPH radical scavenging activity (RSA) was calculated using the following equation [11]:

$$\text{DPPH scavenging activity (\%)} = \frac{Ac - As}{Ac} \times 100$$

where: Ac is the control sample absorbance,

As – absorbance in the presence of the sample.

*Assessment of proximate composition and energy value*

Proximate composition determination for C1 and C2 was carried out using the same ISO methods presented by Dumbrava *et al*., [9].

*Statistical analysis*
The determination of TPC, antioxidant activity, as well as proximate composition was performed in triplicate, and the results were expressed as a mean values±standard deviation (SD). Microsoft Excel 2010 was used for statistical data processing.

*Sensory evaluation*
Sensory analysis of C1 and C2 was performed by a group of 20 people, men and women, aged between 20 and 57 years, using the hedonic scoring scale from 1 to 5, evaluating the external appearance, appearance in the section, color, taste, aroma, smell, texture and overall acceptability. The same sensory analysis methodology was followed as that presented by Dumbrava *et al*., [9].

## RESULTS AND DISCUSSIONS

*Total polyphenols content*
Table 2 shows the results regarding the total polyphenols content in the two finished products as well as in the raw materials

**Table 2.** Total polyphenols content in vegan coconut milk-based cheeses and in the raw materials

| Sample | Total polyphenols (mg gallic acid/g) |
|---|---|
| **Coconut milk** | 2,02±0,12 |
| **Garlic powder** | 4.48 ± 0.16 |
| **White pepper** | 9.14±0.14 |
| **Lemon juice** | 1,81±0,10 |
| **Paprika** | 11,32±0,24 |
| **Turmeric** | 90.16±1.74 |
| **C1** | 3,24±0,06 |
| **C2** | 5,17±0,14 |

Analyzing the experimental data it is found that between raw and auxiliary materials, the highest TPC was recorded in the case of turmeric powder (90.16±1.74 mg gallic acid/g), followed by paprika (11.32±0.24 mg gallic acid gallic/g) and white pepper (9.14±0.14 mg gallic acid/g). The lowest TPC value was obtained for lemon juice (1.81±0.10 mg gallic acid/g). Regarding the two finished products, C2 - with added turmeric and paprika, had a higher TPC (5,17±0,14 mg gallic acid/g) than C1 (3,24±0,06 mg gallic acid/g). From the literature data it is found that dairy cheeses have a lower TPC than the vegan variants presented in this paper. Thus, for example, for different types of fresh cow cheese Sik *et al.* [12] reported TPC values between 0.33 and 1.24 mg GAE/g, for fresh goat cheese only 0.32 mg GAE/g, for cow ricotta cheese 0.11-0.19 mg GAE/g, and for goat ricotta cheese 0.15 mg GAE/g. Ukom *et al*., [13] studying TPC in vegan cheese obtained from a mixture of coconut milk and melon seeds milk, in different proportions, obtained values between 4.15 to 13.15 mg GAE/g, the values increasing proportionally with the percentage of melon seeds milk in the mixture.

*Antioxidant properties*
The DPPH free radical scavenging activity (RSA) determined for the raw materials, respectively for the finished products, is presented in the Table 3.

**Table 3.** RSA (%) for raw materials and finished products

| Sample | Dilution | RSA (%) |
|---|---|---|
| **Coconut milk** | 1:100 | 56.16±0,38 |
| **Garlic powder** | 1:100 | 60.96±0.61 |
| **White pepper** | 1:100 | 61.47±0.56 |
| **Lemon juice** | 1:100 | 60.34±0,42 |
| **Paprika** | 1:100 | 58,12±0,33 |
| **Turmeric** | 1:100 | 95,63±0,51 |
| **C1** | 1:100 | 51,72±0,23 |
| **C2** | 1:100 | 55,86±0,34 |

For 70% (v:v) ethanol extracts of raw and auxiliary materials, diluted 1:100, the strongest RSA was reported for turmeric powder (95,63±0,51%), followed by white pepper (61.47±0.56%) and garlic powder (60.96±0.61%). The finished product C2, with the highest TPC, also had the strongest RSA (55,86±0,34%). Ukom *et al.,* [13] studying the DPPH free radical inhibition activity of vegan cheese from coconut milk and melon seeds milk, but using methanol as solvent and other dilutions, reported RSA values between 0 and 16.17%, these increasing with the percentage of coconut milk added.

*Proximate composition and energy value*

Proximate composition and energy value for the coconut milk - based alternatives to cheese are presented in Table 4.

**Table 4.** Proximate composition and energy values for C1 and C2 alternatives to cheese

| **Parameters** | **C1** | **C2** |
|---|---|---|
| Proteins (g/100g) | 7.02±0.06 | 7.10±0.08 |
| Lipids (g/100g) | 11.65±0.15 | 11.46±0,12 |
| Saturated fatty acids (g/100g) | 9.87±0.11 | 9.71±0.08 |
| Carbohydrates (g/100g) | 8.09 | 8.44 |
| Sugar (g/100g) | 1.18±0.04 | 1.16±0.03 |
| Dietary fiber (g/100g) | 5.50±0.08 | 5.57±0.08 |
| Moisture (g/100g) | 55.04±0.82 | 54.92±0.80 |
| Mineral substances (g/100g) | 1.65±0.01 | 1.64±0.02 |
| Energy value (kcal/100g) | 165.29 | 165.30 |

In terms of proximate composition and energy value, the two products C1 and C2 are extremely close in value. Thus, the water content for C1 and C2 was 55.04±0.82% and 54.92±0.80% respectively, and of the nutrients the largest proportion is represented by lipids (11.65±0.15%, respectively 11.46±0,12%), followed by carbohydrates (8.09%, respectively 8.44%) and proteins (7.02±0.06%, respectively 7.10±0.08%). Also note the high dietary fibre content (5.50±0.08%, respectively 5.57±0.08%). For vegan cheeses obtained from coconut milk and melon seeds milk, Ukom *et al*., [13] found values of moisture content between 56.31 to 59.93% (proportional to the melon seeds milk content), while for cow's milk cheese the moisture value was significantly lower (51.45%). For coconut milk and melon seeds milk cheeses, the same authors [13] reported higher values for protein (10.05 to 20.15%) and carbohydrates (10.76 to 25.77%) due to melon seeds milk content,.but lower lipid (5.32 to 7.47%) and dietary fibre (0.31 to 0.73%). For different types of fresh cow cheese Sik *et al*. [12] reported

higher values of protein and respectively lipid content:16.84 to 22.02%, respectively 14.80 to 26.50% and for fresh goat cheese: 15.22% respectively 19%. Unlike dairy cheeses, which contain cholesterol [14], in the vegan alternatives proposed in this paper it is absent.

*Sensory analysis*

The sensory evaluation of the two coconut milk cheese variants resulted in the overall scores shown in Figure 2.

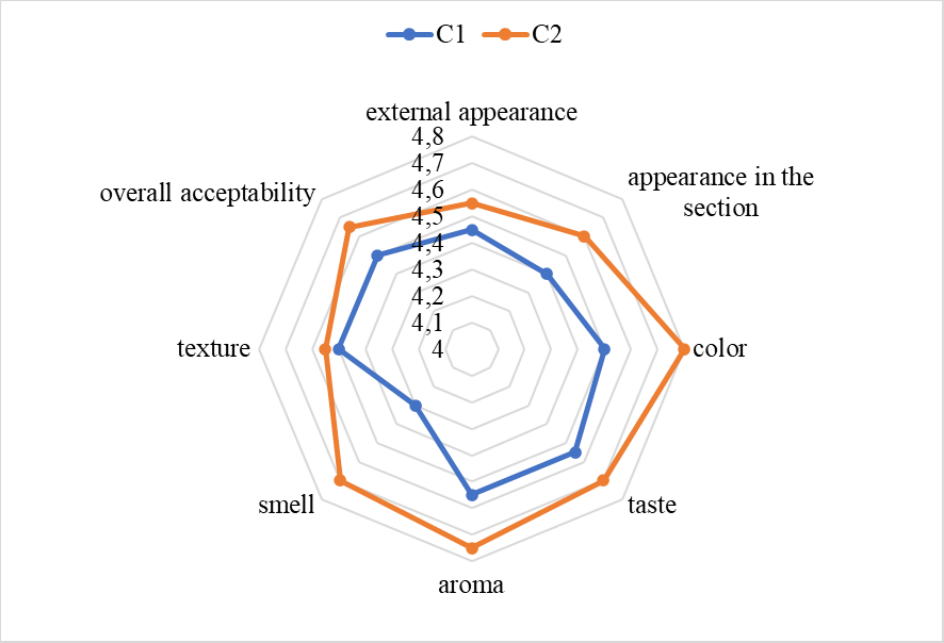



**Figure 2. Global values of the sensory evaluation of C1 and C2**

Following the organoleptic analysis, both products obtained good scores, the C1 variant between 4.3 and 4.55 (acceptable) for all the analysed characteristics, and the C2 variant was evaluated with higher scores between 4.55 and 4.8 (very acceptable).

## CONCLUSION

This paper presented the preparation of two innovative vegan alternatives to cheese using coconut milk as raw material: one with white pepper and garlic powder (C1) and the second with turmeric and paprika (C2). The determination of total polyphenol content by the Folin-Ciocalteu method revealed that, among the raw and auxiliary materials, the highest values were recorded for turmeric, followed by white pepper and garlic powder, and of the two finished products, C2 had the highest concentration of these compounds. The DPPH free radical scavenging activity determined for 70% (v:v) ethanol extracts obtained from raw materials and finished products showed that turmeric extract had the strongest antiradical effect, followed by white pepper and garlic powder extracts; also, the C2 assortment of vegan alternative to cheese was with the strongest DPPH free radical scavenging activity. The two finished product variants had a very similar proximate composition and energy value, and compared to dairy cheeses, they have a much lower energy value, are cholesterol-free, have less protein and fat, but have

more total carbohydrates and contain dietary fibre. In the sensory analysis, both products were well appreciated by panellists, with the highest scores for all sensory characteristics analysed being obtained by the C2 assortment. Whereas the coconut milk-based cheese alternatives proposed in this paper have a high content of total polyphenols, good antiradical activity, good energy value, balanced proximate composition, are cholesterol-free, lactose-free, high in dietary fibre, and being also well appreciated in terms of organoleptic characteristics, they can be recommended as a tasty and healthy alternative to dairy cheeses, both for people with a vegan diet and for those with lactose intolerance, with cholesterol restrictions, or for who want to consume tasty and healthy new products.

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# COMPARATIVE STUDY ON THE BENEFITS OF THE USE OF GAME MEAT IN THE PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF A COMMON CONSUMER PRODUCT

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## ABSTRACT

In recent years, consumers, including domestic meat consumers, have been looking for new products with high biological and nutritional value, plus flavour. Consumer concern for a healthy diet has led to increased demand for low-fat, low-cholesterol products, coupled with growing interest in returning to traditional products as an option to advanced processing. Game meat could meet consumer needs and requirements as an alternative to meat from domestic animals, with the following recommendations: **1.** low fat content; **2.** optimal unsaturated/saturated fatty acid ratio; **3.** high protein content; **4.** low calorie content; **5.** outstanding texture, taste and flavour. The main aim of the work was to obtain a salami-type product from wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*) meat, from the Western area of Romania, followed by a comparative study of quality indicators with a similar advanced processed product from domestic pork. From a physico-chemical point of view, the determination of water, ash, protein, fat, NaCl, and finally the carbohydrate content and nutritional value were calculated. From a sensory point of view, appearance, consistency, taste, smell and general acceptability were investigated.

**Keywords:** game meat, raw-dry-smoked salami, proximate analysis, sensory evaluation.

## INTRODUCTION

Currently, consumer interest in the consumption of hunted meat resulting from hunting is constantly increasing, especially in territories where hunting is a common and sustainable practice. This claim is supported by interdependence of humans → animals → plants → environment, constrained by demographic expansion, industrialization and intensive agricultural practices, culminating in globalization of communicable disease risks [1].
The negative effects of intensive agriculture in general and livestock farming in particular on the environment are causing global concern. They demonstrate the need for sustainable management strategies to meet growing global demand for animal protein. Many game species, from widespread to lesser-known, attract attention for their introduction into the food production chain. However, further investigation is needed to define their physicochemical, nutritional, sensory and food hygiene characteristics [2]. For this

reason, wildlife could be a substitute for pork, beef and poultry meat from the intensive livestock production area. This can be the case for wild boar (*Sus scrofa*), but also for other wild animals (deer (*Capreolus capreolus*)) [3].
However, consumers trend towards healthy eating has led to increased demand for low-fat and cholesterol-rich products, with interest in traditional products. Hunted meat could be the alternative to domestic animal products that meets these needs. Information and comparison is condition to success of diversifying assortment range in the segment of raw-dried-smoked minced meat products (*salami*), a consumer product, with positive potential on health status, but also satisfying sensory (eg texture, taste, aroma, etc.) [4].
In general, it is recognized for hunted meat, compared to similar products from domestic and/or conditioned animals (intensive (chicken, pork, beef)), low fat content, optimal ratio of saturated/unsaturated fatty acids, high protein content, low calorie (energy balanced), special taste and aroma [5, 6]. In this segment, literature records research on quality and safety of hunted meat, studies on consumer preferences in relation to wild hunted meat [7]. However, specialized studies on behavior, perception and preferences of the *European consumer* are relatively limited in relation to the consumption of wild hunted meat products [8]. It is known that consumer behavior (expectations/needs) is dependent on three mutually conditioned factors: **1.** psychological (individual); **2.** sensory (product-specific factors); **3.** Marketing (circumstantial market factors). Behavior influenced by condition, culture and access to information. Statements that require processors to identify the attributes of hunted meat considered important by the consumer, in order to develop policies to promote them, but to satisfy the needs of the individual (nutritional and bioavailability (e.g. amino acids and superior essential organic acids)).
Studies conducted on red deer meat (*Cervus elaphus* = European deer) confirm olfactory intensity compared to beef, intense taste and aroma, properties that are accepted and appreciated by a small segment of consumers, representing a major disincentive to consumption. Research on the chemical composition of some muscle tissues (e.g. m. *Longissimus lumborum*, *Longissimus dorsi*, *Semitendinosus* and *Triceps brachii*), taken from different hunted species (*Cervus elaphus L., Sus scrofa ferus* (South-West of Romania), *Cervus canadensis*, *Capreolus capreolus*, *Cervus capreolinae* (Latvia)), revealed the following (average values): 74% water, 22% protein, 2-3% lipids, respectively 1-2% other soluble non-protein substances [2]. Conditional attributes of age, physiological state, habitat conditions, hunting season.
This "*awakens" interest in the area of animal bioresources*, as the basis of a sustainable agri-food economy.

## MATERIALS AND METHODS

### Materials and recipes used to obtain *salami* (product) formulas

**A)** raw-dry-smoked salami hunting mixture (wild boar + roe deer) (***product***)

■ raw: wild boar meat (pulp), 2000 g (hunting fund S-V Romania); deer meat (pulp) 2000 g (hunting fund S-V Romania); bacon domestic pork from a supermarket (Timișoara, Timiș County, Romania), 1500 g;

■ auxiliaries (from a supermarket (Timișoara, Timiș County, Romania)): salt, 80 g; Blak pepper, 4.4 g; garlic 13 g;

■ materials: natural membrane (sheep intestine) (length, L = 30 cm; diameter, ø = 4 cm), 10 g.

**B)** raw-dried-smoked salami (***control***):

■ raw: domestic pork (pulp), 4000 g, bacon domestic pork (1500 g), from a supermarket (Timisoara, Timis County, Romania);

■ auxiliaries (from a supermarket (Timisoara, Timis County, Romania)): salt, 80 g; Blak pepper, 4.4 g; garlic 13 g;

■ materials: natural membrane (sheep intestine) (L = 30 cm; ø = 4 cm), 10 g.

Reagents used for the chemical analyses were purchased from Sigma-Aldrich (St Louis, MO, USA) and Chimreactiv (Bucharest, Romania) and were of analytical purity.

**Manufacture of salami**

The salami samples were prepared in the butchery laboratory of the University of Life Sciences "King Mihai I" from Timișoara, Romania. Chilled game meat was cut into cubes and minced using a mincer up to ø 5 mm (Maxima Holland MMM 12-229, Mijdrecht, The Netherlands). Four (representative), raw-dry-smoked bars from each sample (product/blank), obtained according to the block scheme of operations proposed in figure 1, were taken to work.

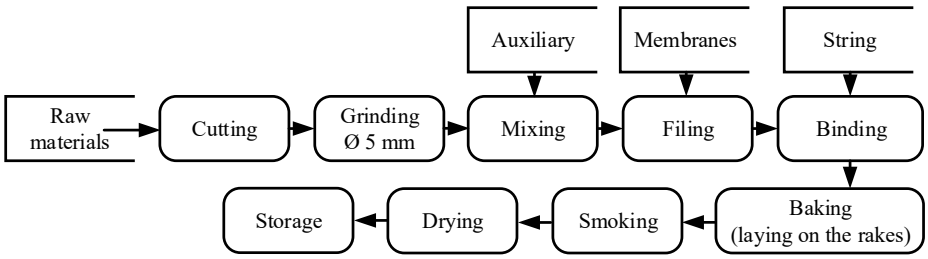



Figure 1. Proposed block diagram of operations for obtaining the two samples: product/control

Ingredients weighed according to recipes shown above were mixed using the meat mixer (La felsinea 10M, Piazzola sul Brenta PD, Italy), the total mixing time was 15 minutes and final temperature of mixture was below 12°C. The resulting mixture was filled into ø 4 cm natural membranes using a salami filler (MSFA15L, Maxima, Mijdrecht, The Netherlands), resulting 30 cm long pieces. Below are briefly described the main operating parameters accessed by process phases: **1.** winding: 40 min.; **2.** cold smoking (hardwood): 45oC, 90 min.; **3.** drying: 10÷12 °C, 72 hours. Resulting salami were stored at 4°C until analysis.

**Proximate composition of salami**

To determine the proximate analysis of the analysed salami samples, the content of water (according to SR ISO 1442:2010), ash [9], total protein (according to SR ISO 936:2009), total lipids (according to SR ISO 1443:2008) and salt (according to SR ISO 91-2007), was determined. The amount of carbohydrates was calculated according to the relationship shown in equation 1, and energy value was calculated using equation 2.

$$\text{Carbohydrates} = 100 - [\text{L} + \text{P} + \text{A} + \text{M}] \quad [\%] \quad (1)$$

$$\text{Energy value} = [(9 \cdot \text{L}) + (4 \cdot \text{P}) + (4 \cdot \text{C})] \quad [\text{kcal/100g product}] \quad (2)$$

**where**: L – lipids, (%); P – proteins, (%); A – ash, (%); M – moisture, (%); C – carbohydrates, (%).

### Sensory analysis

Sensory evaluation of salami samples was carried out after their culinary preparation by roasting according to the method described by [10]. S *The hedonic scale method* was used, with a maximum admissibility score of consumption of ***20*** points. A number of 20 respondents were interviewed (10 men and 10 women, aged 19÷22 years) at the Faculty of Food Engineering (Timisoara, Romania), in natural light (daylight) and at room temperature. salami samples were prepared on plates 5 minutes before the evaluation. The characteristics evaluated by each *respondent* were: external appearance/secțion, consistency, flavor, odor. *The hedonic scale method* establishes five quality classes depending on overall score achieved, as follows: *very good* (18.1÷20.0); good (15.1÷18.0); *satisfactory* (11,1÷15,0); *unsatisfactory* (7.1-11.0); *unsuitable* (0÷7.0) [10].

### Statistical data analysis

Results were expressed as the mean of three determinations ± standard deviation (SD). Statistical differences ($p < 0.05$) between samples were performed by t-test using Microsoft Excel 365 (Version 2208, Redmond, WA, USA).

## RESULT AND DISCUSSION

Hunted meat has a number of benefits, induced by its low fat, high protein content, rich in vitamins, minerals, absence of antibiotics, hormones and growth enhancers. For these reasons, this paper discusses use as raw material of a mixture of hunted meat (wild boar + deer), in obtaining *a raw-dry-smoked* salami in artisanal conditions, subsequently characterized, compared, from a physico-chemical and sensory point of view, respecting the same technological "*trail*", with a similar product for which domestic pork meat was used as raw material (control). Obtained results were compared with maximum admissibility values regulated by *Order no. 210 of 30 August 2006* (Table 1) [11].

**Table 1**. Physicochemical admissibility* requirements for raw-dried-smoked meat products [11]

| Attribute | Admissibility criteria (%) |
|---|---|
| moisture | 45 |
| lipids | 50 |
| chloride content | 6÷9 |
| protein | 20÷22 |
| ash | - |
| Kreiss reaction | negative |

**Order no. 210 of 30 August 2006*

*Proximate analysis* composition of the salami samples is important because it provides information about the quality of the raw materials used and determines the finished products', nutritional value. The main physico-chemical characteristics monitored in the salami samples (table 2, figure 2, 3) were: moisture content, protein, lipids,ash, sodium chloride, carbohydrates, Kreiss reacțion, energy value and reference consumption. The

salami samples were analysed from a physico-chemical point of view using the analysis methods regulated by the legislation in force.

**Table 2.** Proximate analysis of the salami samples

| Sample | Attribute | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Moisture (g/100 g) | Protein (g/100 g) | Lipids (g/100 g) | Ash (g/100 g) | Chloride content (g/100 g) | Carbohydrates (g/100 g) | Kreiss reaction | Energy value (kcal/100 g) | Benchmark consumtion (%) |
| limit | 45 | 16 | 50 | - | 6.00 | 0.5 | - | 386 | - |
| control | 43.04± 1.25$^{b}$ | 19.59± 0.56$^{a}$ | 31.91± 0.92$^{b}$ | 1.73± 0.04$^{a}$ | 2.87± 0.07$^{a}$ | 0.86 | - | 368.99 | 18.45 |
| product | 41.55± 1.19$^{a}$ | 24.13± 0.69$^{b}$ | 28.54± 0.81$^{a}$ | 2.53± 0.06$^{b}$ | 2.83± 0.07$^{a}$ | 0.42 | - | 355.06 | 17.75 |

$^{a-d}$ different letters between samples signify statistical differences acording to t-test

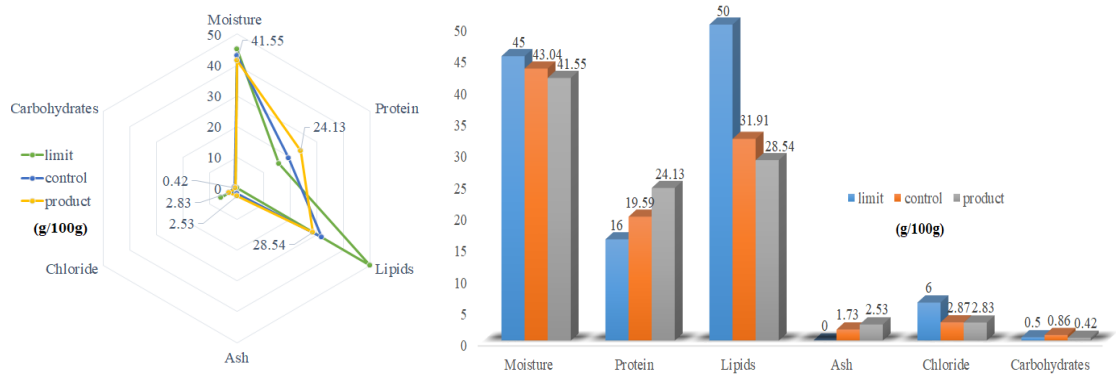



Figure 2. Integrated graphical interpretation of evolution for physico-chemical attributes in parameters: limits – control – product (table 2)

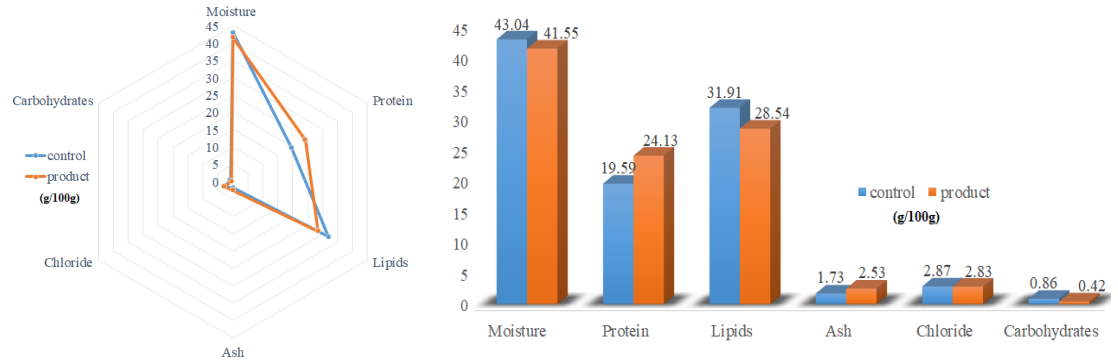



Figure 3. Integrated graphic interpretation the evolution of physico-chemical attributes in parameters: control – product (table 2)

*Moisture* (table 2). *Moisture* (table 2). The greater weight of the animal, the lower the total water share of the pieces of meat. The amount of water present in muscle tissue greatly influences the texture, color and flavor of the product. Share of water (max.) in raw-dry-smoked products is 45%. Value recorded for the analyzed product was 41.55%, compared to 43.04% recorded for the blank sample. Values respecting the maximum admissibility limit (45%), corresponding to the studies conducted by *Bordea, S.A. et al.*, (**2017**), *Cocan, I. et al.*, (**2015**) [12, 13].

*Protein* (table 2). Meat protein provides information on nutritional value of the product. A high protein content supports growth, regeneration and maintenance of tissues, having a positive influence in regulating vital functions of the body. Barrier against diseases and infections. Protein content for smoked and dried meat products is 22%. The value obtained in terms of protein content of study product was 24.13%, higher than the control sample (19.59%). The weight of protein in the studied salami sample corresponds to the values reported in the literature [12].
*Lipids* (table 2). ). Hunted mixture is characterized by low fat content, compared to meat from domestic animals. Initially, fat content in mixture (wild boar + roe deer) was 7%. Finally, addition of bacon reached 28.54%, below permissible limit (50%), compared to the blank sample of 31.91%.
*Chloride content* (NaCl (%)) (table 2). Chloride content in analyzed sample was 2.83%, and in the blank sample 2.87%, values that meet the admissibility conditions (maximum 9%) [11].
*Ash* (table 2). Mineral substances are energy suppliers, involved in regulating and maintaining normal parmetries of vital body functions (e.g. regulating amount of water in tissues; mediators of protein metabolism, growth function, nervous system balance, hemoglobin synthesis processes, etc.). Mineral substances have been shown to be involved in cell growth. In this case, no maximum or minimum limit is regulated.
*Carbohydrates* (table 2). Share of carbohydrates in product was 0.42% and 0.86% respectively for the blank. Results in terms of carbohydrate content are similar to other values reported in literature for different types of raw-dried-smoked meat products (e.g.: 2.1% for smoked pork sausage; 2.7% for pork and beef sausages) [14].
*Energy value* (table 2, figure 4). In this case, the legislation does not regulate a "threshold" (minimum/maximum) for the energy value. However, literature recommends an average intake of 386 kcal/100 g product [14]. Calculated energy value for the product was 355.06 kcal/100 g, compared to the blank sample of 368.99 kcal/100g (*benchmark consumption* = daily requirement of 100 g product that provides the energy/24 hours requirement of an adult (recommended 2000 kcal)).

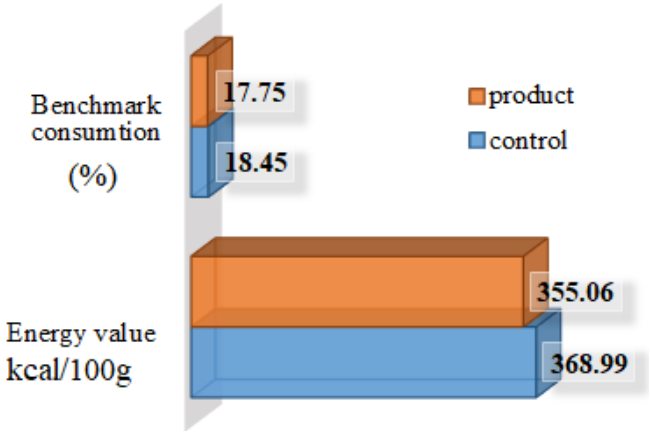



Figure 4. Graphical interpretation of the evolution of the weight of energy developed by 100g product for the two samples (product/control)

*Reacția Kreiss* (table 2). This indicator is measure of degree of freshness of product, correlated with degree of oxidation of lipids present in food of animal origin. Following the determinations made, the Kreiss reaction was negative, which translates that the

product is fresh. Results according to SR ISO 17025 – General requirements for the competence of testing and calibration laboratories [15].

**Sensory analysis**

Sensory analysis followed the influence of raw/auxiliary materials, correlated with technological process, which imprints sensory attributes. Results of sensory analyses of salami samples are shown in figure 5.

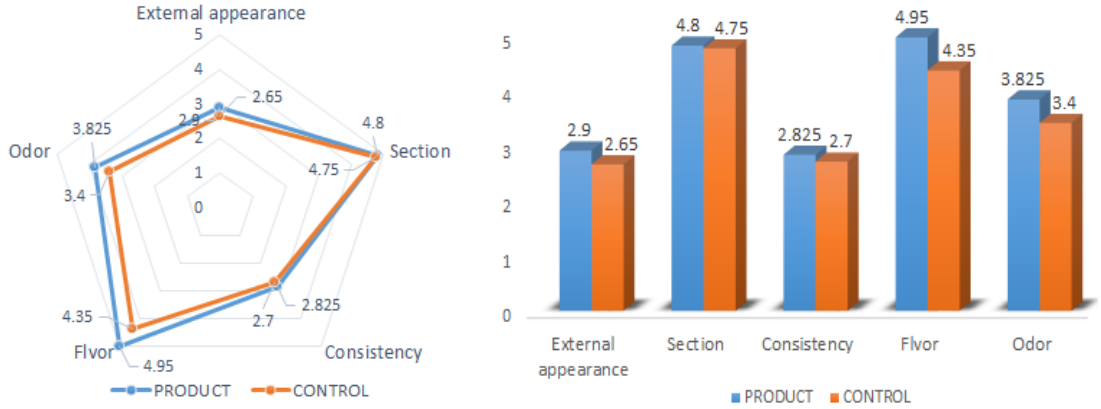



Figure 5. Centralised, graphic interpretation, of the scores obtained (weighted average score) for the five sensory attributes of the two samples (product/control)

Total values (mean) recorded for the studied samples (figure 5) are within upper limits (17.85÷19.30), which correspond to and recommend the products in very high quality class. Highest value was recorded for the product (19.30) and 17.85 for control. In terms of *external appearance*, maximum score was also recorded for the product (2.90), and for blank salami sample an average score of 2.65. When evaluating *aspect* in section, average score obtained is a balanced one, 4.8 recorded for the product (4.8) and 4.75 recorded for control. The *consistency* assessment of the analysed samples recorded following values: a maximum for the product (2.825) and a minimum for control (2.70). Taste *analysis* follows the same trend. Upper value recorded for the product (4.95) compared to the blank test (mean score 4.35). *The smell* scored an average of 3.825 for the product and 3.40 for the control. Finally, sensory analysis performed compared for the two samples of salami (product/control), admits upper score, individual/total, for each attribute, to the raw-dry-smoked salami product made of *wild boar* and *deer* meat mixtures.

## CONCLUSION

This study brings to attention the possibility of capitalizing on the hunting fund in the South-West area of Romania, even seasonally, through informational arguments regarding the qualitative characteristics of consumption of a ***common salami product***, having as raw material a mixture of game meat (wild boar + deer), in order to stimulate the consumption of processed products of this class (***hunted***). Compared physical-chemical/sensory attributes, product/witness/admissibility conditions, gave confidence to respondents and recommend the product as a *consumer good*.

**ACKNOWLEDGEMENTS**

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# CONTROL OF PHYTOPHAGOUS APHIDS IN URBAN GREEN SPACES THROUGH ENVIRONMENTALLY FRIENDLY WAYS

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## ABSTRACT

Aphids have grown significantly both in number of species and in number of individuals in populations, especially in green spaces in urban environments. By feeding, they affect the plants in the structure of the ecosystem at the foliar and floral level, therefore it is necessary to manage them so that they are numerically reduced but at the same time to avoid the application of pesticides. Through this work, 2 ways of management were tried for 5 months (April-August): by exploiting existing natural enemies and by using sticky traps. The 4 study sites in western Romania (Timișoara city) consisted of green spaces composed of various mixtures of ornamental plants, usually shrubs and trees of different species. As a result, 4 aphid species (*Macrosiphum rosae, Aphis gossypii, Aphis pomi* and *Myzus cerasi*) were identified whose populations had high values (x= 468.7-1246.5) and which were considered as the target organism in the study. The effectiveness of the most common natural enemies (beetles, syrphids and parasitic wasps) was analyzed and yellow sticky traps were placed for dual-role capture (aphid detection and their immortalization). It was found that both methods are effective, the harmful insects being visibly reduced compared to the initial value. Natural enemies vs. phytophagous aphids had a good effectiveness ratio (1:18.25), while colored sticky traps retained and killed 78% of captured aphids. The management of aphids in the agro-horticultural spaces between people's homes, although it seems difficult to achieve, we consider that it can be done within reasonable limits through non-pheromonal traps and existing natural enemies, somehow keeping the harmful populations under control and not affecting humans and the environment.

**Keywords:** aphids, friendly strategies, control, green spaces.

## INTRODUCTION

Environmental pollution, whether it comes from pesticides or other sources, affects human health and life [10]. The measurement of pollution is relative and debatable, mainly using animal organisms as bioindicators [15], but it is clear that both air and water as vital sources for humans must be protected. As such, it is important that in our strategic choices for managing pests near homes and beyond, we have an environment-friendly attitude [4]. For example, in morpho-biology studies and evaluation of

individuals in a population, some researchers used non-polluting coloured traps that attract and immortalize insects at the same time [8] or natural enemies [11] in order to replace pesticides [6]. Another less applicable but potential way is non-promotion of big global companies producing only pesticides, which in many cases have proven to be sources only interested in profit without taking into account the environment and human health [12] or these not exposing the negative unwanted effects [3].
Globally, for aphids, the most numerous treatments are done given the large number of generations that is increasing [13], [14], therefore the assessment of the population level is usually made through periodic observations and monitoring. At the same time, aphids are among the insect pests that are controlled by many natural enemy species, and among these we can mention parasitic wasps and predatory species such as ladybugs or syrphids [7]; [2]; [5].
Monitoring studies carried out in several parks in western Romania (close to the current study site) in 2020 showed that *Aphis gossypii* and *Aphis rosae* had the highest frequency among the aphid species [9].
Recently, the diversity and quantity of aphid species and their predators have increased numerically [13], so that a continuous check of the ecosystems is necessary.
As such, in the following we consider the monitoring of aphids in the green spaces of the urban environment as well as the abundance of predators in order to evaluate the effectiveness of some non-polluting methods.

## MATERIALS AND METHODS

**Places of study.**
The study sites in the city of Timisoara located in the west of Romania consisted of 4 green spaces formed by various mixtures of shrubs, trees and decorative plants of different species. Of these, 2 were recreational parks and 2 were green spaces between buildings or boulevards located in different areas (Table 1).

Table 1. Description of study places

| Places | Name of places | Location area in Timisoara City | Type of plants |
|---|---|---|---|
| Place 1 | University Park ULST | Northern part | Trees, shrubs and annual decorative plants |
| Place 2 | Roses Park | Central part | Trees, shrubs and roses |
| Place 3 | Green space between houses | Eastern part | Trees, shrubs and annual decorative plants |
| Place 4 | Boulevard- green space | Western part | Trees and shrubs |

**Observations and traps used.** As a first step, the species of phytophagous aphids and their natural enemies were identified. The identification of aphid species was generally done according to Blackman and Eastop's guide [1]. Then the numerical level of both categories was quantified by placing 2 yellow sticky traps/place and free observations and also the efficiency of the most frequent natural enemies (beetles, syrphids, parasitic wasps). The observation period lasted for 5 months (April-August) from 2023, in total 10 readings, 2 each month.
**Statistical analyses.** The data obtained from the experiments were processed using the program IBM SPSS Statistics Version 21. For statistical analysis, the numerical characteristics were calculated (mean, standard deviation, minimum value, maximum

value, confidence intervals for the mean) and to establish significant differences, the ANOVA test, and the LSD post hoc test.

## RESULTS

The species of aphids caught on the traps were 4, namely *M. rosae, A. gossypii, A. pomi* and *M. cerasi.* Depending on the place of capture, it can be seen that the maximum average of catches was recorded in Place 2 (Roses Park), namely 104.1750 individuals, with a standard deviation of 84.505 individuals, respectively, the minimum average was recorded in Place 4 (Boulevard- green space) with a value of 61.9750 and a standard deviation of 41.11553 individuals (table 2, figure 1).

Table 2. Numerical characteristics associated with the number of phytophagous aphids depending on the study places

| Study place | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| Place 1 | 40 | 77.9000 | 51.93823 | 61.2893 | 94.5107 | 2.00 | 211.00 |
| Place 2 | 40 | 104.1750 | 84.50516 | 77.1489 | 131.2011 | 1.00 | 302.00 |
| Place 3 | 40 | 67.0000 | 51.40438 | 50.5601 | 83.4399 | 0.00 | 200.00 |
| Place 4 | 40 | 61.9750 | 41.11553 | 48.8256 | 75.1244 | 0.00 | 148.00 |
| Total | 160 | 77.7625 | 61.18335 | 68.2095 | 87.3155 | 0.00 | 302.00 |

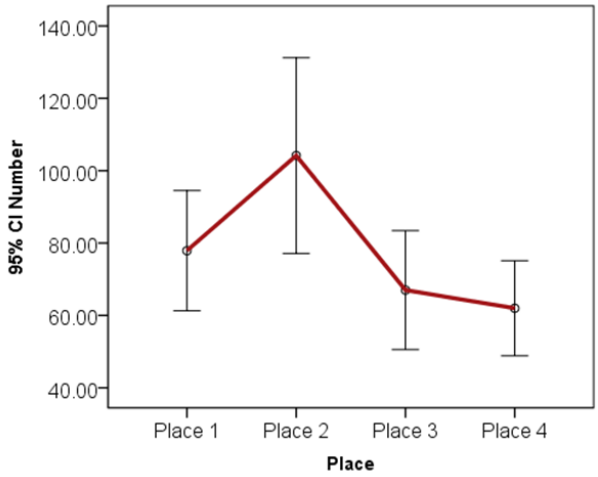



Figure 1. Species Mean and 95% Confidence Interval for Mean of aphid catches depending on the study places

The statistical analysis of the values recorded in the 4 places where individuals-phytophagous aphids were recorded shows us that there are significant differences between these values (ANOVA test F=3.999, p=0.009<0.01). By applying the LSD test, it is observed that there are significant differences in the number of phytophagous aphids recorded in Place 1 vs Place 2 (p=0.049<0.05), Place 2 vs Place 3 (p=0.006<0.05) and Place 2 vs Place 4 (p=0.002) (table 3).

Colored sticky traps retained and killed 78.02% of captured aphids at all sites. The highest kill percentage was on the traps in Place 2, with 80.62% dead aphids (table 3a).

Table 3. Multiple comparisons of individuals of phytophagous aphids caught on traps depending on the study places

| Place vs Place | | Mean Difference | Std. Error | p | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Place 1 | Place 2 | -26.27500* | 13.30958 | 0.049 | -52.5653 | 0.0153 |
| | Place 3 | 10.90000 | 13.30958 | 0.414 | -15.3903 | 37.1903 |
| | Place 4 | 15.92500 | 13.30958 | 0.233 | -10.3653 | 42.2153 |
| Place 2 | Place 3 | 37.17500* | 13.30958 | 0.006 | 10.8847 | 63.4653 |
| | Place 4 | 42.20000* | 13.30958 | 0.002 | 15.9097 | 68.4903 |
| Place 3 | Place 4 | 5.02500 | 13.30958 | 0.706 | -21.2653 | 31.3153 |

Table 3a. The percentage of dead aphids from those captured on traps

| Study places | Mean of all aphids captured | Mean of all aphids immortalized | Effectiveness Percent (% dead aphids) |
|---|---|---|---|
| Place 1 | 97.8400 | 77.9000 | 79.60 |
| Place 2 | 129.2000 | 104.1750 | 80.62 |
| Place 3 | 91.0000 | 67.0000 | 73.62 |
| Place 4 | 79.0000 | 61.9750 | 78.44 |
| | | | 78.02 |

Natural enemies observed in the study sites belonged to the categories of ladybugs, syrphids and parasitic wasps (figure 2). The maximum number of natural enemies observed was 22 in Place 2, which also has the maximum average of 5.2 with a standard deviation of 3.02 (table 4). By applying the ANOVA test, it is observed that there are no significant differences in the number of individuals observed depending on the study site (F=1.272, p=0.287) (table 5).

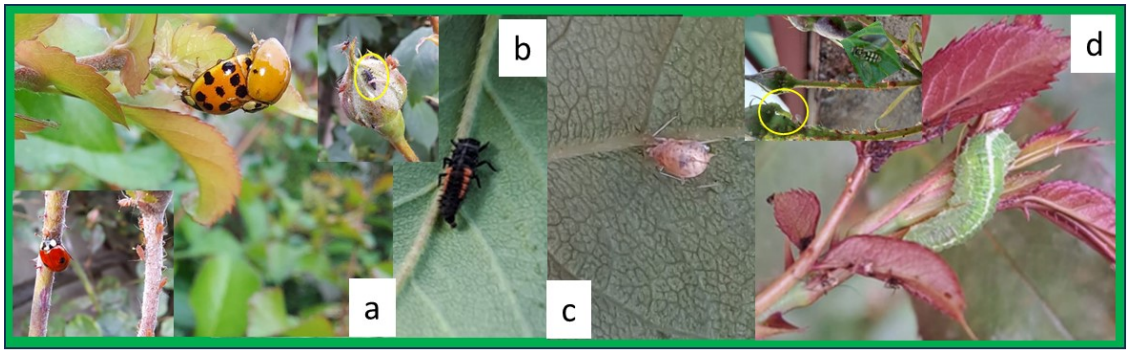



Figure 2. Species of natural enemies of aphids found in places P1-P4, at the time of predation (a, b, d) or after parasitism (c-formation of mummies)

Table 4. Numerical characteristics associated with natural enemies depending on the study places

| Place | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| Place 1 | 30 | 4.7667 | 4.81867 | 2.9673 | 6.5660 | 0.00 | 19.00 |
| Place 2 | 30 | 5.2000 | 5.83332 | 3.0218 | 7.3782 | 0.00 | 22.00 |
| Place 3 | 30 | 3.4667 | 3.45147 | 2.1779 | 4.7555 | 0.00 | 11.00 |
| Place 4 | 30 | 3.3667 | 3.33718 | 2.1205 | 4.6128 | 0.00 | 12.00 |
| Total | 120 | 4.2000 | 4.49575 | 3.3874 | 5.0126 | 0.00 | 22.00 |

Table 5. Analysis of variance (ANOVA) for groups of natural enemies

| | Sum of Square | df | Mean Square | F | p |
|---|---|---|---|---|---|
| Between Groups | 76.600 | 3 | 25.533 | 1.272 | 0.287 |
| Within Groups | 2328.600 | 116 | 20.074 | | |
| Total | 2405.200 | 119 | | | |

The maximum number of natural enemies observed was from the Ladybirds category (22 individuals), with an average of 9.1 and a standard deviation of 4.46 (tabel 6).

Table 6. Comparisons between species of natural enemies taking into account the sites

| Daunatori | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| Syrphids | 40 | 1.7500 | 1.72091 | 1.1996 | 2.3004 | 0.00 | 6.00 |
| Ladybirds | 40 | 9.1000 | 4.46525 | 7.6719 | 10.5281 | 0.00 | 22.00 |
| Parasitic wasps | 40 | 1.7500 | 1.35401 | 1.3170 | 2.1830 | 0.00 | 5.00 |
| Total | 120 | 4.2000 | 4.49575 | 3.3874 | 5.0126 | 0.00 | 22.00 |

There are significant differences between the number of natural enemies observed (ANOVA, F=87.368, p=0.000), i.e., Syrphids vs Ladybirds (p=0.000<0.05) and respectively Ladybirds vs Parasitic wasps (p=0.000<0.05) (table 7).

Table 7. Comparisons between all groups of natural enemies

| Natural enemies | | | Mean Difference | Std. Error | p | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Type | Syrphids | Ladybirds | -7.35000* | 0.64205 | 0.000 | -8.8742 | -5.8258 |
| | | Parasitic wasps | 0.00000 | 0.64205 | 1.000 | -1.5242 | 1.5242 |
| | Ladybirds | Syrphids | 7.35000* | 0.64205 | 0.000 | 5.8258 | 8.8742 |
| | | Parasitic wasps | 7.35000* | 0.64205 | 0.000 | 5.8258 | 8.8742 |
| | Parasitic wasps | Syrphids | 0.00000 | 0.64205 | 1.000 | -1.5242 | 1.5242 |
| | | Ladybirds | -7.35000* | 0.64205 | 0.000 | -8.8742 | -5.8258 |

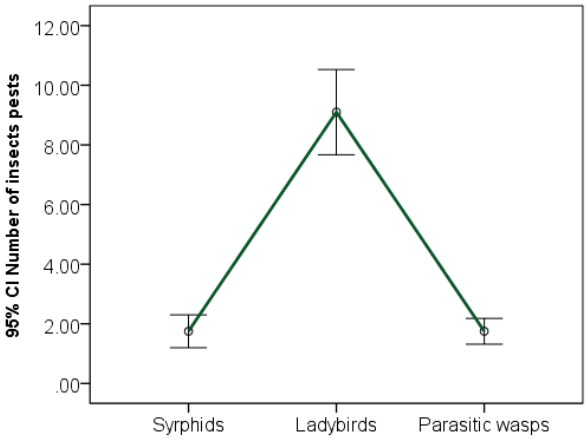



Figure 3. Species Mean and 95% Confidence Interval for Mean of natural enemies depending on the place of observation

The effectiveness ratio between the harmful phytophagous aphids and the natural enemies in the analyzed period shown in table 8 is located at good values from 1 natural enemy to 16 aphids/Place 1 to 1 natural enemy to 20 aphids/Place 2. The best results have been recorded in Place 1 and Place 4 (1:16 and 1:18) (Table 8).

Table 8. The interaction and effectiveness ratio between phytophagous aphids and natural enemies

| Study places | Mean of all aphids regardless of species | Mean of all natural enemies regardless of species | Effectiveness ratio |
|---|---|---|---|
| Place 1 | 77.9000 | 4.7667 | 1: 16 |
| Place 2 | 104.1750 | 5.2000 | 1: 20 |
| Place 3 | 67.0000 | 3.4667 | 1: 19 |
| Place 4 | 61.9750 | 3.3667 | 1:18 |
| | | Ratio average | 1:18.25 |

## DISCUSSION

In all 4 places (Place 1, Place 2, Place 3 and Place 4) the aphids had different levels of abundance expressed by the numerical level. Area type somewhat influenced aphid abundance, such that more aphids were trapped in parks than in green spaces between buildings. Therefore, their abundance is different and it is not clear whether the type of area, the combination of host plants or the location are the influencing factors, as Wang also found [14]. The attractiveness of coloured sticky traps is also proven in this work, as it has been proven on other plants in previous research [8]. With a capture percentage of over 75% and immortalization of over 60%, it can be said that this method is quite effective, and has a double role, monitoring and killing aphids. It is not possible to make a comparison with other researchers of the immortalization rate because this approach is our own, guiding us and adapting to what we found at a given moment in the analysed parks and green spaces. Aphids, regardless of species, clearly attracted natural enemies [2],[14] and had a reasonable consumption ratio, 1 natural enemy (either weevil, syrphid or parasite wasp) apparently can prey on up to 20 aphids. It is clear that if the 2 methods are combined, the reduction of phytophagous aphids can be achieved below the level of damage that causes obvious quantitative or qualitative losses and of course without intervening with other polluting methods in spaces where humans and pets find a place to relax and of clean air [10] [3].

## CONCLUSION

As a result, the 2 management systems addressed proved to be effective both through the use of colored sticky traps and through the exploitation of natural enemies. Thus, an effectiveness ratio between 73 and 80% was obtained at 2 traps/100m$^2$ for the 4 species of aphids found in all the study sites. They belonged to the genera Aphis (2), Macrosiphum (1) and Myzus (1) and had average values between 79 and 129 individuals/place. Among the natural enemies, syrphids, ladybirds (as predatory species) and parasitic wasps have been identified and considered as control agents. The effectiveness/places ratio was good (1 to 18.25), which means that a natural enemy (be it ladybug, parasitic wasp or syrphid) can consume/parasite 16-20 aphids, depending on the place. It can be concluded that both the ratio of immortalization/traps and the rate of

consumption/parasitism can ensure the reduction of phytophagous aphids in natural conditions, without chemical intervention. However, it is necessary to periodically observe and monitor the numerical level of aphids and their natural enemies, without relying on previous research, because each year or study area may have specificity and particular management needs.

## ACKNOWLEDGEMENTS

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# CONTROL TECHNIQUES USED TO MANAGE AND MITIGATE FOOD FRAUD

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## ABSTRACT

This paper aims to synthesize in a logical and coherent approach the main control techniques used in managing and combating food fraud, as well as to discuss their effectiveness.

Given the impact that food fraud can have on consumers' health and well-being, anti-fraud control techniques used throughout the food chain are an important and relevant topic, still insufficiently addressed and accepted in the relevant economic environment.

The objectives of the article include presenting the current context of food fraud, different anti-fraud control techniques and relevant examples, as well as drawing conclusions and recommendations for the future. By addressing this topic, the article aims to contribute to raising awareness about the importance of preventing and combating fraud of any kind in the food industry.

Preventing and combating food fraud involves a multidimensional approach that includes food monitoring and control, enforcement of regulations and standards, and international cooperation. By implementing these measures, authorities can help protect consumers against fraudulent practices in the food industry.

In conclusion, monitoring and controlling food supply chains is a proactive and essential approach to effectively combat food fraud. By applying these measures, it can be ensured that food reaches consumers with guaranteed authenticity and safety, thereby protecting public health and strengthening trust in industry.

**Keywords:** food fraud, control technics, food safety

## INTRODUCTION

Food fraud dates back to ancient Rome and Athens, where there were laws on counterfeiting wines with flavours and colours [7]. Even though food fraud is an old problem, it is still topical. Food fraud is a problem that affects all food supply chains and therefore the entire food industry, customers and consumers. Food supply chains are becoming increasingly vulnerable due to globalisation and its prolongation [1], [11]

With greater awareness of food safety and quality, consumers are increasingly demanding reassurance about the origin and content of food consumed, while producers must be able to confirm the authenticity of components of marketed products in compliance with government legislation. Therefore, protecting the rights of consumers and processors of genuine food products and preventing fraudulent or misleading practices relating to food adulteration is an important and challenging issue in the food industry. As a result, scientific and technological advances have taken place in recent years in determining food authenticity. [11]

Modern food authentication techniques focus on newly developed techniques and their recent applications in authenticating and combating food fraud.

Techniques specific to the identification of falsifications include various spectroscopic technologies, methods based on isotopic analysis and chromatography and other DNA-based techniques, enzymatic analysis, electrophoresis and thermal methods.

Within this general framework for addressing quality, food occupies a particularly important place in EU policy; The quality requirements imposed on these products are due, on the one hand, to the prospects of increasing the number of consumers with the acceptance of new members (and implicitly to the substantial increase in food trade), and on the other hand to the numerous and costly crises in the agri-food sector it has faced (BSE crisis – bovine spongiform encephalopathy, nitrofurans, hormones, mycotoxins, etc.) and which have jeopardized confidence in quality and safety food from the EU. [12]

In this context, the European Union recommends monitoring food products from the stage of obtaining raw materials to the final consumer. This strategy aims to protect and regain the confidence of consumers in the Member States by adopting concrete legislative actions, based primarily on the precautionary principle.

Throughout its existence, the European Union has sought to provide its full members with advantages that they alone would not have obtained, namely the four free movements: for persons, goods, capital and labour. [5]

Products accepted on the market circulate freely, without being subjected to multiple and costly checks in terms of time and money. In order to achieve this desideratum, however, compliance with harmonized standards at European level must be achieved and proven, which give the presumption of seriousness in terms of achieving quality and especially maintaining its constancy. In the European Union, national quality specifications will be replaced by directives that are common. EU directives set minimum conditions that certain products and services must meet in order to be sold on the European market. For products covered by these directives, suppliers must demonstrate compliance with current European Union standards.

The food industry is in the unique position of being a victim of food fraud and also largely responsible for food fraud incidents [12]. Therefore, the food industry needs a clear understanding of food fraud, as well as guidance on preventing and mitigating food fraud.[13]

However, within these documents, definitions of food fraud often differ, as well as the types of food fraud that may occur. Therefore, prevention and mitigation strategies are pervasive and not specific to a particular supply chain, which increases ambiguity. The authenticity of agri-food products has not been officially defined, nor is there a European or national regulatory framework: there are no sanctions for economic operators /

individuals who intervene in the food chain with inauthentic / falsified products, product characteristics, procedures / technologies for obtaining are not regulated, there is no validated, unanimously recognized definition - there are nuances between the approach of specialists in Europe and those in the United States when the definitions of food fraud, integrity, and economically motivated adulteration of food are brought up.

Food information shall not mislead the purchaser, in particular as regards the characteristics of the food, its nature, identity, properties, composition, quantity, date of minimum durability, country of origin or place of provenance, method of manufacture or production - it shall not attribute to the food effects or properties which it does not possess. Label declarations must comply with what is laid down by regulations and what the food actually contains.

In this context, we emphasize the need for public policies in the food field, even if the very concept of public policies in the field of food (food policy) is an "ambiguous concept" that requires debates on the interrelation with other areas of public policies or the need for an integrative approach of food policy with public policies related to consumer health, agriculture, environment, security, research and education, tourism, culture, transport, etc.[8]

The responsible authorities, in collaboration with other stakeholders, must ensure appropriate conditions so that safe (harmless/wholesome), quality (intrinsic nutritional value, energy supply due to biomineral compounds, vitamins and other bioactive compounds, bioavailable nutritional value) and authentic food products reach consumers' meals.

Simply, food regulations address the control of food businesses with local, regional, national and international rules and regulations for food production and sale, including food safety and quality and industry lobbying activities. Out of the referred aspects of the food industry, food regulations become pivotal in the food trade as those are to ensure food safety, quality and honest offering keeping food trade comfortable for the economic growth.

Due to technical and legal complications and the involvement of different institutions with responsibilities in food safety and security, food regulatory systems are complex in nature. A well-structured, efficient and accessible regulatory system is key to the development of the food industry.

In 2003, WHO and FAO made a joint publication on ensuring food safety and quality: guidelines for strengthening national food control systems. With a great interest in promoting national food control systems, especially in developing countries, WHO and FAO presented this publication aimed at national authorities concerned with ensuring food safety and quality in the interests of public health and consumer protection. Its guidelines are also helpful to a number of other stakeholders, including consumer groups, industrial and commercial organisations, farmer groups and any other groups or associations influencing national policy in this area.[6]

As the WHO and FAO identified common factors undermining national food control systems, its guidelines sought to advise national authorities, particularly in developing countries, on strategies to strengthen food control to protect public health, prevent fraud and deception, avoid food adulteration, and facilitate trade. These guidelines therefore appear to help formulate recommendations on food fraud and ways to prevent and combat

it, considering the local food regulatory environment, policy feasibility and socio-cultural elements.[8]

FAO published Strengthening National Food Control Systems: Guidelines for Assessing Capacity Building Needs (2006). It provides robust assistance in developing food regulations and standards in the field of food fraud and food control at the level of each state. FAO recalls the five essential components of working in a food control system as follows:

- Food Control Management
- Food law
- Food inspection
- Official Food Control Laboratories
- Information, education and communication on food safety and quality

## 2. CONTROL TECHNIQUES USED TO PREVENT AND COMBAT FOOD FRAUD

In general, control techniques used in managing and combating food fraud are divided into two categories: detection techniques and prevention techniques.

### 2.1. Food fraud detection techniques

Food fraud detection techniques are used to identify food that has been contaminated or adulterated.

Detection techniques include DNA analysis, near-infrared spectroscopy (NIR), high-performance liquid chromatography (HPLC), and tandem mass spectrometry (MS/MS). These techniques are used to detect contaminants and adulterants such as pesticides, heavy metals, food additives and allergens. [1],[5]

- **Mass Spectrometry**

Identifies chemicals present in food by measuring the masses of molecules. It can detect illegal additives or contaminants. [2]

- **Liquid or Gas Chromatography**

Chromatographic techniques in their various forms are among the most important methods used in food analysis. The term 'chromatography' covers techniques based on the adsorption and/or division of analytes between a mobile and a stationary phase. Thus, gas chromatography (GC), either as gas to liquid chromatography (GLC) or gas-solid chromatography (GSC), is a method in which the mobile phase is gaseous. In liquid chromatography (LC), commonly referred to as high-performance liquid chromatography (HPLC), the solid stationary phase is applied to a column and the mobile phase is pumped through the column. Thin layer chromatography (TLC) systems consist of a flat solid phase and a liquid mobile phase. Due to the superior capability of GC and HPLC, the two techniques have often been used for authentication purposes - i.e. identifying forgeries in natural juices. [5]

- **PCR (Polymerase Chain Reaction)**

Used to identify and quantify specific DNA or RNA. It helps detect fraud regarding species labeling or food origins.

In food authentication, species identification is a predominant scope. Apart from methods based on immunoassays, the so-called polymerase chain reaction (PCR) is of utmost importance for the purpose of species authentication and has a number of advantages over immunoassays - such as the relatively high stability of DNA and its predictable behavior [7]. PCR allows millions of times amplification of a DNA fragment that is framed by two primers. [5]

- **Atomic Absorption Spectrometry**

Determines the concentrations of metals or minerals in food. Useful for identifying unwanted metal contamination.

Spectroscopic techniques, especially infrared (IR) spectroscopy and nuclear magnetic resonance spectroscopy (NMR), as well as UV-Vis spectrophotometry, are widely used in food authentication. [9]

- **Infrared spectroscopy**

Identifies chemical compounds by analyzing infrared radiation absorption. It can be used to authenticate the origin of food. [6],[12]

These laboratory techniques are essential for ensuring food quality and safety, identifying potential fraud and helping to maintain consumer confidence in food.

### 2.2. Techniques to prevent and combat food fraud

Prevention techniques include supply chain tracking, food labeling, risk analysis and internal audit. These techniques are used to prevent food fraud from occurring by identifying and eliminating risk factors in the supply chain, as well as ensuring compliance with food safety regulations and standards.

- **Quality Certifications and Standards**

Adopt and comply with internationally recognized certifications and quality standards certifying the origin, production process and quality of food. [10]

- **Supply Chain Monitoring**

Supervise the entire food production and distribution chain to identify vulnerabilities to fraud and ensure product integrity throughout the process.

- **Batch Tracking Techniques**

Implement batch tracking systems to monitor every stage of production and distribution, making it easy to quickly identify and fix any issues.

- **Certification and Labelling**

Adopt and implement certification and labelling standards certifying product quality and origin, providing transparency to consumers and reducing the risk of fraud. Using modern

labelling technologies such as QR or RFID codes to provide consumers with access to detailed information about the product and its origin. [7]

- **Food control and health claims on food labelling**

Health claims on food labels are claims by food manufacturers that their food will reduce the risk of developing a disease or certain conditions. In today's world, food manufacturers use these health claims through a marketing communication tool to promote their products to competitors in their aggressive market sales campaigns. However, it has been widely experienced that most of these health claims have no scientific basis and has therefore been a violation of the honest supply of food to the consumer. Therefore, these misleading and fraudulent health practices must be controlled in the food industry, both for consumer protection and for fair commercial competition. [3]

- **Cross-sectoral collaboration**

Cooperation between government authorities, industry organisations and consumers to share information and develop joint strategies to combat food fraud.

- **Rigorous Control at Borders and Points of Entry**

Step up controls at borders and points of entry to prevent fraudulent or non-compliant food from being placed on the market.

- **Consumer education**

Providing information, education and advice to all stakeholder groups along the entire food chain is an increasingly important role for food control systems [4].

Informing consumers about signs of recognition of authentic products and promoting awareness of the risks of food fraud.

Raising consumer awareness of the risks associated with food fraud and promoting responsible behaviour in purchasing and consuming food.

In general, control techniques are effective in managing and combating food fraud, but there are still many challenges and issues that need to be addressed. These include the lack of standardisation of detection and prevention techniques, as well as the need to develop new techniques to deal with new threats and risks.

These techniques properly combined and applied, help ensure food safety and prevent fraud in the food industry.

To prevent food fraud, a number of techniques and practices are used throughout the food industry.

## 3. CONCLUSIONS

The main conclusions that can be drawn and that can contribute to combating and preventing food fraud are:

Strengthen monitoring and control of food products at different stages of the supply chain to detect and prevent fraudulent practices.

Improve the enforcement of regulations and standards in force, including by applying sanctions for violations found.

Promote international cooperation to combat cross-border food fraud, including through the exchange of information and collaboration with international organisations.

Encourage the use of advanced technologies to detect food fraud, such as enzyme techniques, biosensor techniques, immunochemical techniques, DNA-based techniques, chromatography methods and spectroscopy.

By implementing these measures, authorities can contribute to increasing the safety and quality of food products, thus protecting consumers against the risks associated with the consumption of counterfeit or adulterated food.

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# CYTOLOGICAL DIAGNOSTIC TECHNIQUES FOR ENHANCING RESILIENCE IN BREEDING PROGRAMS

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## ABSTRACT

Since the inception of modern plant breeding, especially with the discovery of heterosis and the development of hybrids, which are known for their ability to enhance crop yields, plant breeders have consistently strived to advance and identify an efficient method for developing inbred lines. In this endeavor, breeders have encountered various challenges, including the complexity of requiring multiple generations to develop inbred lines and the struggle to find effective ways to optimize this process. In our research, we aim to present a method for cytological diagnosis and the identification of high levels of inbreeding.

Our study involves the analysis of sunflower genotypes, both hybrids and inbred lines, regarding the mitotic index and its correlation with leaf area and root development in an automatic rhizotron.

During our research, we observed significant differences between hybrid genotypes and inbred lines in terms of the mitotic index and variations in leaf area and root development. Therefore, this research provides insight into the applicability of cytology for timely identification and elimination of inbred genetic material, thereby contributing to increased resilience and economic efficiency in breeding programs through the adoption of cytological diagnostic techniques.

**Keywords:** Cytological diagnostic, Mitotic index, heterosis, Inbreeding, Plant breeding.

## INTRODUCTION

Breeding programs around the world have focused on the timely creation and adaptation of new genotypes to counteract various factors, whether abiotic or biotic, to make crop production more efficient by promoting high-performance varieties or hybrids [10]. The exploitation of the heterosis phenomenon has changed the course of research on some crops by replacing varieties with hybrids. Their introduction into breeding programs has also resulted in the development of inbred genotypes that made it possible

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to develop hybrid seeds. Polymorphism levels between inbred lines have played an important role in the development of high-performance hybrids. The inbreeding effect results in genotypes with decreased yield as well as increased susceptibility to biotic or abiotic factors, depending on the level of inbreeding [5].

Inbred lines developed due to gene homozygosity are uniform in terms of yield, flowering time, height, and other morphological characteristics. Although the development of hybrids has made a considerable contribution and has fully replaced varieties in the market and in culture, their creation requires a very long time due to the large number of generations that a new inbred line must reach before full development.

Thus, to shorten the development time of hybrids and improve varieties, biotechnological methods have been introduced in most crops [9], such as introducing in vitro cultures [2, 4, 12], such as the rescue of immature embryos, to go through a considerable number of generations in one year, up to five generations in some cases. Haploidy is also an innovative method that has shortened the creation time of consanguineous lines [1].

Cytological diagnosis, however, is a less widely used method in the development and involvement of plant breeding. Nevertheless, cytological analyses can provide accurate information regarding the tolerance of genotypes to different abiotic or biotic stresses, as well as the identification of mitotic indices and division capacity [8].

The applicability of cytological investigations can support the development of inbred lines, as the early identification of genotypes with low growth capacity due to high levels of inbreeding can reduce the number of offspring selection in synthetic populations created by breeders.

In our study, we investigated the relationship between mitotic index levels in hybrid genotypes and inbred lines by performing and identifying the cell division rate. Additionally, to assess growth levels, we implemented real-time monitoring using an automated rhizotron, which enabled us to identify growth differences between the hybrid and the inbred line. The utilization of the cytological method in breeding for inbred line development is an innovative approach that can significantly reduce costs in breeding programs.

## MATERIALS AND METHODS

### *Gemoplasma*

Sunflower genotypes, inbred line HA-89 (Pl 599773) and hybrid HA112, were used for the experiment. Line HA-89 has a flowering period of 65 days and a yield per caladium of 60.2 g with an oil content of 41%. Hybrid HA112 was created by crossing CMS line HA-89 with restorer line RHA 274.

### *Cytological analysis*

For cytological analysis, 100 seeds of each genotype were germinated on filter paper by adding water. After harvesting, meristematic tissue from the root tips was fixed with Carnoy's reagent (3:1) and kept at 4°C for 24 hours. Acid hydrolysis with a 1 N HCl solution for 5 minutes at 60°C in a water bath followed this step. Roots were stained using the Feulgen technique with Carr's reagent, and the staining time was 24 hours, followed by color intensification in distilled water for 20 minutes [15].

To determine the mitotic index (MI), preparations were made, and microscopic investigations were performed at 400x magnification. The dividing capacity was determined by calculating the number of dividing cells relative to the total number of cells in the microscopic field (figure 1).

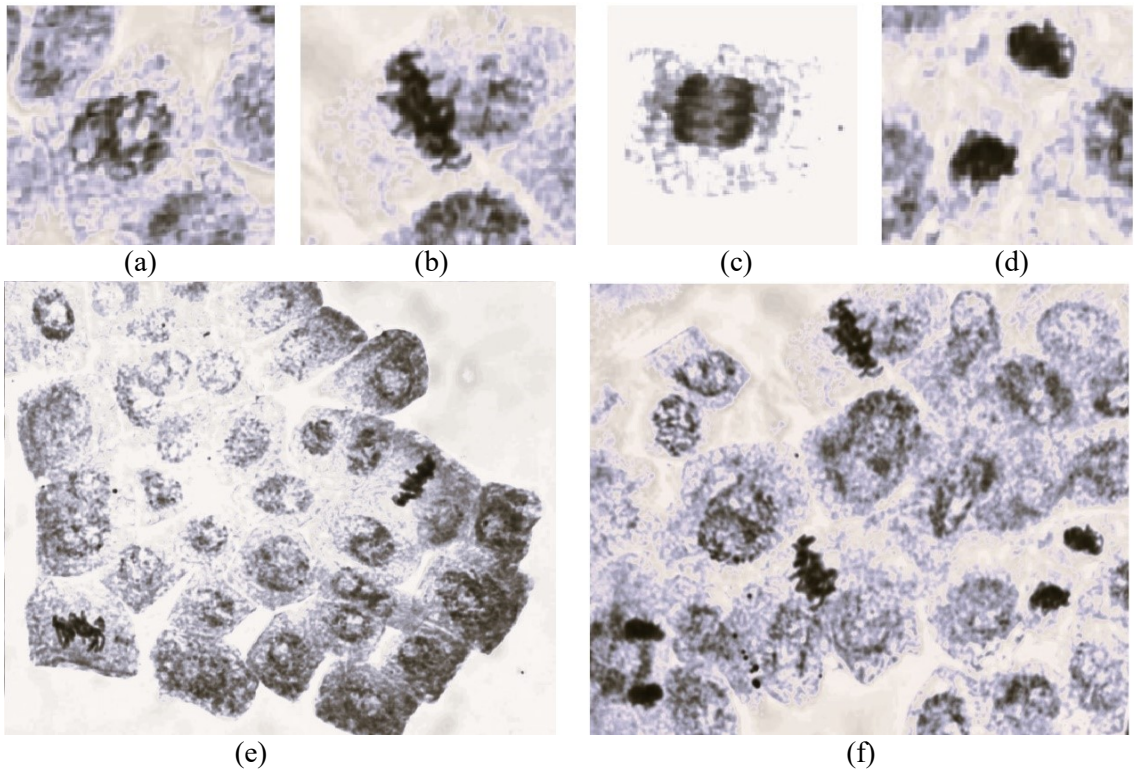



**Figure 1.** Cell division phases in *Helianthus annuus* (2n=34), (a)-Propase, (b)-Metapase, (c)-Anapase, (d)- Telopase, (e)- Inbred line, (f)- Hybrid

For the calculation of the mitotic index (MI), cells in the cell division phases (prophase, metaphase, anaphase and telophase) were quantified for both the hybrid genotype and the inbred line (figure 1). After counting the dividing cells in the microscopic field, the formula was used to calculate the mitotic index (MI):

$$\text{Mitotic index (\%)} = \frac{Number\ of\ cells\ in\ divizion}{Number\ of\ cells} \text{ X } 100$$

Root collections were carried out in intervals of 24, 48 and 72 hours, analyzing 15 microscopic fields for each experimental variant, both in the hybrid genotype and the inbred line.

*Analysis in automatic rhizotron*

For leaf and root area analyses, the two genotypes were sown in automatic rhizotron plates at the Biological Research Institute in Szeged (BRC). The experiment was conducted over a period of 30 days, with monitoring at 3-day intervals after the seeds germinated. Seeds of both the hybrid genotype and the inbred line were placed in automatic rhizotron plates in 3 replicates. Leaf and root area growth was quantified by

monitoring green pixels for leaf area and white pixels for root area growth assessment. All results obtained were compared to determine if there were significant differences in measurements over 30 days between the two genotypes.

*Statistical interpretation*

The results obtained for both cell division capacity and mitotic index (MI) were compared between the two genotypes using Tukey's test for comparison of means at significance levels of $p< 0.05$. The same comparisons were performed for leaf and root area. Results were highlighted using RStudio software.

**RESULT AND DISCUSSION**

Microscopic investigations identified significant differences in cell division capacity between the hybrid and the inbred line ($p< 0.05$).

The observed differences suggest that in inbreeding, compared to heterosis, cellular activity is diminished.

Measurements made on different time units indicated that for the hybrid genotype, the values of mitotic index (MI), regardless of the time unit analysed, were higher than those from the inbred line (table 1).

**Table 1**. Mitotic index values depending on the genotype used: hybrid and inbred line.

| Variant | | Mean Difference (I-J) | Std. Error | Mr | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Inbred 24h | Inbred 48h | -0,4659 | 0,75522 | 0,990 | -2,6685 | 1,7367 |
| | Inbred 72h | 0,6047 | 0,75522 | 0,967 | -1,5979 | 2,8074 |
| | Hybrid 24h | -2,4252* | 0,75522 | 0,022 | -4,6278 | -0,2225 |
| | Hybrid 48h | -2,7279* | 0,75522 | 0,007 | -4,9306 | -0,5253 |
| | Hybrid 72h | -2,7599* | 0,75522 | 0,006 | -4,9625 | -0,5572 |
| Inbred 48h | Inbred 72h | 1,0706 | 0,75522 | 0,716 | -1,1320 | 3,2733 |
| | Hybrid 24h | -1,9592 | 0,75522 | 0,110 | -4,1619 | 0,2434 |
| | Hybrid 48h | -2,2620* | 0,75522 | 0,041 | -4,4647 | -0,0594 |
| | Hybrid 72h | -2,2940* | 0,75522 | 0,036 | -4,4966 | -0,0913 |
| Inbred 72h | Hybrid 24h | -3,0299* | 0,75522 | 0,002 | -5,2325 | -0,8273 |
| | Hybrid 48h | -3,3327* | 0,75522 | 0,000 | -5,5353 | -1,1300 |
| | Hybrid 72h | -3,3646* | 0,75522 | 0,000 | -5,5672 | -1,1620 |
| Hybrid 24h | Hybrid 48h | -0,3028 | 0,75522 | 0,999 | -2,5054 | 1,8998 |
| | Hybrid 72h | -0,3347 | 0,75522 | 0,998 | -2,5373 | 1,8679 |
| Hybrid 48h | Hybrid 72h | -0,0319 | 0,75522 | 1,000 | -2,2346 | 2,1707 |

*Notes: *. The mean difference is significant at the .05 level.*

Comparisons showed that, regardless of the harvesting interval, the mitotic index variations (%) did not show significant changes according to the time periods harvested ($p> 0.05$) in both the hybrid genotype and the inbred line. Also, comparing the mitotic

index, a greater difference was observed at all time intervals in favour of the hybrid compared to the inbred line. This suggests that the level of inbreeding has a negative effect on cell division capacity compared to the hybrids.

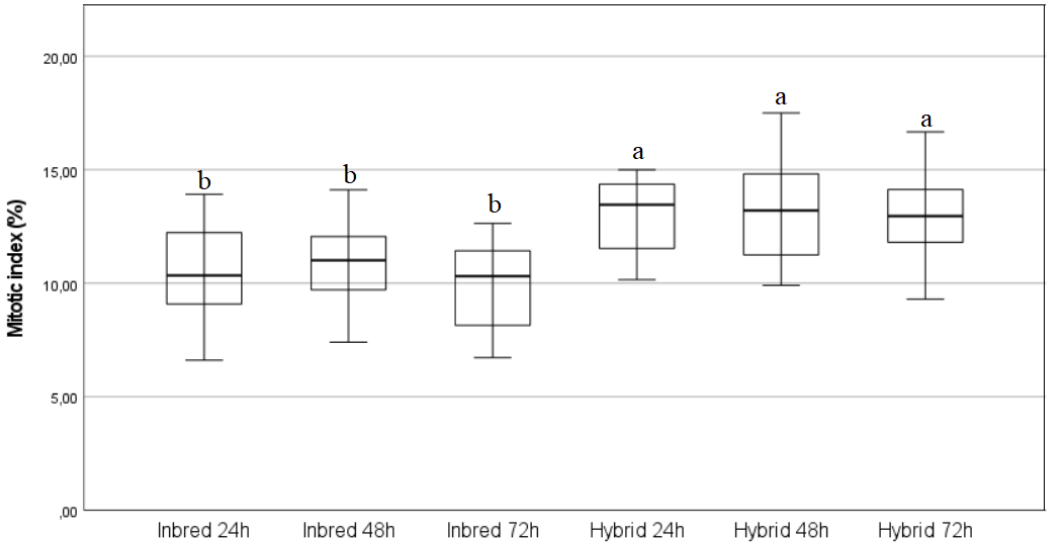



**Figure 1**. Comparing the Mitotic Index (%) in inbred lines and hybrids at different time intervals.

The variations observed at the microscopic level, as indicated by the mitotic index (MI),correspond to differences at the macroscopic level (figure 1 and 2). Measurements conducted using the automated rhizotron confirmed a notable increase in the root system between the two genotypes. These distinctions were also noticeable at the leaf area surface level.

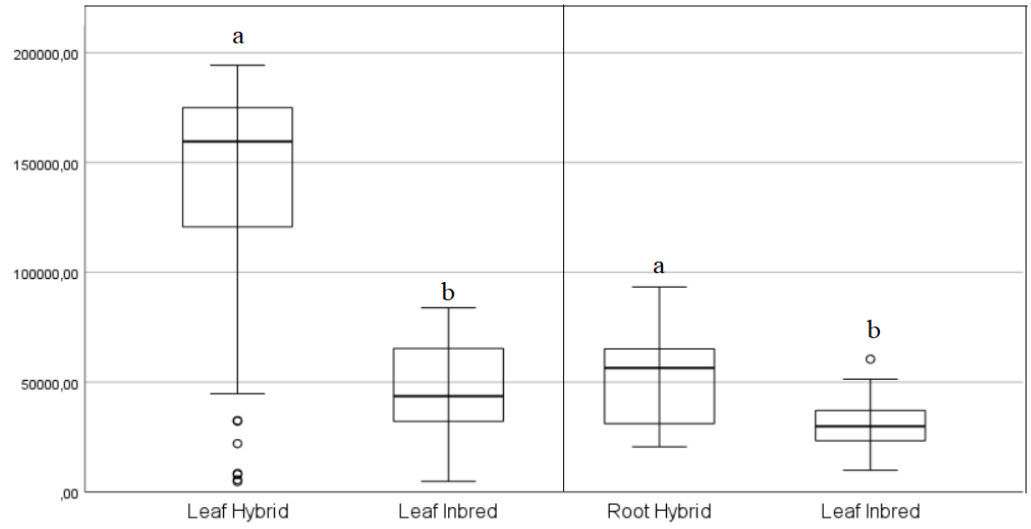
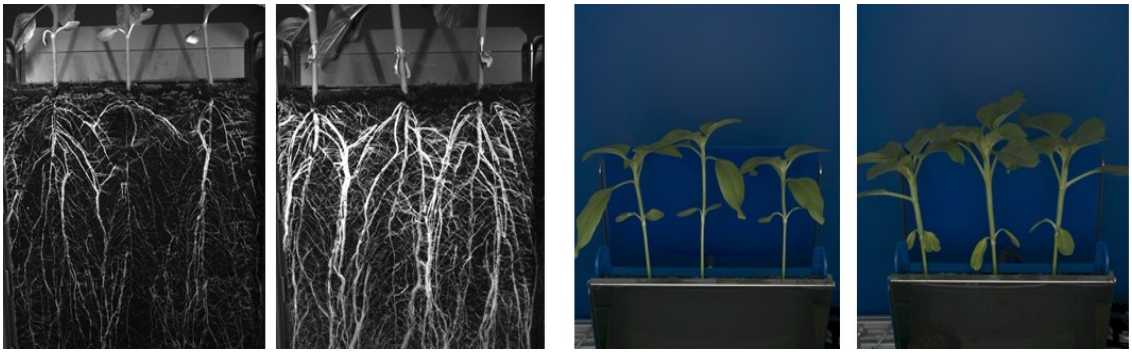



**Figure 2**. Variation in leaf area and root measurements in the automatic rhizotron for the inbred line and hybrid.



(a) (b) (c) (d)

**Figure 3.** Automatic rhizotron images of leaf and root areas taken 30 days after the start of the experiment. (a, c) - Inbred line, (b, d) - Hybrid.

The study of the mitotic index (MI) is also confirmed at the macroscopic level (figure 3), allowing us to conclude that it can serve as a reference point for analyzing the level of inbreeding. Indicators of this level include a reduced mitotic index (MI) as well as a significant reduction in leaf and root area ($p< 0.05$).

Based on the results obtained from both the cytological method and the automatic rhizotron, the hybrid outperforms the inbred genotype. Cytological tests are valuable for monitoring salt stress tolerance [3, 11], identifying toxicity levels of heavy metals in soil [6,], and obtaining genotypes adapted to constantly changing climatic conditions, making them more adapted [7]. Other studies show at the cellular level a decrease in the mitotic index and an increase in chromosomal aberrations as a result of various stress factors, whether abiotic or biotic, as well as the appearance of specific toxicity effects such as vacuolization [13,14].

The integration of cell diagnosis into sunflower breeding programs, as well as in other crop species, can enhance the process of identifying inbred forms in the early generations.

Cell diagnosis also empowers breeders to eliminate a substantial amount of genetically unstable material at an early stage, reducing the need for additional cultivation. This optimization improves the production of valuable materials and enhances the economic resilience of research programs.

In perspective, the automatic rhizotron offers the potential to identify hybrid vigor that can be utilized for early testing of various progeny. Furthermore, the utilization of both macroscopic and microscopic analyses in the early stages can greatly enhance breeding programs, facilitating the development of inbred lines.

## CONCLUSION

The analyses conducted with the automated rhizotron represent an essential tool in plant studies, offering the possibility to closely examine plant growth processes, including the development of leaves and roots. The use of this method has shown that the effect of inbreeding in sunflower genotypes is manifested by a reduction in leaf and root surface area. This decrease in leaf and root surface area in inbred lines can have significant consequences on photosynthesis and nutrient absorption, thus affecting the vigor of the plant.

The growth capacity of plants can be observed at the cellular level, where cytological diagnosis involves the examination of cells to determine the mitotic index.

Cytological diagnosis can be used to distinguish between inbred and hybrid genotypes, highlighting that the effects of inbreeding differ significantly from those of heterosis, especially regarding the capacity for mitotic division. Studies conducted over different time units, from 24 to 72 hours, have shown that the rate of cellular division remains constant in both hybrid genotypes and inbred ones. The cytological diagnostic method, by establishing the mitotic index, provides an effective means of determining the level of inbreeding in new populations obtained by breeders in research programs. The effect of heterosis is a phenomenon that exhibits traits of superiority over the parental forms. This phenomenon becomes visible in the development of leaves and roots and can be quantified by analyzing mitotic index.

By combining cytological diagnosis with analyses conducted through the automated rhizotron, breeders can more clearly identify levels of inbreeding and heterosis, thereby contributing to the improvement of new biological materials and increasing the resilience of breeding programs.

## ACKNOWLEDGEMENTS

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# CYTOTOXIC EFFECT OF CHELIDONIUM MAJUS FREEZE DRYED EXTRACT: IN VITRO STUDY ON SKIN CANCER LINE

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## ABSTRACT

The multiple therapeutic properties of *Chelidonium majus* are known and well documented in the bibliography, being used for a long time in Chinese homeopathic medicine [1]. At the level of the skin, in traditional folk medicine, *C. majus* is used to treat warts, condylomas, and papillae, which are due to human papillomavirus (HPV) infections [2, 3]. In this context, the aim of this study was to assess the potential antitumor effects of *C. majus* extracts on human melanoma cells - A375 - and to evaluate it in terms of cell viability and morphology changes. Thus, an extract of *C. majus* (C_ex) was obtained in order to assess its potential antitumor effects. This extract was then tested on human melanoma cells - A375 - and evaluated in terms of cell viability and morphology changes. The results of the study highlighted the potential cytotoxic effect of C_ex on melanoma cells. It was observed that cell viability decreased in a dose-dependent manner after 24 hours. Moreover, the morphological changes are consistent with those observed following a cell viability test, exhibiting the characteristic signs of cell death.

**Keywords:** *Chelidonium majus*, plant extract, antitumor effect

## INTRODUCTION

Medicinal plants are a continuous source of therapeutic solutions, some being well-known since ancient times, others being in continuous research. *Chelidonium majus* L. (*C. majus*) is a representative of the *Papaveracea* family. The multiple therapeutic properties of the plant are known and well documented in the bibliography, being used for a long time in Chinese homeopathic medicine [1, 4]. *C. majus* has been intensively used in folk medicine as a choleretic, diuretic, and analgesic drug, especially efficient in peptic ulcer,

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abdominal and menstrual pain, and also as an anti-inflammatory, spasmolytic, and anti-tumor compound. It has been also observed that the herb promotes diuresis in ascites and edema, treats jaundice, and relieves hypnotic effects and cough. It has been widely used to treat liver disorders, gallstones, skin diseases, and ulcers. It is also used as an anti-virus, anti-fungal, antibacterial, and anti-protozoa representative [5]. The most encountered secondary metabolites in *C. majus* are benzylisoquinoline alkaloids, including protoberberines (coptisine, berberine, stylopine), benzophenanthridines (sanguinarine, chelerythrine, isochelidonine, chelidonine), and protopines. Chelerythrine and sanguinarine are some of the most prominent alkaloids found in roots while chelidonine, coptisine, and berberine are usually found in the aerial parts of the plant [6]. Different *in vitro* studies put forward that the presence of chelidonine, sanguinarine, berberine, and chelerythrine determine the antitumor effect of the *C. majus* extract (C_ex). Sanguinarine was found one of the most potent antitumor agents, which is capable to intercalate strongly with DNA [7]. Berberine was found to manifest antiproliferative properties in various cancer types and to induce apoptosis in vitro, in human cancer cells, such as pancreatic, breast [8, 9], prostate [10] and lung [11]. At the level of the skin, in traditional folk medicine, *C. majus* is used to treat warts, condylomas, and papillae, which are due to human papillomavirus (HPV) infections [2]. In this context, the aim of the study was to assess the potential antitumor effects of *C. majus* extracts on human melanoma cells - A375 - and to evaluate it in terms of cell viability and morphology changes.

## MATERIALS AND METHODS

### *Reagents*

A-375 cell line (CRL-1619™) was purchased from ATCC (American Type Cell Collection, Lomianki, Poland). The specific media Dulbecco's Modified Eagle's Medium (DMEM) (P04-03550) was acquired from PANBiotech, dimethyl sulfoxide (DMSO) (67-68-5), penicillin/streptomycin mixture (P4333), phosphate saline buffer (PBS) (P30-193306), and trypsin-EDTA solution (P4333) were acquired from Sigma-Aldrich (Darmstadt, Germany analytical grade forms) and MTT cell viability kit 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (11465007001) was purchased from Roche Applied Science.

### *Extract preparation*

Whole plants of *Chelidonium majus L*. was obtained from a national bio-producer. The plants were freeze-dried with the use of a Kryptonstraat 11 (ilShin Europe) freeze-dryer. In order to prepare the extracts, 15 grams of the dried and grounded plants have been mixed with a 70% ethanol solution in a ratio (plant material:EtOH) of 1:10. The complex thus prepared was fully mixed (30' at 200 rpm) with a Panaonic MIR-S100-PE orbital shaker and filtered through a 90 mm filter paper. The extracts where again freeze-dried in order to obtain a standardized extract powder. The plant material was dissolved in DMSO to obtain the stock solution, from which the final concentrations (25, 50, 100, 150 and 200 mcg/mL) were obtained by dissolving in the culture medium.

### *Cell culture*

A-375 was isolated from the skin of a 54-year-old, female with malignant melanoma. Cells were cultured in their specific medium, DMEM, supplied with 10% FBS and 1% of penicillin/streptomycin mixture, to prevent contamination. Cell growth was carried out in

a humidified incubator, following standard conditions: 5% $CO_2$ and a temperature of 37 °C. Cells were morphologically observed using an IX73 inverted microscope (Olympus, Tokyo, Japan).

### *Viability test*

The cell viability was analyzed following the MTT assay. The experimental protocol was applied according to our previous studies [12]: (1) cells were cultured in 96-well plates ($1x10^4$ cells/200 µL medium per well), and when a uniform confluence of approximately 80-90% was reached, they were incubated in increasing concentrations of extract (25, 50, 100, 150, respectively 200 µg/mL) for a period of 24 hours; after the incubation period, the medium was replaced with fresh culture medium (100 µL/well) and a volume of 10 µL/well of MTT solution (5 mg/mL) (kit I) was added to each well, followed by a 3-hour incubation; (3) the formed formazan crystals were dissolved in 100 µL of solubilization buffer provided by the manufacturer (kit II), and the interaction time was 30 minutes, at room temperature and in the dark; (4) the reduced MTT was analyzed spectrophotometrically at 570 and 630 nm using the Cytation 5 microplate reader (BioTek Instruments Inc., Winooski, VT, USA) [12]. All experiments were performed in triplicate.

### *Statistical analysis*

All the experimental data are displayed as means ± standard deviation. One-way ANOVA analysis and Dunett's multiple comparisons post-test were used to analyze de results. The used software was GraphPad Prism version 9.4.0 for Windows. The statistically significant differences between data were noticed with * (*** $p<0.001$; **** $p<0.0001$).

## RESULTS AND DISCUSSIONS

Melanoma represents a multifactorial disease, reflecting genetic and environmental factors that interact, apart from the crosstalk between melanocytes and keratinocytes. Around the world, a continuous increase in the incidence of melanomas has been observed, varying according to sex, age, skin color, and geographic location. Between 2008 and 2018, there was an increase of 44% in the number of cases and 32% in the number of deaths. In addition, it was observed that the incidence and mortality rate from skin melanoma varies significantly between women and men, so males are 10% more liable to develop the disease and 4% more prone to die from melanoma than females. It is estimated that in three years, the number of people diagnosed with melanoma and the number of melanoma-related deaths will increase by approximately 20% [13]. Although the treatments are continuously evolving, it seems that they are not sufficiently selective and effective, so it is important to identify new therapeutic solutions. Phytotherapy has the great advantage of lower costs and significant therapeutic properties [14].

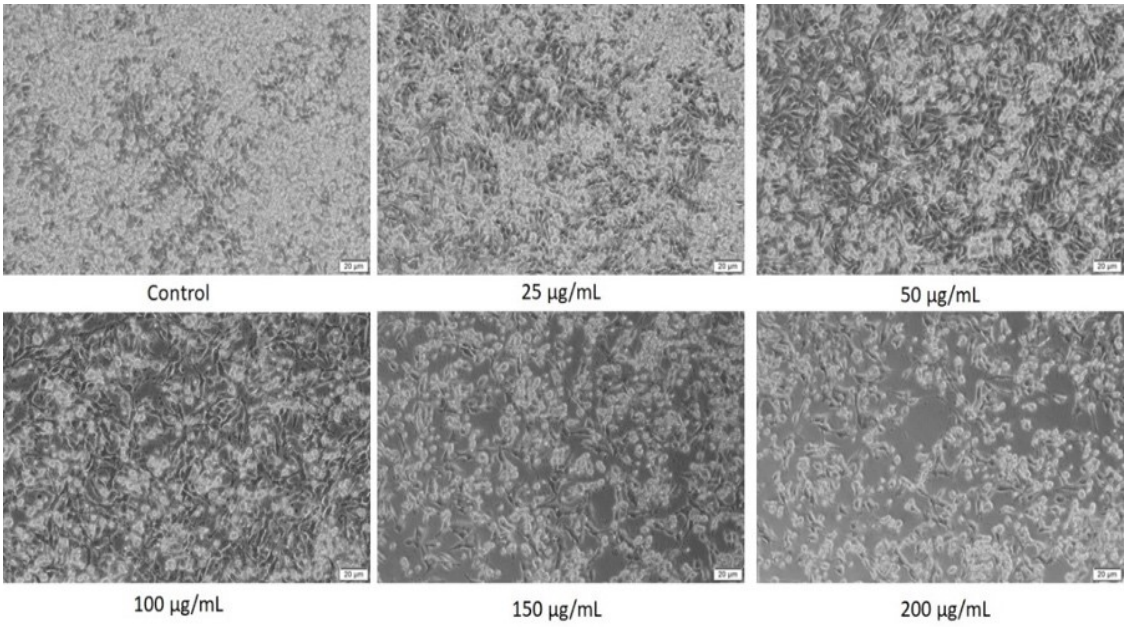



**Figure 1.** The morphological aspect of A-375 cells after treatment for 24 h with C_ex at 25, 50,100,150, and 200 µg/mL concentrations

Thus, the aim of the study was to prepare and analyze the extract on the A-375 melanoma cell line, in order to identify its toxicological profile. In order to analyze the cytotoxicity of C_ex, morphological changesets and viability were observed. As shown in Figure 1, the morphology and confluence of the cells were direct proportional with the dose. At the concentration of 25 µg/mL) cells easily lose their elongation and confluence. Thus, at these phenomena can be better observed at the highest concentration. Following the observation of the impact on cell morphology, the next step in the study was to evaluate the viability of the cells. It was found that the extract revealed a cytotoxic effect in a concentration-dependent manner. As a consequence, at the concentration of 25 ug/mL the viability of the cells was reduced by approximately 87%, while at the concentration of 200 ug/mL the viability decreased by almost 45% (Figure 2).

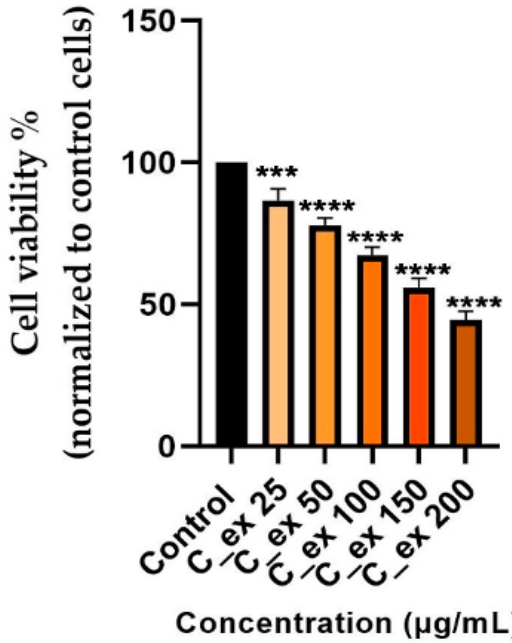



**Figure 2.** Viability assessment of C_ex (25, 50, 100, 150, and 200 µg/mL) in A-375 cells at 24h rapport with control group followed by Dunnett's multiple comparisons post-test (*** $p<0.001$ and **** $p<0.0001$)

Similar results were observed on A 431 cells, where the viability was inhibited in a dose- and time-dependent manner, thus at a concentration of 200 µg/mL it was significantly reduced [3]. In the same study, it was observed a pronounced pro-apoptotic potential [3]. When tested on B16F10 (mouse melanoma cells), isoquinoline alkaloids from *C. majus* also significantly reduced cell viability. The meaning antiproliferative activity of isoquinoline alkaloids in melanoma cells was as well highlighted by J. Hammerova et al. in their recent research [15]. Thus, A-375 cell treatment with chelidonine resulted in a dose-dependent manner cell viability decrease.

## CONCLUSIONS

In conclusion, the results of the study highlighted the potential cytotoxic effect of C_ex on melanoma cells. It was observed that cell viability decreased in a dose-dependent manner after 24 hours. Moreover, the morphological changes are consistent with those observed following a cell viability test, exhibiting the characteristic signs of cell death.

## ACKNOWLEDGEMENT

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# EFFECT OF CABBAGE INTERCROPPING ON SOIL MICROBIAL AND ENZYME ACTIVITIES

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## ABSTRACT

Intercropping increases biodiversity in vegetable fields. It affects both above-ground and soil communities. The aim of the research was to study and analyze the soil microbiological and enzymatic activity in intercropping of cabbage with leek, green bean, tagetes, flower mix, and dill. The studies were carried out in two directions: 1) of microflora, as a sensitive indicator of the microbiological status of soils; 2) enzyme research, such as functional characterization and expression of microbial activity. Soil biogenicity increases at the end of the growing season in all variants. In the middle of the growing season, the biogenicity is highest with "cabbage and flower mix", while at the end of the growing season with "cabbage and tagetes". For the entire vegetation period, the biogenicity and catalase are consistently the lowest when intercropping cabbage and leeks, as well as cabbage and dill in the middle of the growing season. A regrouping was found in the composition of the general microflora in the variants: "cabbage and leek", "cabbage and beans" and "cabbage and flower mix" the bacilli began to dominate the non-spore-forming bacteria. Catalase and cellulase have the highest values at the end of the growing season in most combined variants.

**Keywords:** catalase, cellulose, intercropping vegetables, soil microflora

## INTRODUCTION

Cultivation patterns, intercropping, and soil nutrients affect soil microbiota and soil enzyme activity. Intercropping is based on the biodiversity–ecosystem functioning relationship (well studied in natural ecosystems, less well studied in agrogenic ecosystems) and is the practice of growing two or more crops during the same growing season, in the same field, usually in small area systems to increase yields and reduce inputs [1].

Increasing spatial diversity by plant intercropping is positively connected with higher biomass production and yield [2],.

Plant diversity positively affects soil microflora – directly, such as promoting plant growth, suppressing pathogens, and forming symbiotic relationships involving mycorrhizal fungi and rhizobial bacteria [3] indirectly, by increasing the supply of soil nutrients (respectively soil fertility) from the activity of the soil microbial community [4, 5].

The number of microbes (bacteria, fungi, and actinomycetes) in the rhizosphere soil in a sugarcane+soybean intercropping model increased significantly compared to those in monoculture models [6]. The combined cultivation of walnut and tea improves the

nutrient profile of the soil, increases the amount and diversity of soil microorganisms (Proteobacteria, Bacteroidetes, Firmicutes, Chlamydiae, Rozellomycota, Zoopagomycota), and increases soil enzyme activity [7].
In polycultures, plants that encourage microbial activities that boost soil fertility or decrease harmful organism populations can also increase yields [8]. Increased plant diversity in cropping systems has the potential to increase soil physical stability, microbial abundance, and resistance to microbially mediated nutrient cycling processes [9; 10]. Plant diversity affects the extracellular enzyme activity of soil microorganisms. When enzyme activity was compared between monoculture and intercropping in studies, the results were extremely inconsistent, with intercropping treatment exhibiting higher [11], similar [12], or lower [2, 13] enzyme activity.
According to Curtight and Tiemann [14], intercropping considerably enhanced enzyme activity by an average of 13%, but the effect varied depending on the kind of enzyme, the plant species employed as the main crop or intercrop, as well as other experimental and environmental conditions.
The aim of the present scientific study is to investigate the influence of the joint cultivation of agricultural crops on the microbiological and enzymatic activity of soil microorganisms, in the context of ensuring sustainable and productive agriculture.

## MATERIALS AND METHODS

The experiment was carried out at the educational and experimental field "Vrajdebna" at the Faculty of Agronomy of the University of Forestry – Sofia (42°7′ N, 23°43′E and 552 m above sea level) on fluvisol soil type. The climatic zone in which Sofia enters, including the experimental field, is classified as the Marine west coast, warm summer climate zone.
The field experiment was intercropping with a main crop of cabbage (*Brassica oleracea* var. *capitata*) with companion crops: leek (*Allium porrum* L.), green beans (*Phaseolus vulgaris* L.), tagetes - French marigold (*Tagetes patula* L.), seed mix of flowering crops as flower mix (*Calendula officinalis* L., *Borago officinalis* L., *Centaurea cyanus* L., *Fagopyrum esculentum* Mill., *Anethum graveolens* L., *Phacelia* sp. Jus.) and dill (*Anethum graveolens* L.).
There were developed six variants: Control cabbage (Cc) – solely growing; Cabbage + Leek (C+L); Cabbage + Green bean (C+Gb); Cabbage + Tagetes (C+T); Cabbage+Flower mix (C+Fm); and Cabbage + Dill (C+D).
Cabbage is for late field production, with a planting date in the third ten days of July. The companion plants were direct-seeded or transplanted (leek and French marigold). The scheme of cabbage growing is row spacing of 70 cm and 50 cm between plants in a row. The companion plants were sowed or transplanted between the rows of cabbage.
The microbiological analysis of soil samples covers the period before the sowing of vegetable crops (control sample) and in the vegetation phases of development of the investigated crops - phase "leaf period" and phase "productive period", respectively day "0", day 50 of the growing season and at the end of the growing season. Sampling was carried out in the root-inhabited layer (depth of about 25 cm). The study variants and the corresponding analysis scheme are presented in Table 1.

Table 1. Variants and scheme for microbiological and enzymatic analysis

| Variant | Analysis period | | |
|---|---|---|---|
| | Day "0" | Day "50" | End of vegetation |
| Control before planting ($C_0$) | + | - | - |
| Cabbage control (Cc) | + | + | + |
| Cabbage+Leek (C+L) | + | + | + |
| Cabbage+Green bean (C+GB) | + | + | + |
| Cabbage+Tagetes (C+T) | + | + | + |
| Cabbage+Flower mix (C+FM) | + | + | + |
| Cabbage+Dill (C+D) | + | + | + |

Note: "+" sign indicates a performed analysis; sign "–" indicates absence of analysis

Soil samples for microbiological analysis were examined by the dilution method, cultivation in a thermostat, and counting the number of colony-forming units (CFU) in 1 g of abs. dry soil by inoculation of solid nutrient media (Mesopeptone agar for the isolation of non-spore-forming bacteria and bacilli; Actinomycetes isolation agar – for the isolation of actinomycetes and mineral nitrogen-assimilating bacteria; Chapek-Dox agar for the isolation of mold fungi (Micromycetes)).

Representative groups of microbes from the composition of the microcenosis were analyzed - non-spore-forming bacteria, bacilli, actinomycetes, micromycetes, and bacteria using mineral nitrogen sources. Their amount and ratio within the heterotrophic block of the microflora are reliable indicators of the functioning of the microbial population in the soil under natural conditions or under conditions of external influence.

Total microflora and mineralization coefficient indicating the rate of decomposition of organic substances in the soil were determined.

The rate of mineralization of organic substances in the soils of the studied variants is represented by determining the activity of microbial enzymes that decompose carbohydrates (cellulase) and a respiratory enzyme - catalase, which catalyzes the decomposition of highly toxic hydrogen peroxide released during the mineralization of organic compounds.

The following methods were used:

- Cellulase activity - Laboratory method - to follow the enzyme activity in dynamics: in a petri dish with a diameter of 10 cm, soil with a thickness of about 7 mm is poured, maintaining 60% limit field moisture. In each Petri dish, 3 strips of sterile filter paper, measuring 10/50 mm, are placed on the soil and cultivated at 25 ºC. In 15 days, the area of the degraded area is counted with a grid standard [15];
- Catalase activity – manganometric titration method [15].

Statistical processing of the results includes: the calculation of an average value of three repetitions for the studied groups of microorganisms and correlation analysis, presented using MS Excel.

## RESULT

The biogenicity of the investigated soils was highest in the sample before planting the crops compared to the variants in the middle of the growing season (Tab. 2), which also correlates with the highest mineralization activity at $C_0$ (Fig. 1).

Table 2. Quantity and qualitative composition of soil microorganisms (CFU/g)

| Variant | Total microflora | Non-spore-forming bacteria | Bacilli | Actino-mycetes | Micro-mycetes | Bacteria assimilating mineral nitrogen |
|---|---|---|---|---|---|---|
| Day „0“ - Control | | | | | | |
| $C_0$ | 1 909 940 | 1 085 800 | 76 540 | 694 200 | 53 400 | 4 722 933 |
| Day “50“ | | | | | | |
| Cc | 540 940 | 350 880 | 133 300 | 38 700 | 18 060 | 250 260 |
| C+L | 528 900 | 180 600 | 282 080 | 61 920 | 4 300 | 301 000 |
| C+GB | 649 440 | 267 520 | 322 080 | 46 640 | 13 200 | 1 115 840 |
| C+T | 671 950 | 368 460 | 222 500 | 66 750 | 14 240 | 1 060 880 |
| C+FM | 780 880 | 309 600 | 417 960 | 43 000 | 10 320 | 1 420 720 |
| C+D | 314 480 | 250 260 | 4 300 | 46 000 | 13 920 | 925 680 |
| End of vegetation | | | | | | |
| Cc | 1 980 500 | 1 207 000 | 680 000 | 68 000 | 25 500 | 4 335 000 |
| C+L | 1 657 500 | 714 000 | 841 500 | 85 000 | 17 000 | 1 742 500 |
| C+GB | 4 006 800 | 1 696 800 | 2 074 800 | 142 800 | 92 400 | 6 115 200 |
| C+T | 4 947 600 | 3 402 000 | 1 318 800 | 142 800 | 84 000 | 3 259 200 |
| C+FM | 4 000 600 | 3 062 700 | 838 300 | 58 100 | 41 500 | 937 900 |
| C+D | 4 922 400 | 3 603 600 | 1 209 600 | 25 200 | 84 000 | 2 990 400 |

Compared to the second control (cabbage solely growing), the total microflora has lower values in C+L (cabbage and leek) for the entire vegetation period and in C+D (cabbage and dill) in the middle of the vegetation.

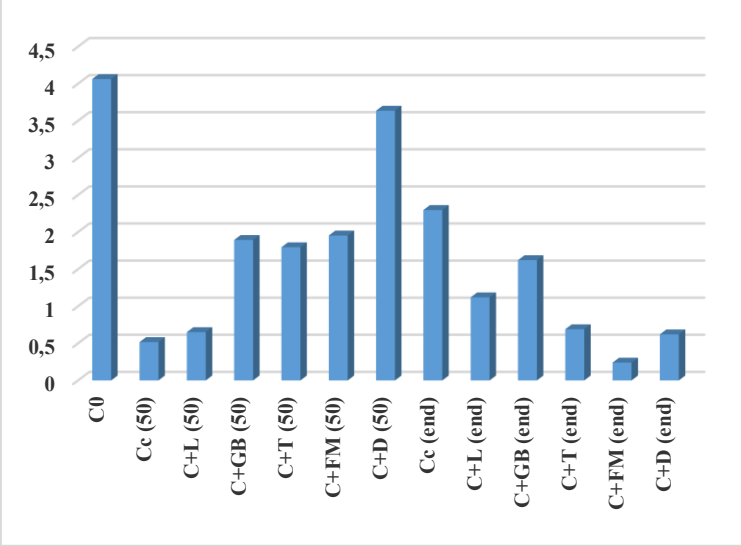



Figure 1. Mineralization coefficient

During the vegetation period, higher values of the total microflora were found at C+GB, C+T, C+FM, and C+D at the end of the vegetation compared to the 50th day from the beginning of the vegetation; C+L at the end of the vegetation, and the controls. The opposite trend is found for the other three variants – C+GB, C+T, and C+D. In the middle of the growing season, the biogenicity is the highest in variant C+FM, while at the end of the growing season, the total microflora is the highest in variant C+T.

For the entire vegetation period, the lowest values of the total microflora were found in C+L which did not correlate with the lowest mineralization activity at the end of the growing season.

Catalase activity was lowest in C+L for both study periods during the growing season, as well as in C+D in the middle of the growing season, which correlated with the lowest total amount of microorganisms.

However, the same catalase activity was also found in variants with different biogenicity, which, in addition to the listed factors affecting biogenicity and mineralization activity, probably also depends on the presence of catalase of plant origin (Fig. 2).

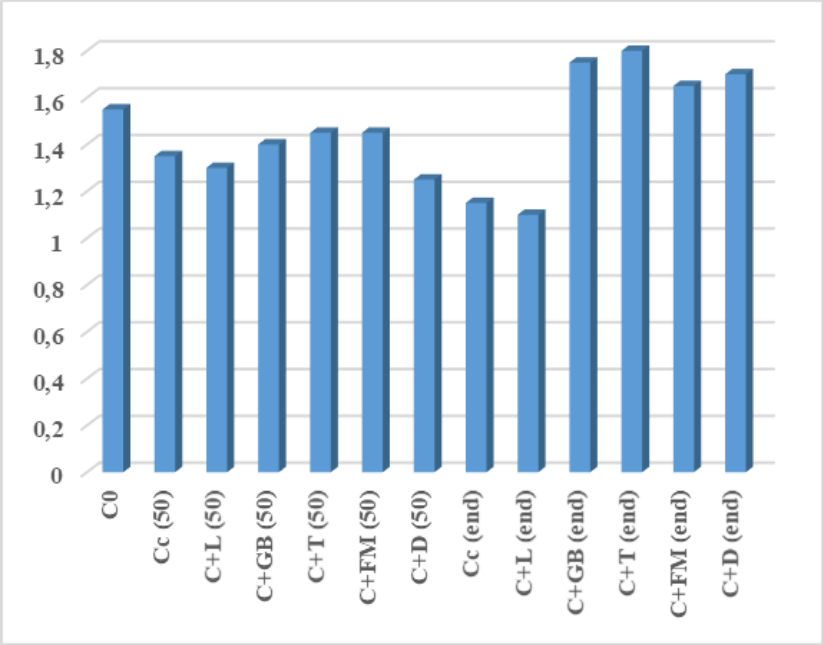



Figure 2. Catalase activity of soil microorganisms (ml $O_2$/30 min)

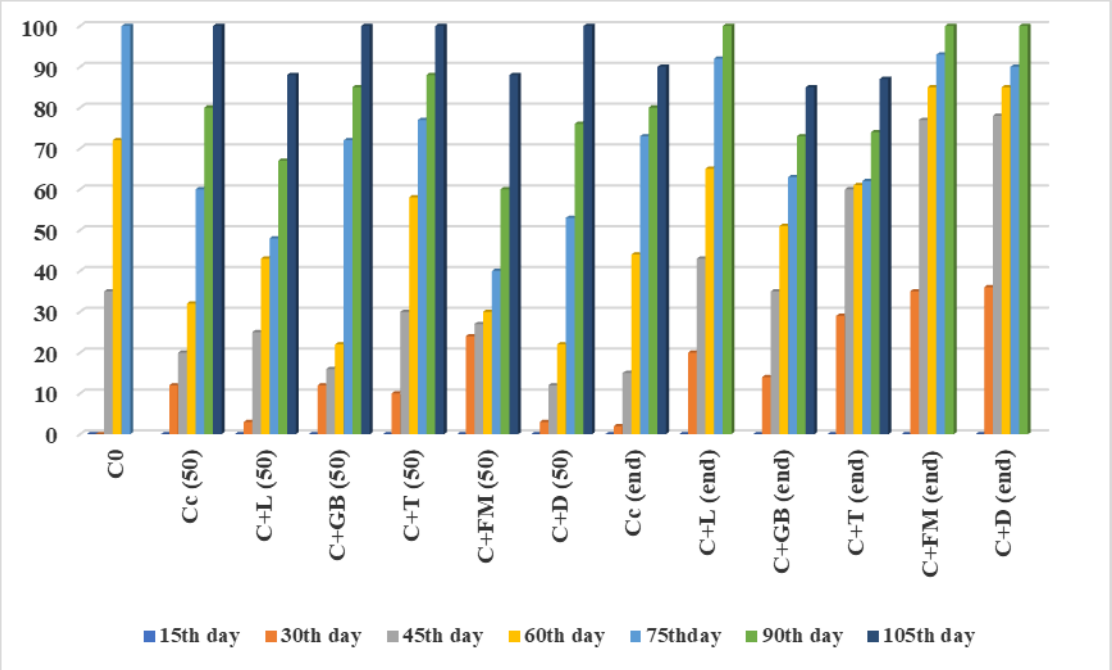



Figure 3. Cellulase activity of soil microorganisms (% degraded area)

The results showed that complete cellulose degradation (100%) was achieved on day 75 in the control before planting the cultures, while in the cabbage control (Cc), C+Gb,

C+T, and C+D such complete activity was achieved one month later. This trend correlates with the highest biogenicity in the first control.
The correlation dependences between the studied indicators showed that the catalase activity most strongly depends on the total microflora. No strong positive correlation was found between the general microflora and the other investigated indicators (Tab. 3).

Table 3. Correlation coefficient

| Indicator | Total microflora | Mineralization coefficient | Catalase | Cellulase |
|---|---|---|---|---|
| Total microflora | 1 | | | |
| Mineralization coefficient | -0,39135 | 1 | | |
| Catalase | 0,761185 | -0,24753 | 1 | |
| Cellulase | -0,23387 | 0,144757 | -0,23247 | 1 |

## DISCUSSION

The lower biogenicity in the combined cabbage and leek variant (C+L) is a sustainable trend during entire growing period, while in the combination of cabbage and flower mix (C+Fm), a sustainable trend for higher biogenicity was established during the entire vegetation period of the crops. Probably this trend in C+Fm is also determined by the combination of more crops (7 plants). Some authors found an increase in the biogenicity of the soil microflora [6; 7], biomass, and plant yield in the combined cultivation of several crops compared to the cultivation of a monoculture [1; 2].
The quantity of microorganisms does not always determine their activity - it depends on a complex of factors: quantity and qualitative composition of microorganisms, type of soil and its treatment, the mechanical composition of the soil, humidity, and temperature of the soil, pH of the soil, nutrient supply in the soil, type of vegetation and other factors.
In the middle of the growing season, the soil microorganisms for C+L are somewhat hindered in terms of mineralization activity compared to the end of the growing season.
In the composition of the total microflora in the controls, as well as in C+T; C+Fm (end of vegetation), and C+D, the highest percentage share is occupied by non-spore-forming bacteria, while in C+L; C+Gb, and C+Fm (50th day) a regrouping and change of the dominant group – the bacilli are more than the non-spore-forming bacteria and, as a spore-forming group, are more resistant to external influences and participate most actively in the decomposition of organic substances in the soil.
In all variants, the least represented in the composition of the total microflora are micromycetes. Plant diversity has a positive effect on the soil microflora - symbiotic relationships between mycorrhizal fungi and rhizobial bacteria are formed [3], and soil fertility increases [4; 5].
The catalase activity of the soil microorganisms generally correlates with the biogenicity of the studied soils – the highest values of the enzyme are found at the end of the growing season in C+Gb, C+T, C+Fm, and C+D.
The trend of cellulose degradation correlates with the highest biogenicity in the first control. In dynamics and in the control before the start of the experiment, the

degradation of cellulose begins slowly - there is no degradation one month after planting, after which the adapted microorganisms in the cultivation conditions are activated. In intercropping variants, in the middle of the growing season, the cellulase activity was highest in C+Gb, C+T, and C+D. At the end of the growing season, cellulase activity was highest in C+L, C+Fm, and C+D. Only in C+D, a sustainable trend was outlined in terms of higher cellulase activity, increasing at the end of the growing season compared to the mid-growing season of the crops.
In studies where enzyme activities were measured in monoculture compared to interculture, results were highly variable, with the interculture treatment showing greater [11], similar [12], or reduced [2, 13] enzyme activity.
Crop intercropping increases enzyme activities with the effect of intercropping varying depending on the enzyme category, the type of plant used as a main crop or catch crop, and other experimental and environmental factors [14].

## CONCLUSION

The biogenicity of the studied soils is highest in the sample before planting the crops, which correlates with the highest mineralization activity. Sustainable low biogenicity was established in the intercropping of cabbage and leeks, and with the combination of cabbage and flower mix was established sustainable high biogenicity of the soil.

The highest percentage share in the composition of the total microflora is occupied by non-sporulating bacteria and bacilli. The less represented are micromycetes and actinomycetes. A regrouping was found in the composition of the total microflora in some of the combined variants (50th day), where the dominant group of non-spore-forming bacteria is replaced by the bacilli, which participate most actively in the decomposition of organic substances.

The catalase activity of soil microorganisms depends most strongly on the general microflora. The highest values of the enzyme are found at the end of the growing season in the intercropping variants: cabbage and beans, cabbage and tagetes, cabbage and flower mix, and cabbage and dill. Catalase activity was lowest in cabbage and leek intercropping for both study periods during the growing season.

Cellulase activity was highest in the control before planting the crops, which correlated with the highest biogenicity compared to the other variants in mid-vegetation and with the highest mineralization activity. Only in the intercropping of cabbage and dill a sustainable trend is outlined in terms of higher cellulase activity, increasing at the end of the growing season, regardless of the lowest biogenicity in this variant.

## ACKNOWLEDGEMENTS

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# EFFECT OF EXTRUSION PARAMETERS ON THE EXTRUDED PRODUCTS FEATURES

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## ABSTRACT

The extrusion process is recognized as the most versatile processing technology, and it can be used to produce food with higher nutritional, functional, and sensory qualities. The extrusion process involves forcing a mixture of ingredients through a small die under controlled conditions of temperature, pressure, and shear. This review highlights the main effects of the process parameters (feed rate, feed moisture, screw speed, barrel temperature, raw material characteristics, etc.) and system parameters (specific mechanical energy, residence time, product pressure, and temperature, etc.) on the features of extruded products (expansion ratio, bulk density, texture and color parameters, water absorption capacity, swelling capacity, thermal properties, sensory characteristics, etc.). Extrusion conditions can decrease the expansion ratio and increase bulk and apparent density. When humidity decreases and temperature increases, the Maillard reaction improves, while screw speed negatively affects color change because it influences residence time. Product final porosity, texture, and moisture content are also subject to various changes. The extrusion process can lead to changes in particle size distribution, influencing the mouthfeel and sensory attributes of the product. At high temperatures, screw speed and low moisture, water absorption capacity can decrease, whereas, at low screw speed, it increases. The effects of extrusion parameters on these properties can vary depending on the specific ingredients and formulations used, and the interactions between different parameters can be complex, leading to intricate changes in the features of extruded products. Thus, more advanced research needs to be done.

**Keywords:** extruded snack, barrel temperature, screw speed, feed moisture, expansion, textural properties

## INTRODUCTION

Food extrusion stands out as one of the most suitable technologies for food processing, offering versatility and innovation in crafting a diverse array of foods with distinctive textures, shapes, and flavors [1]. This method subjects' ingredients to heat, pressure, and mechanical shear forces, transforming them into a cohesive and uniform mass.

Extrusion, as a technique, can modify the functional properties of food ingredients through processes like mixing, cooking, kneading, shearing, shaping, and forming [2]. This process induces a range of physical, chemical, and structural transformations in raw materials, particularly noticeable in the food processing industry. Examples include starch, protein, vitamin, and pigment hydrolyzation, as well as the formation of complex products between amylose and lipids [3]. The evolving snack food industry has seen a notable trend in extruded snacks with various health benefits [4]. Consequently, raw materials introduced into the extrusion process typically have high starch contents to achieve the expansion of the final product. To optimize extrusion processes and attain desired product features, manufacturers frequently conduct experiments and trials, varying parameters to identify the ideal combination for their specific product goals. These material and process variables contribute to various macromolecular transformations during extrusion, impacting pasting properties in extruders and, subsequently, the quality of extrudates [2]. This optimization allows for the production of extruded products with diverse textures, shapes, flavors, and nutritional properties. The quality of the final product is also influenced by process responses or extruder system parameters, including specific mechanical energy (SME), product pressure, product temperature, and residence time [2]. These extruder system parameters result from various combinations of extrusion conditions such as feed moisture, screw speed, and barrel temperature [5]. This article aims to provide a systematic review that consolidates recent research on the primary variables involved in the extrusion process and their impact on the ultimate quality of products.

## 1. EXTRUSION PROCESS PARAMETERS

The extruded feed is significantly influenced by moisture content, screw speed, die temperature, and feed rate—these are the most important adjustable parameters during the extrusion process [6]. The starch from raw materials undergoes thermo-mechanical treatment during extrusion, leading to biochemical reactions such as gelatinization, degradation, and the Maillard reaction [7]. Achieving the desired characteristics of the extruded product is possible only through the proper control of these parameters [2].

Figure 1 illustrates the primary extrusion process and system parameters, along with the corresponding changes in product characteristics.

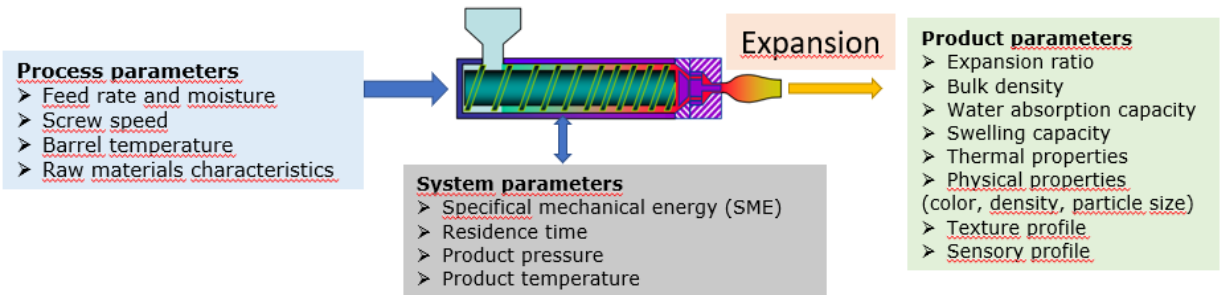



**Figure 1**. Principal variables implied in the extrusion process

**Feed moisture** content is crucial for product expansion and texture. Higher moisture content can result in greater product expansion and a lighter texture, while lower moisture content can lead to denser, crunchier products [9].

The **screw speed** affects residence time and shear in the extruder. Higher screw speeds often lead to increased shear and frictional heat, raising the product temperature, which can impact product texture, flavor, and nutritional content [5]. Lower screw speeds may result in denser products.

**Barrel temperature** is almost the most critical parameter that affects product cooking, starch gelatinization, protein denaturation, and more [3]. Controlling barrel temperature profiles can influence the final product's texture, color, and flavor. Different zones of the barrel can be set to specific temperatures to achieve the desired product characteristics [3]. High barrel temperature and screw speed with low feed moisture during feed material extrusion led to structural degradation of starch due to high shearing, resulting in a higher extrusion ratio and lower bulk density. However, this situation had an inverse trend when using a low barrel temperature [7,8].

The **type of raw material**, including its composition and particle size, has a significant impact on product features. For example, the protein content and starch content in grains can influence the texture and nutritional content of the extruded products. The incorporation of additives, flavorings, colors, and other ingredients can also influence product features. These may be added at different points in the extrusion process, and their type and amount can affect taste, appearance, and nutritional properties [7]. Some authors demonstrated that fiber from raw materials prevents expansion due to starch content dilution, inducing lower gelatinization, breaking the air cell, and favoring viscosity [11]. Furthermore, poor expansion can be explained by the small connection between starch and insoluble fiber, and the ability of the fiber to increase shear viscosity and decrease elasticity [11].

The feed rate, referring to the speed at which raw materials are introduced into the extruder, can affect product density and texture [2]. A higher feed rate may yield a more compacted product with potentially higher density, whereas a slower feed rate could result in a less dense and potentially more porous product [8].

## 2. EXTRUSION SYSTEM PARAMETERS

The ratio of the total mechanical energy derived from the transport of material due to screw rotation within the extruder to the mass flow rate of feed to the extruder is known as specific mechanical energy (SME) [7]. The final product may exhibit better mixing and dispersion if a higher SME leads to greater shearing and deformation. The physical attributes of the extruded goods, such as expansion, hydration properties, density, and texture, are affected by this system parameter [11]. The screw speed has a linear relationship with the increase in specific mechanical energy. Higher SME values can be correlated with the components of raw materials, with high protein and low starch content that can influence the decrease in blend viscosity [2]. Many studies have demonstrated that SME has a good correlation with extrudate attributes such as expansion, density, and texture parameters [2,10].

The stay of the material in the extruder is known as residence time. Material mixing and homogenization can be improved with longer residence times. Thermal

degradation or over-processing can occur due to extended residence times, especially for heat-sensitive materials [7]. At different temperatures, a high residence time improves water absorption capacity, whereas high temperature and high screw speed enhance the number of soluble solids by reducing the polymeric bonds due to shear force [7].

Material flow and compaction are influenced by product pressure inside the extruder. Increasing product pressure can result in higher density and better material consolidation. Die swell or die deflection can result from excessive pressure, impacting product dimensions and quality. Extrusion requires temperature control as it has a direct impact on material viscosity and flow behavior [2]. Higher temperatures can reduce the viscosity of the material, making it easier to process. However, excessive temperatures can lead to thermal degradation or scorching of the material. Temperature profiles (varying temperatures at various zones along the extruder) are frequently employed to manage material properties [2]. Screw speed and barrel temperature were the main determinants producing an increasing effect on product temperature, while feed moisture content had a decreasing effect on product temperature [5].

## 3. FUNCTIONAL AND RHEOLOGICAL PROPERTIES

The positive and negative correlations between extruder system parameters and product features are presented in Table 1.

**Table 1.** Correlation between process parameters and system and product features

| System parameter | Process parameter | | | | | References |
|---|---|---|---|---|---|---|
| | FR | FM | SS | BT | RMC | |
| **SME** | ↓ | ↓ | ↑ | ↓ | ↓ | [2] |
| **RT** | ↑ | ↓ | ↓ | ↓ | ↓ | [5] |
| **PP** | ↑ | ↓ | ↓ | ↓ | ↓ | [5] |
| **PT** | ↑ | ↓ | ↑ | ↑ | ↓ | [5] |
| **Product features** | | | | | | |
| **ER** | ↑ | ↓ | ↑ | ↑ | ↑ | [5] |
| **BD** | ↑ | ↓ | ↓ | ↓ | ↓ | [10] |
| **WAC** | ↑ | ↓ | ↓ | ↓ | ↑ | [9] |
| **SC** | ↑ | ↓ | ↓ | ↑ | ↑ | [5] |
| **TP** | ↑ | ↓ | ↑ | ↑ | ↑ | [7] |
| **Color** | ↓ | ↓ | ↓ | ↓ | ↓ | [7] |
| **Texture** | ↑ | ↑ | ↑ | ↑ | ↑ | [5] |
| **Sensory profile** | ↑ | ↓ | ↑ | ↑ | ↑ | [7] |

↑, ↓ - indicate an increase and a decrease, respectively. SME - Specifical mechanical energy; RT – residence time; PP – product pressure; PT – product temperature; ER – expandion ratio; BD – bulk density; WAC – water absorption capacity; SC - swelling capacity; TP – thermal properties; FR- feed rate; FM – feed moisture; SP – screw speed; BT - barrel temperature; RMC - raw material characteristics.

A rise in **bulk density** is a consequence of a reduction in the expansion ratio and vice versa [10]. The **expansion ratio** in extrusion is a critical factor that influences the final properties and characteristics of the extruded product, depending mainly on mold and vapor pressures [2]. It identifies the difference in cross-sectional area between the die (extrusion die) and the extruded product's cross-sectional area. Extrudate expansion has been proven to have a strong correlation with moisture content and extrusion temperature [10]. The melting flow and viscosity of the material being extruded can be influenced by the die's temperature. Lower viscosity can be caused by higher die temperatures, leading to higher expansion ratios [11]. Higher screw speeds can reduce residence time and result in higher expansion ratios [10].

The porosity of a product is determined by its bulk density, impacting its quality. High shearing and expansion caused starch structural breakdown, resulting in lower density during extrusion at high screw speed and low moisture. A reverse trend was observed when products were extruded at low temperatures [12]. Texturized rice had a higher bulk density due to a low screw speed [9], possibly indicating reduced starch gelatinization. The expansion ratio was impacted by increased screw speed, contributing to low melt viscosity and high shear [12].

Starch's ability to retain water after swelling is determined by its **water absorption capacity** (WAC), indicating the extruded product's behavior during water interaction as processing continues and starch conversion increases [2]. WAC is significantly affected by all extrusion process variables: raw materials, expansion level, and processing conditions [13]. The water content of extruded foods is significantly influenced by material humidity and barrel temperature [11]. Starch degradation reduces molecular size, hindering water absorption. In a weak gluten network, water is absorbed more easily, but in a strong protein network, it could reduce water penetration and starch swelling [14].

Raw material starch, crystallinity, and molecular structure are key factors in extrudate **thermal properties**. A recent study found that extruding commune cereals and pseudocereals decreased peak viscosity, suggesting starch granule breakdown impacting swelling ability when heated. This indicates that starch particles did not achieve the highest swelling rate under experimental conditions [14]. Extruded semi-cooked flour has a higher peak and final viscosity, and setback compared to its raw form, possibly due to increased starch crystallization and uncontrolled interactions between amylose molecules in branched crystalline regions [15]. The lower viscosity of extruded pasta was attributed to amylose molecule rearrangement during the cooling stage, causing a decrease in viscosity and weakening of rearranged amylose molecules [15]. Increases in temperature and feed rate lead to increased gelatinization, while an increase in feed moisture leads to a decrease [7].

## 4. PHYSICAL PROPERTIES, TEXTURE, AND SENSORY PROFILE OF FINITE PRODUCT

The **color** of a product in an extrusion process can be influenced by several factors related to the extrusion parameters, such as the structure of the introduced mixture and the state of the process used to prepare the product [7]. Nonenzymatic browning, such as the Maillard reaction, is the most common reaction. Barrel

temperature, feed moisture, and rate were the main factors that influenced the lightness (L*) and redness (a*) of extrudates, while the yellowness parameter (b*) was influenced by extrusion parameters [7]. The breakdown of pigments, product expansion, and chemical reactions, such as caramelization of carbohydrates, have been reported to be responsible for color modification in extruded products [11].

**Texture** is crucial when it comes to the structural integrity of products and affects the acceptability of extruded foods by consumers. A compact texture and higher hardness value in snacks were achieved by lowering the expansion ratio caused by higher feed moisture. The excess water in the materials acts as a lubricant for the screw, leading to a reduction in shear force and friction between the food material and the extruder screw [16]. The texture of extruded products is determined by the chemical composition. Feed moisture, temperature, and screw speed play a significant role in determining hardness, while softer extrudates are influenced by higher temperature and screw speed [11]. Crispiness is a vital parameter for snacks and correlates with the density of cell structure [2,16]. Previous studies demonstrated that when the screw speed increased, it created a bubble and turned the melted starch into a continuous foam, while the high temperature reduced the thickness of the cell wall and expanded the radius of the air cell [11,16]. Expansion can be negatively impacted by the fiber and protein content in raw materials, resulting in low crispness [11].

The **sensory profile** was associated with some textural properties, such as toughness, hardness, crushing strength, and crispness, which are affected by the extrusion process [17]. Expanded snacks have air bubbles enclosed in a crunchy structure that contains starch, fiber, and protein [18,19]. The Maillard reaction gives specific aroma compounds. Snacks extruded from pea and oat fractions resulted in better sensory and texture attributes at the highest values of SME, pressure, and drive load when production was increased [18,19]. The extrudates with a higher score for color, appearance, crispness, and flavor were the most accepted in terms of overall acceptability and were greatly impacted by the interaction between barrel temperatures [19,20].

## CONCLUSION

Extrusion technology is becoming increasingly important in food production, necessitating the modification of organoleptic properties. The food industry is experiencing a rise in the number of extruded products, and extrusion has been a key method for producing new and creative products for years. Extrusion cooking is a process that generates a variety of final goods with low processing time and inexpensive raw materials. Extrusion parameters, such as screw speed, temperature, and feed moisture content, can be managed to influence the expansion, texture, and color of the product. These variables affecting the extrusion process are functionally related to each other, so further investigation should be directed toward modifying extrusion parameters to create products with the desired characteristics, given the significant impact of extrusion parameters on their physicochemical properties. This information is useful for predicting how the extruded products will meet sensory and nutritional requirements. Therefore, additional research is needed to develop methods that can incorporate other food ingredients (such as vegetables and fruits) to enhance the vitamin content of the product, aiming to optimize the extrusion processing parameters and

interactions between process parameters and raw materials. In future articles, it will be crucial to examine the effect of product and processing parameters on the product's aroma and its mechanism.

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# EFFICACY OF ENTOMOPATHOGENIC MICROORGANISMS FOR ECOFRENDLY MANAGEMENT OF *HELICOVERA ARMIGERA* (HUB) LARVAE IN CORN CROP

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## ABSTRACT

In the western part of Romania, *Helicoverpa armigera* larvae cause significant damage to several types of agricultural and horticultural crops leading to considerable economic losses, so the purpose of this paper was the use of some ecofrendly agricultural practices, namely the use of entomopathogenic microorganisms. The field experiment was conducted at the Research farm of West Land Company SRL, Jimbolia, (Timis County). The trial was laid out during 2022 vegetation season, in randomized block design with seven treatments: control plot, *Bacillus sphericus* + *Glomus* (2%) – 5 l/ ha, *Metarhizium anisopliae* + *Glomus* (2%) – 5 l/ ha, *Bacillus thuringiensis* var. *kurstaki* – 1 kg/ ha, chlorantraniliprole – 125 ml/ ha, indoxacarb – 250 ml/ ha (withdrawn from the market in 2022) and chlorantraniliprole 50 EC - 150 ml/ ha; replicated thrice having a plot size of 3042 sqm. The larval population of *H. armigera* was recorded before spraying and at 48 hours, 7 and 14 days after spraying. All treatments were significantly superior to control plots in reducing larval populations. Among the chemicals, percent larval reduction over control was highest at 7 days after spraying in chlorantraniliprole - 125 ml/ha plots, with an efficiency coefficient of 87.88%. Looking into the efficacy of the bioinsecticides, it was highlighted that the treatments with *Bacillus thuringiensis* var. *kurstaki* proved to be the most effective in minimizing the larval population of *H. armigera* in corn crop, showing an efficiency coefficient of 87.88% at 7 days, respectively 81.96% at 14 days after spraying.

**Keywords:** efficacy, entomopathogenic microorganisms, management, *Helicovera armigera,* corn crop

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## INTRODUCTION

Maize (*Zea mays* L., also commonly known as corn) is one of the most productive and common crop plants, being an economically important sources of human food, and also a key ingredient in animal feed, being widely used in industrial and energy products [1], occupying the second place worldwide, in terms of cultivated surfaces [2]. The agricultural corn crop represents an important income for many farmers, both from Romania and for those from the EU countries, Romania being at the same time one of the representative EU cereals producers [3], harvesting almost 14.445 million tons of corn in 2021 [4]. Over the past five years, the extreme increase in abiotic (e.g. heat, drought, crowding) and biotic (e.g. disease and insect pests) stress factors has had many undesirable consequences that have impacted the security of crop production. Under the influence of these decisive factors, the dynamics of an insects pest population could oscillate, and therefore, it is very important to monitor pest's appearance and abundance, since the conditions of their occurrence can change at a high rate. [5, 6].

In a constantly changing climate, new adaptive management strategies are needed to reduce the impact of agricultural pests on crops [7]. In this context, the aim of this paper was to highlight the efficacy of ecofrendly agricultural practices, namely the use of entomopathogenic microorganisms, in controlling corn insect pests' species.

Among the most economically important pest species with frequent outbreaks, in corn crops from western Romania, is *Helicoverpa armigera* (Hübner), which in favorable climatic conditions or under inappropriate management approaches produces significant yield losses [8]. Being a cosmopolitan species with strong migratory abilities [9], rapid reproductive rates - going through 2-3 generations/year in most of the growing areas of Eastern Europe - and with an extraordinary ability to adapt [10], *H. armigera* has become a pest almost impossible to control using classical methods. Acquired resistance to most chemicals led to the use of ecological management practices, where we tested entomopathogenic microorganisms and mycorrhizal fungi simultaneously.

Despite the long-term effectiveness of conventional insecticides, a number of factors currently threaten their effectiveness and continued use, including the development of resistance to certain active substances, the withdrawal of certain substances from use in the European Union or globally due to increased toxicity to human and environmental health. Therefore, the ecological alternative is the last-minute solution. This requires the continued development and testing of biological control agents that are effective, biodegradable and have no environmental impact [11].

## MATERIALS AND METHODS

### General characteristics of the experimental field:

To conduct the research in 2022, the experimental field was located on a private farm (WestLand Company) in the Jimbolia area (45°49′44,7″N latitude and 20°48′24,6″E longitude), Timiș County (Romania).

The Jimbolia locality is situated in the Jimbolia-Bulgăruș Plain, which has the character of a low tubular plain, covered with sedimentary materials of eolian origin, and simultaneously represents the most fertile area in the western part of the country. The gleyed chernozem soil with medium and fine granulometric composition and large

humus and nutrient reserves characterizes the study area. The climate is a temperate continental one. The average annual temperature is around 10ºC, with the warmest month being July and the coldest being February, which results in an average thermal amplitude of 22.7ºC. Between 7 May and 26 September, there are 143 days a year with temperatures favorable to optimum crop development, i.e. with an average of more than 15ºC.

The field trial to evaluate the efficacy of entomopathogenic microorganisms in controlling the pest *Helicoverpa armigera* in corn crop was conducted in a randomized complete block design with seven treatments, each consisting of three replicates (21plots). The total area of the experimental field was 64.134 $m^2$, with a plot size of 169 m length x 18 m width (3042 $m^2$/plot) and a 1.0 m path maintained around each plot. DEKALB DKC 5830 hybrid, FAO 450 - 490 group was used in the trial.

**The treatments used in the experiment**

Three bioinsecticides were tested for their efficacy against *H. armigera*. In addition, three chemical control treatments, namely clorantraniliprole, indoxacarb, lambda-cyhalothrin and an untreated control were included for comparison. The treatments were as follows: T1 = *Bacillus sphericus* + *Glomus* mycorrhizal fungi (2%) - 4 l/ ha/ 200 L water, T2 = *Metarhizium anisopliae* + *Glomus* mycorrhizal fungi (2%) - 5 l/ ha/ 200 L water, T3 = *Bacillus thuringiensis* var. *kurstaki* - 1 kg/ ha/ 200 L of water, T4 = chlorantraniliprole - 125 ml/ ha/ 200 L of water, T5 = indoxacarb - 250 ml/ ha/ 200 L of water, T6 = lambda - cyhalothrin - 150 ml/ ha/ 200 L of water and T7 = untreated control. The Pantera self-propelled sprayer was used for foliar application of all the insecticides tested.

**Survey on *Helicoverpa armigera***

Between May and September 2022, the species *Helicoverpa a.* was monitored by installing Csalomon-RAG pheromone traps in the experimental field in order to determine the population dynamics and identify the optimal time for bioinsecticide application (fig. 1 a). The traps were installed on 23 May 2022 at a rate of 2 traps per hectare, they were read every 7 days and the sex pheromone was changed every 4 weeks.



a b

Figure 1. Aspects from the experimental fields: a - Csalomon-RAG pheromone traps and b – L 2 larvae attack on corn cob

In order to evaluate the efficacy of different entomopathogenic insecticides, the EPPO Standard 1/109 (2) method was used to assess the efficacy of some products with larvicidal activity. Treatments were applied when the PED of 5 larvae (L 1 or L 2) present per 100 corn cobs was exceeded. On 6 August 2022, after reading the

pheromone traps (the maximum flight curve increases exponentially), the time to apply the treatments was determined. Prior to the application of the treatments (24 hours before - the first assessment), observations were made on the larvae attack on the corn cobs, using the numerical quantitative method. The method consists of highlighting live first or second instar larvae per 200 randomly selected cobs (fig. 1 b); the level of infestation should be greater than 1% per 200 cobs. Entomopathogenic bioinsecticides were applied on 8 August 2022, and chemicals on 9 August. Efficacy was assessed 48 hours, 7 and 14 days after application.

**Data analysis**

Statistical calculations were performed using IBM-SPSS Statistics. Average values, standard deviations, coefficients of variation were calculated and the Duncan test was applied to determine significant differences between batches. The Abbot test was used to calculate the efficacy coefficient for reducing the *Helicoverpa a.* larval population.

## RESULTS

In western part of Romania, the species develops two complete generations in the corn crop, with highly mobile adults whose spatial distribution takes place at several levels: between crops, between fields, between areas in the same region; these extensive movements also contribute to the complexity of the population dynamics of this pest.

The sample incidence of *Helicoverpa a*. adults in the corn crop in the Jimbolia area in 2022 started in the week 23-27.05 (6-8 leaves) with an average of 8.6 adults/trap/day and reached the maximum threshold for the first generation on 23 June (11.1 adults/trap/day). The maximum threshold for the second generation was reached on 6 August, averaging 13.8 adults/trap/day.

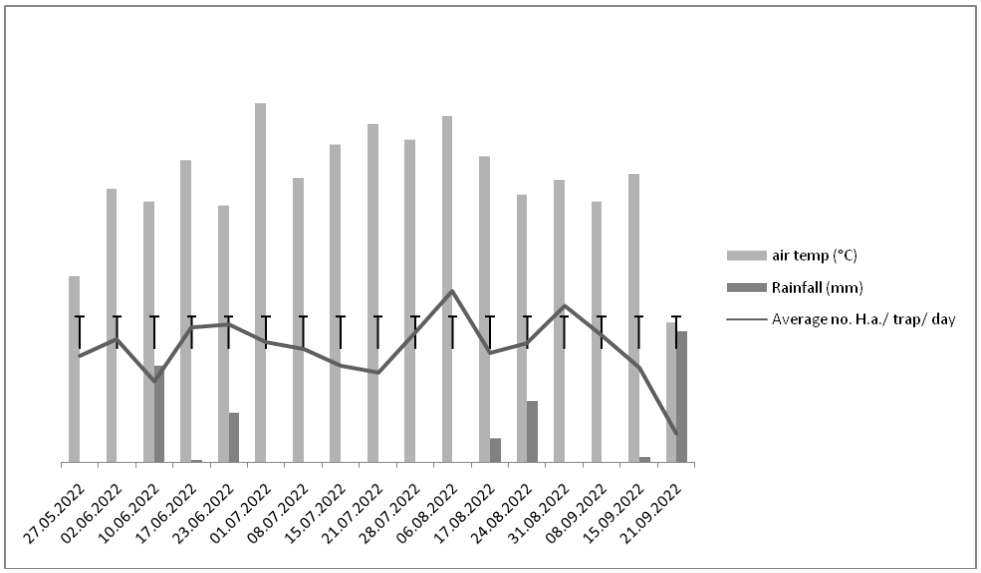



Figure 2. Population dynamics of *Helicoverpa armigera* Hub. on corn crop, Jimbolia, 2022

The statistical analysis revealed significant variations, the high number of adults in the period from 1 July to 31 August being due to the constant but very high air temperatures, which, together with the lack of rainfall, were the main reasons for the increase in the number of adults sampled.

Due to the very high incidence in the samples, but mainly to be able to establish a correct management practice through reduction of the pest pressure, a number of products, both biological and chemical, have been tested in 2022.

Table 1.

Assessment of insecticide response on mean *Helicoverpa armigera* larvae at Jimbolia, 2022

| Treatments | Average no. larvae / 200 cobs | | | | | | |
|---|---|---|---|---|---|---|---|
| | after 48 hours | E% | after 7 days | E% | after 14 days | E% | Total experim |
| T1 = *Bacillus sphericus* + *Glomus* mycorrhizal fungi (2%) | 29.33 a,b | 2,23% | 27.33 b,c | 8,9% | 7.33 a | 75,57% | 23.5 a,b |
| T2 = *Metarhizium anisopliae* + *Glomus* mycorrhizal fungi (2%) | 40.33 b | -11,01% | 33.33 c,d | 8,26% | 13.33 a | 63,31% | 30.83 b |
| T3 = *Bacillus thuringiensis* var. *kurstaki* | 23.67 a | 1,42% | 14.67 a,b | 38,92% | 4.33 a | 81,96% | 16.67 a |
| T4 = chlorantraniliprole | 25.67 a | 22,24% | 4.00 a | 87,88% | 17.33 b | 47,49% | 20 a,b |
| T5 = indoxacarb | 31.67 a,b | 7,78% | 13.00 a | 62,13% | 22.67 c | 34,00% | 25.42 a,b |
| T6 = lambda – cyhalothrin | 27.33 a, b | 3,53% | 9.33 a | 67,07% | 24.33 c | 14,12% | 22.33 a,b |
| T7 = untreated control | 54.67 c | | 42.33 d | | 18.67 b | | 42.58 c |
| Mean | 33.24 | | 20.57 | | 15.43 | | 25.9 |
| CV% | 142.19 | | 214.25 | | 124.65 | | 217.19 |
| Sig | * | | * | | * | | * |

*Values in columns followed by the same letter(s) are not significantly different to each other according to Duncan's multiple range tests at $p \leq 0.05$, CV- coefficient of variation, Sig- significance level, *Significant at $p < 0.05$, E% - efficiency coefficient

The cumulative mean results showed that larval populations of *H. armigera* ranged from 4.33 to 54.66 individuals/ 200 cobs. In terms of larval population reduction, all insecticide and bioinsecticide treatments were significantly superior to the untreated control.

Statistic analysis of the data (table 1) revealed a significant difference between the treatments. The mean values corresponding to the number of larvae recorded after the application of the treatments were significantly different in all three periods analysed ($p < 0.05$).

At 48 hours after application, all treatments applied had a significant effect on population reduction, ranging from 2.23% to 22.24%, with the exception of the *Metarhizium anisopliae* + *Glomus* mycorrhizal treatment (2%). For this bioinsecticide, no population reduction was reported, on the contrary, larval numbers increased significantly. Chlorantraniliprole was the most effective of all the treatments applied, with 25.67 larvae per 200 cobs and a coefficient of efficacy of 22.24%.

Seven days after application, statistically analysed data showed that mean *H. armigera* larvae numbers varied between 4.00 and 33.33 larvae/200 cobs. The insecticide with chlorantraniliprole as the active ingredient proved to be extremely effective in controlling this pest; it had a mean number of 4 larvae/200 cobs and an efficacy coefficient of 87.88%. The product containing lambda-cyhalothrin with a coefficient of 67.07% was the next most effective. A similar level of efficacy was shown by indoxacarb, with a mean number of 13 larvae per 200 cobs and a coefficient of efficacy of 52.13%. Plots treated with *Bacillus sphericus* + *Glomus* (2%) and *Metarhizium anisopliae* + *Glomus* (2%) differed significantly from those treated with chemical insecticides, with low efficacy ranging from 8.26% to 38.92%. Among the biopesticides, *Bacillus thuringiensis* var. *kurstaki* showed the best results with an average number of 14.67 larvae per 200 cob and an efficiency of 38.92%.

At the end of the period, after 14 days, plots treated with entomopathogenic insecticides differed significantly from plots treated with chemical insecticides and untreated ($p<0.05$), with efficacy coefficients ranging from 63.31% to 81.96%, compared to chemical products, where efficacy coefficients ranged from 14.12 to 47.49%. Among the biological insecticides, those based on *Bacillus thuringiensis* var. *kurstaki* showed a mean number of 4.33 larvae/200 cobs and 81.96% efficacy, followed by *Bacillus sphericus* + *Glomus* (2%) - 75.57% and *Metarhizium anisopliae* + *Glomus* (2%) - 63.31%. In the case of chemical insecticides, the best efficacy was achieved by the chlortraniliprole-treated plots: 47.49% efficacy coefficient.

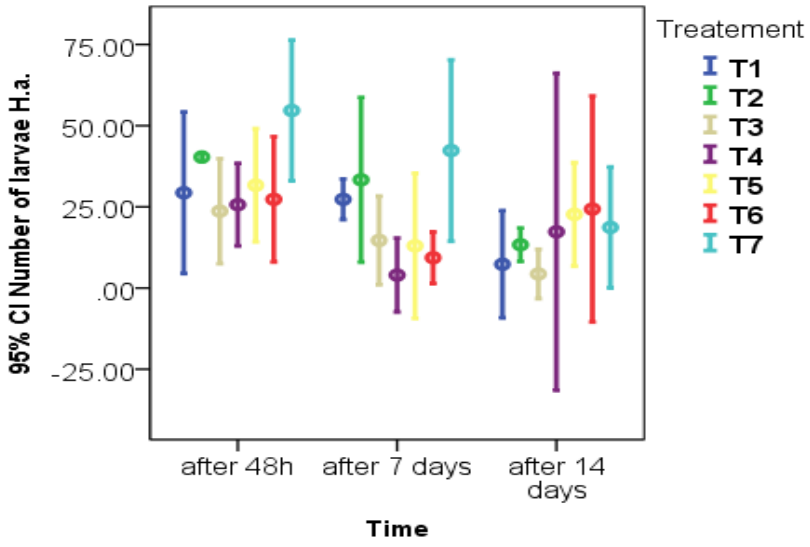



Figure 3. Efficacy of chemical and biological insecticides against *Helicoverpa armigera* larvae, Jimbolia, 2022

The present research highlight that in 2022, in the Jimbolia area, the populations of *H. armigera* larvae infesting the corn crop were significantly lower in the plots chemically treated with chlortraniliprole and in those treated with entomopatogenic *Bacillus thuringiensis* var. *kurstaki*, with a coefficient of efficacy of 87.88% at 7 days after treatment and 81.96% at 14 days after treatment. The following insecticides were used, in order of efficacy: *Bacillus sphericus* + *Glomus* (2%) - 75.57% and - lambda-cyhalothrin with a coefficient of efficacy of 67.07%. *Metarhizium anisopliae* + *Glomus* (2%) and indoxacarb were the products with the lowest results (figure 3).

## DISCUSSION

The current study was conducted to assess the effect of entomopatogenic microorganisms against 1st and 2nd instar larvae of *H. armigera*. Both larval stages showed varied mortality responses to various bio – and chemical insecticides used in this study. In the present findings, the efficacy of bioinsecticides was in invers relation with chemicals one, this meaning that *Bacillus thuringiensis var. kurstaki* and *Bacillus sphericus* + *Glomus* (2%) were the two most effective microorganisms in controlling *H. armigera* larvae, with efficacy demonstrated 14 days after applying the treatments, invers then chlortraniliprole whose effectiveness was proven 7 days after application. Similar downfall in the efficacy of *Bacillus* sp. and *Metarhizium anisopliae* against *H. armigera* larvae was reported by Rijal et al., 2008 [12] and Nguyen et al., 2007[13].

Furthermore, Mantzoukas, 2018 [14] reported good to excellent larval control using low to high concentrations of *B. thuringiensis* and *Metarhizium sp*. respectively, and that the combination of two pathogenic microorganisms increased *H. armigera* larval mortality over time.

## CONCLUSION

The experimental research clearly showed that the larval mortality rate of Helicoverpa argimera was significantly higher in all treatments compared to the untreated control.

Of the six insecticides evaluated, only three were found to be effective in controlling more than 75% of the larvae: chlortraniliprole, *Bacillus thuringiensis* var. *kurstaki and Bacillus sphericus* + *Glomus* mycorrhizal fungi (2%).

Our study points to the potential of entomopathogenic microorghanisms as biological control agents, especially when used synergistically to control *H. armigera* species.

In addition, intensive research will be necessary to determine the most appropriate timing of application of biocontrol agents in relation to pest activity and to develop better formulations and application methods for entomopathogenic insecticides, especially as production in 2022 was not assessed because it was an extremely dry year with high production losses not caused by the pest under study.

## ACKNOWLEDGEMENTS

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# *ELETTARIA CARDAMOMUM*: CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY. AN IN VITRO STUDY

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## ABSTRACT

Recent years have seen an increase in microbial resistance, which has led to great challenges for the medical community in terms of treating diseases and the food industry in terms of contamination and the loss of shelf life. This research aimed to evaluate the chemical composition and antimicrobial activity of the essential oil of Elettaria cardamomum against the following reference strains: *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* ( ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* type B (ATCC 10211), *Listeria monocytogenes* (ATCC 19114), *Bacillus cereus* (ATCC 10876) *Clostridium perfringens* (ATCC 13124), *Candida parapsilopsis* (ATCC 22019) and *Candida albicans* ( ATCC 10231). The chemical analysis revealed 24 GC-MS-identified compounds using a Shimadzu QP 2010 Plus apparatus (Columbia, SC, USA) equipped with an AT WAX 30 m 0.32 mm 1 µm capillary column. The antimicrobial activity and MIC evaluation using the microdilution method suggested a higher efficacy against Gram-positive bacteria and fungi than Gram-negative bacteria.

**Keywords:** *Elettaria cardamomum*, antimicrobial, GC-MS

## INTRODUCTION

Recent years have seen an increase in microbial resistance, which has led to great challenges for the medical community in terms of treating diseases and the food industry in terms of contamination and the loss of shelf life.

Plants offer a rich reservoir of bioactive compounds with promising antimicrobial properties. Extracts and essential oils derived from various parts of plants, including leaves, stems, roots, flowers, and seeds contain various chemical compounds such as

alkaloids, phenols, terpenoids, flavonoids, and essential oils [1]. These compounds exhibit significant antimicrobial activity against fungi, bacteria, and viruses. Research into plant-derived antimicrobial compounds continues to explore their potential applications in various fields, such as food preservation, drug development or alternative medicine. Scientists are exploring plant-derived compounds as potential leads for developing new antimicrobial drugs to combat antibiotic resistance and treat infectious diseases.

Cardamom, scientifically known as Elettaria cardamomum, derives its name from various linguistic roots. The word "cardamom" comes from combining the Malabar term "elettari" and the Greek word "kardamomon," which describes this Indian spice [2].

Cardamom belongs to the *Zingiberaceae* family, commonly known as the Ginger family. It is renowned as one of the oldest and most prized spices globally, traded for centuries. The spice comes from the seeds and pods of the cardamom plant and is used extensively to add flavour to a wide range of dishes and beverages.

The *Elettaria* genera belonging to the *Zingiberaceae* family have been largely explored for their biological properties. Various pharmacological properties, such as anti-inflammatory, analgesic, antioxidant, and antimicrobial effects, have been related to this genus[2]. Cardamom contains various chemical constituents such as proteins, minerals, lipids, carbohydrates, terpenoids and carotenoids, flavonoids, and essential oils, and its capsules are widely used as a spice and flavouring ingredient in foods.

This research aimed to evaluate the chemical composition and antimicrobial activity of the essential oil of *Elettaria cardamomum* against ATCC microbiological strains.

## MATERIALS AND METHODS

### Materials

*Elettaria cardamomum* essential oil (ECEO) was purchased from doTERRA (Pleasant Grove, Utah, USA).

The reference microbial strains (ATCC) used in this study were obtained from the culture collection of the Microbiology Laboratory within the Interdisciplinary Research Platform of the University of Life Sciences "King Mihai I" From Timisoara. The essential oil sample was tested on the following reference strains: *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* type B (ATCC 10211), *Listeria monocytogenes* (ATCC 19114), *Bacillus cereus* (ATCC 10876) *Clostridium perfringens* (ATCC 13124), *Candida parapsilopsis* (ATCC 22019) and *Candida albicans* (ATCC 10231).

### Gas Chromatography-Mass Spectrometry (GC/MS)

GC-MS analysed ECEO using Shimadzu QP 2010 plus apparatus (Columbia, SC, USA) equipped with an AT WAX 30 m 0.32 mm 1 µm capillary column. The discharge rate of the carrier gas, helium, was 1 ml/min, and the injector and ion source temperatures were 250 °C and 220 °C, respectively. For compound separation, a temperature gradient was utilised with an initial oven temperature of 40 °C maintained for 1 minute, followed by an increase to 210 °C at a rate of 5 °C/min and a subsequent 5-minute hold at this

temperature. The sample injection volume was 1 µl of a 2% BEO hexane solution, and a split ratio 1:50 was utilised. The GC-MS analysis was executed in triplicate. The volatile components of the essential oil evaluated were identified using the NIST 5 wiley 275 library database.

Microbiological method

A dilution of $10^{-3}$ of fresh strain was used to perform the test, an inoculum equivalent to a standard of 0.5 McFarland using BHI broth and evaluated with a McFarland densimeter (Grand-Bio, England). The dilutions were pipetted 100 µL into each well of the 96-well microdilution plate using a Calibra 852 digital multichannel pipette. The tested ECEO was added at 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.8 mg/mL, 1.6 mg/mL. The plates were covered and left for 24 hours at 37 °C. After 24 hours, the DO was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Tripled tests were performed for all samples. Strain suspensions and BHI were used as positive controls.

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent to inhibit the visible growth of a microorganism after overnight incubation. Our previous research has described the method as a microbial mass loss by measuring OD by spectrophotometry by ISO 20776-1:2019[3,4].

## TABLES, GRAPHS & FIGURES

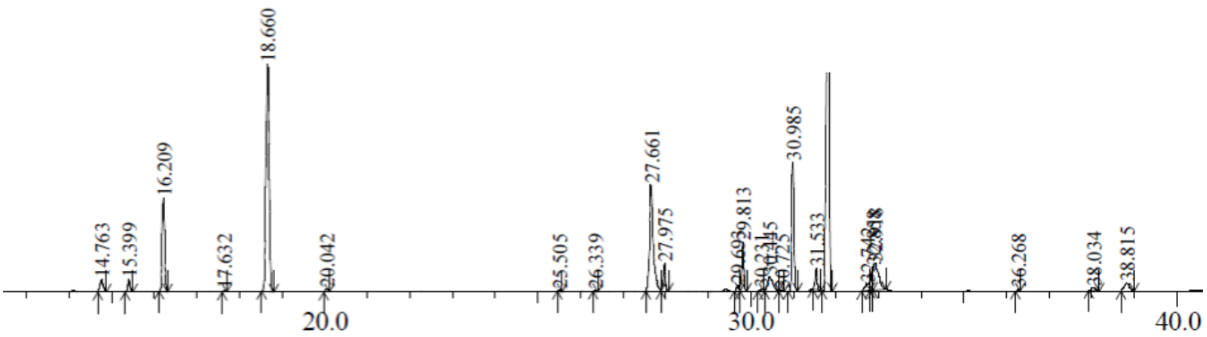



**Figure 1.** GC-MS chromatogram of ECEO

Figure 1 present the results of the GC-MS analysis, as chromatograms revealing the peaks identified for each compound and the constituents identified.

The results obtained as OD (optical density) values were used in calculations with the formulas (1) and (2), and the values are shown in Figures 2, 3, and 4. All the results obtained and calculated are presented as follows in graphical form. The negative values suggest a potentiating effect on the ATCC strain, which could have a positive or a negative evolution trend in correlation with the increased concentration of the tested ECEO.

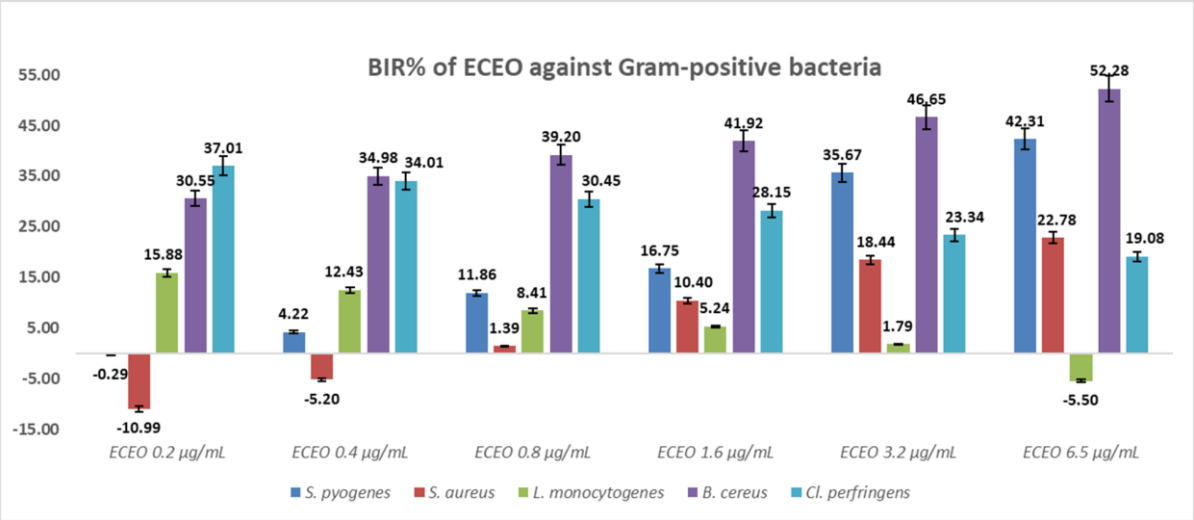



Fig. 2. BIR% of ECEO against Gram-positive bacteria

Figure 2 represents the inhibition rates of ECEO against Gram-positive bacteria at 0.2µg/mL, 0.4 µg/mL, 0.8 µg/mL, 1.6 µg/mL, 3.2 µg/mL and 6.5 µg/mL.

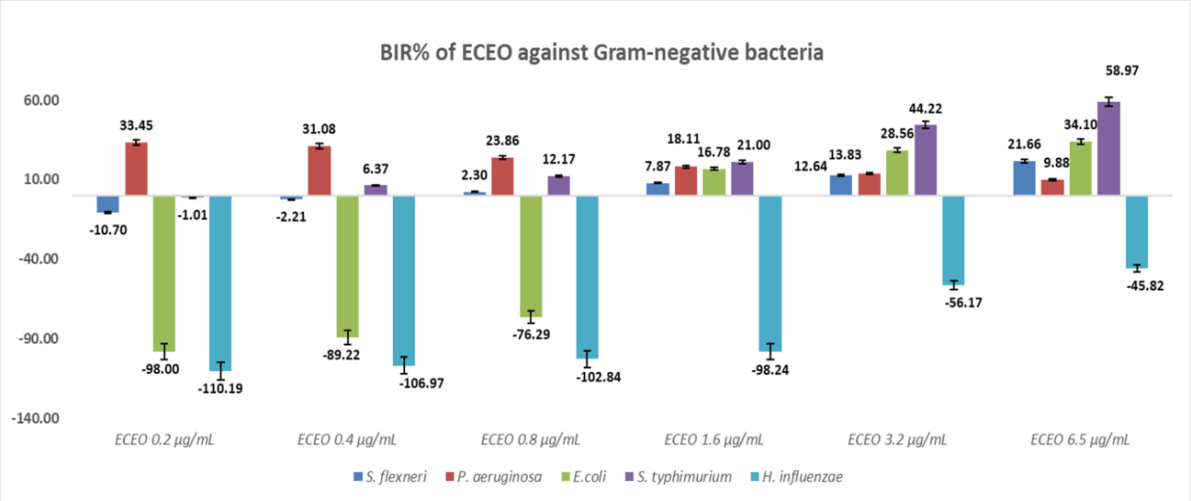



Fig. 3. BIR% of ECEO against Gram-negative bacteria

Figure 3 represents the inhibition rates of ECEO against Gram-negative bacteria at different concentrations tested.

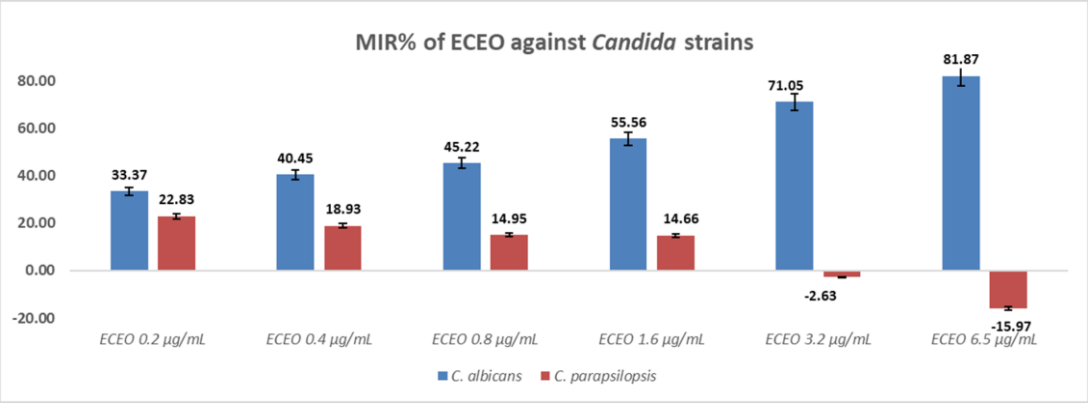



Fig. 4. MIR% of ECEO against fungal *Candida* strains

Figure 4 represents the mycelial inhibition rates of ECEO against *C. albicans* and *C. parapsilopsis* at 0.2µg/mL, 0.4 µg/mL, 0.8 µg/mL, 1.6 µg/mL, 3.2 µg/mL and 6.5 µg/mL.

| | *S. pyogenes* | *S. aureus* | *L. monocytogenes* | *B. cereus* | *Cl. perfringens* |
|---|---|---|---|---|---|
| ECEO 0.2 µg/mL | | | | | |
| ECEO 0.4 µg/mL | | | | | |
| ECEO 0.8 µg/mL | | | | | |
| ECEO 1.6 µg/mL | | | | | |
| ECEO 3.2 µg/mL | | | | | |
| ECEO 6.5 µg/mL | | | | | |

Fig. 5. BIR% of ECEO against Gram-positive bacteria

Figure 5 represents the inhibition rates of ECEO against Gram-positive bacteria at 0.2µg/mL, 0.4 µg/mL, 0.8 µg/mL, 1.6 µg/mL, 3.2 µg/mL and 6.5 µg/mL.

| | *S. flexneri* | *P. aeruginosa* | *E.coli* | *S. typhimurium* | *H. influenzae* | *C. albicans* | *C. parapsilopsis* |
|---|---|---|---|---|---|---|---|
| ECEO 0.2 µg/mL | | | | | | | |
| ECEO 0.4 µg/mL | | | | | | | |
| ECEO 0.8 µg/mL | | | | | | | |
| ECEO 1.6 µg/mL | | | | | | | |
| ECEO 3.2 µg/mL | | | | | | | |
| ECEO 6.5 µg/mL | | | | | | | |

Fig. 6. BIR% of ECEO against Gram-negative bacteria and fungy

## RESULTS

Concerning the chemical composition, the main components identified by GC-MS were alpha-Terpinyl acetate (36.99%), eucalyptol (19.78%), beta linalool (10.97) and D-limonene (6.37%). The microbiological analysis showed the following results: *S. aureus* (Fig.2) proved negative BIR% values at the first two concentrations tested, but the maximum inhibition was reached at 22.78%, demonstrating a medium efficacy. *S. pyogenes* and *B. cereus* were the strains most sensitive, with BIR% values ranging from -0.29% to 42.31% and 30.55% to 52.28%, respectively. *L. monocytogenes* and *Cl. perfringens* proved to be the strains that were negatively inhibited, the inhibition rates

being negatively correlated with the increase in quantity tested. Therefore, the BIR% has a descendent evolution, with BIR% rates higher at the first concentration tested (15.88% and 37.01%) than the values obtained at the last (-5.5% and 19.08%, respectively).

Regarding Gram-negative bacteria (Fig.3), ECEO proved to be less effective, with *P. aeruginosa* demonstrating a descendent evolution, negatively correlated with the increase in concentration. *S. flexneri* and *E. coli* demonstrated low sensitivity with positive values recorded starting from 0.8 µg/mL (*S. flexneri*) and 1.6 µg/mL (*E.coli*) while in the case of *S.typhimurium,* the concentrations tested did not inhibit the bacterial growth.

The *Candida* strains (Fig.4) proved very different evolution patterns, with *C. albicans* demonstrating inhibitory values ranging from 33.37% MIR% to 81.87%, compared to *C. parapsilopsis* that showed a negative correlation with the increase in concentration, the MIR% ranging from 22.83% to -15.97%.

Figure 5 and Figure 6 present the MIC values recorded by ECEO for the tested ATCC strains, in which the first concentration was MIC for *L. monocytogenes, B. cereus, Cl. perfringens, P. aeruginosa, E. coli, H. influenzae, C. albicans* and *C. parapsilopsis*. The light grey colour represents the samples in which the MIC was found, but subsequent concentrations showed a potentiating effect, and therefore, the effect decreased together with the concentration. The red colour highlights the samples where the MIC was determined, and the yellow highlights negative values that decrease with the increase in concentration, the subsequent concentrations achieving MIC.

## DISCUSSION

The chemical composition of ECEOs varies in different regions of the world, as demonstrated by previous studies, knowing that the geographical area, the season, and the method of obtaining essential oil influence this composition. This aspect was highlighted by Alam et al., [5], which demonstrated that ECEO from India contained 83.24% monoterpenes, while the one obtained from Guatemala contained 73.03%. Regarding sesquiterpenes were present in a higher concentration in ECEO from Guatemala (18.35%) than in the one from India (9.27%). In their study, Abdullah et al., indentified by GC-MS twenty-six compounds int the tested ECEO, with α-terpinyl acetate (38.4%), 1,8-cineole (28.71%), linalool acetate (8.42%), sabinene (5.21%), and linalool (3.97%) as major bioactive components [6]. Instead, Tarfaoui et al., found the 1.8-cinéol (43.47%) was the predominant compound in their tested ECEO [2]. Other studies demonstrated that 1.8-cinéol is the second-most abundant compound, the predominant being α-terpinyl acetate [7,8].

ECEO seems to have an antimicrobial activity against various strains, such as *Bacillus subtilis, Candida albicans, Enterobacter aerogenes, Enterococcus spp., Escherichia coli, Klebsiella pneumoniae, Listeria spp., Pseudomonas spp., Salmonella spp*. and *Staphylococcus spp* [7]. The minimum inhibitory concentration is different depending on the chemical composition of the tested ECEO and on the tested bacterial strain. Abdullah et al., 2017, showed that the MIC) values of ECEO against tested strains were 10 ± 0.00 mg/mL against *S. typhimurium, S. aureus* and 5 ± 0.00 mg/mL against *S. mutans, C. albicans* strains, respectively. *P. aeruginosa* was found most resistant strain as ECEO did not inhibited its growth [6]. However, another study highlighted that MIC

for *Pseudomonas aeruginosa* was 0.25 - 0.5 mg/mL, depending on the tested ECEO, whereas MIC for *Escherichia coli* was higher, respectively, 0.5 - 1 mg/mL [5].

## CONCLUSION

As a result of our research, we can say that the essential oil of *Elletaria cardamomum* produced by doTERRA due to the synergistic activity of its constituents causes a strong inhibiting effect, especially on the Gram-positive bacteria, as well as the tested fungi.
The inhibitory effect against the biofilm formation on the Gram-positive bacteria is increasing: *B. cereus* > *S. pyogenes* > *Cl.perfringens* > *S. aureus*> *L. monocitogenes*.
Regarding the Gram-negative bacteria, the sensitivity starts with *S. typhimurium* > *P. aeruginosa* > *S. flexneri* > *E. coli* > *H. influenzae*. The same analysis shows that *C. parapsilopsis* is more resistant than *C. albicans*.
The demonstrated effect of *Elletaria cardamomum* oil recommends it as a potential future candidate in natural products with antimicrobial activity. Our findings could allow oil blends in many areas, such as products for bacterial and fungal dermatological treatments and the food industry, which are strongly affected by the increase in anti-biofilm resistance to standard, commercial antimicrobial products.

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# ENHANCING THE ANTIOXIDANT ACTIVITY OF CHAMOMILE: EXPLORING THE IMPACT OF ULTRASOUND ON PROTEIN EXTRACTION

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## ABSTRACT

Chamomile flower, a well-known medicinal plant, possesses significant antioxidant properties attributed to its diverse array of bioactive compounds, including antioxidant proteins. These proteins are vital in combating oxidative stress and neutralizing harmful free radicals. To fully harness the antioxidant potential of chamomile, it is imperative to optimize the extraction methods. This study evaluates the effectiveness of non-conventional mechanical extraction methods using ultrasound to extract antioxidant compounds from chamomile, comparing it with a traditional agitation-based technique. The extracts obtained using green solvents such as basic aqueous solutions or phosphate-buffered saline (PBS) showed that while the ultrasound extraction requires a shorted extraction time compared to traditional methods, it yields a more active extract in terms of antioxidant activity. These active constituent-rich extracts were then subjected to thorough characterization and quantification using advanced analytical techniques, such as gel electrophoresis, mass spectrometry, UV-vis, and FTIR spectroscopy. Finally, the antioxidant capacity of the aqueous extract was determined using the DPPH free radical method and the FRAP assay.

**Keywords:** Chamomile, antioxidant activity, proteins, ultrasound extraction.

## INTRODUCTION

The global interest in medicinal plants is on the rise, driven by a shared recognition among researchers and the public that natural products, especially those derived from plants, offer significant health benefits. The preference for natural products as drugs, being biocompatible and free from side effects, has grown, especially in contrast to chemical and synthetic drugs [1]. Natural extracts with biological and medicinal properties have gained attention in recent years, serving as food components or specific

pharmaceuticals for humans and animals. Medicinal plants are considered valuable resources for potential drugs targeting various diseases, although many plants' biological and pharmacological properties remain undiscovered [2].

Chamomile (*Matricaria chamomilla L.*), is a medicinal plant that contains a diverse range of bioactive compounds, such as flavonoids, terpenoids and coumarins. Chamomile has consistently demonstrated its antioxidant prowess in numerous studies. For example, apigenin, a compound found in chamomile, exhibited potent inhibition of free radical production, protecting against damage in an $H_2O_2$-induced cell model [3]. Moreover, hydroalcoholic chamomile extracts exhibited notable reductions in reactive oxygen species levels when applied to human adenocarcinoma HT29 cell lines and rat colon specimens treated with lipopolysaccharide [4]. Although the antimicrobial effects of chamomile have been associated with its elevated phenol concentration [5], the precise antioxidant mechanism remains a subject of ongoing investigation [6].

Ultrasonic-assisted extraction (UAE) is acknowledged as an eco-friendly and highly efficient extraction technology with the potential to reduce or eliminate the need for organic solvents, thereby minimizing environmental impact [7]. Ultrasounds are successfully applied to extract polyphenols, carotenoids, aromas, and polysaccharides from plant matrices, including both whole plants and by-products [8-11]. Precise control of variables such as frequency, power, duty cycle, temperature, time, solvent type, and liquid-solid ratio is crucial for achieving optimal extraction in UAE. Researchers have extensively studied the individual and interactive effects of these variables on extracting bioactive compounds from fruit and vegetable by-products [12].

The aim of this study was to investigate how ultrasound-assisted extraction influences the antioxidant properties of chamomile aqueous extracts. The investigation comprised not only quantitative assessments of total phenolic compounds and proteins but also qualitative analyses using FTIR and MS. Furthermore, the antioxidant capacity of the ultrasound-obtained extract was compared with that of the agitated sample. The study also explored the influence of the solvent on the antioxidant activity of the extract, employing two solvents in the extraction process: a phosphate buffer solution and a 0.05M NaOH solution.

## MATERIALS AND METHODS

Chamomile plant (*Matricaria recutita*) was collected from Suceava County, Romania in July 2023 and left to dry in a cool and well-ventilated place. All reagents were of analytic purity and used without further purification. They were purchased from Sigma–Aldrich (Taufkirchen, Germany) and Merck (Darmstadt, Germany).

*Sample preparation*: The plant was mechanically minced using a kitchen robot and sieved for 5 minutes on a laboratory vibrating sieve. Samples smaller than 1 mm diameter were collected and stored at 4°C until use.

*Extraction:* Milled samples (1 g) were suspended at room temperature (RT) in 20 mL of solvent (NaOH 0.05 M; PBS 20 mM pH 7.4) and **(a)** ultrasonic-assisted (Bandelin Sonorex bath) for 45 minutes before were filtered on a Buchner funnel. The filtrate was collected and the residue was resuspended in 20 ml of solvent and sonicated for another 45 minutes. **(b)** conventional shaken at 250 rpm (DLAB MX-M shaker) for 12 hours before were filtered. The filtrate was collected and the residue was resuspended in 20 ml

of solvent and stirred for another 12 hours. After filtration, the collected extract was centrifuged for 15 min at 4500 rpm (Hettich, MIKRO 220 R). The solid residue was stored in the freezer for possible reuse while the supernatant was stored in the fridge for one week while performing the analysis.

*Determination of protein content:* The protein extracted amounts were determined by Bradford colorimetric method (Bradford Assay Kit, Sigma). Briefly, 30 µL of samples extract were mixed with 970 µL of protein assay dye reagent and incubated for 5 min at RT before reading the absorbance at 595. Albumin was used as a standard at 21.8 - 1400 µg/ml to generate a calibration curve.

*Total phenolic content:* The levels of polyphenol compounds were estimated with using a modified Folin-Ciocalteu method. Briefly, 20 µl extract were mixed with 780 µl water, 50 µl Folin-Ciocalteu reagent and 150 µl sodium carbonate solution (200 g/L). Samples were incubated for 60 min at 37°C and absorbance was measured at 765 nm using gallic acid (GA) as a standard (200 - 800 µg/ml). Total soluble phenolic content was expressed as gallic acid equivalent (GAEq) per ml of extract.

*ABTS free radical-scavenging activity*: The antioxidant activity of pollen extract was assessed using the 2,2‘-azino-di[3-ethylbenzthiazoline sulfonate (ABTS•) free radical method. Briefly, 2 µL sample was incubated with 8 µL water and 490 µL reagent in the dark for 30 min at RT. The absorbance was read at 517 nm. The inhibition activity was calculated as a percentage of the ABTS discoloration using the following equation: % Inhibition = $[(A_{ABTS}-A_{Sample})/A_{ABTS}] \times 100$.

*Ferric reducing antioxidant power (FRAP) assay*: was performed according to Benzie and Strain method, with small modifications. 25 µL of extract solution were added to a mixture containing 25 µL water and 550 µL of FRAP reagent freshly prepared (10:1:1, acetate buffer: 10 mM TPTZ: 20 mM $Fe(NO_3)_3$). The samples were incubated for 30 min at 37°C before reading the plate at 593 nm. A calibration curve was prepared using an aqueous solution of $FeSO_4$ (0.25-3 mM). FRAP values were expressed as millimoles of ferrous equivalent per ml of extract.

*Fourier-transform infrared spectroscopy (FT-IR)* analysis was performed using a Nicolet i-20 spectrophotometer (ThermoScientific, USA). The acetone precipitated samples were placed on the ATR crystal and the spectra were recorded in transmission mode within the wave number range of 4000–400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. SpectraGryph–spectroscopy software (Version 1.2.11) was used for spectra analysis.

*Sodium dodecyl sulfate polyacrylamide gel electrophoresis:* Acetone precipitation of proteins was performed prior to SDS-PAGE analysis. The pellet was redissolved in water for a final concentration of 50 µg/15µl before loading it into the gel. Approximatively 15 µL of protein solution (50 µg protein) were mixed with 7 µL of sample loading buffer and heat-denatured at 55 °C for 5 min. The SDS gel was run for 45 min at 40 V and at 100 V for another 1.5 h. Proteins were silver stained using a standard protocol. For evaluating the molecular of the weight of unknown proteins, a protein ladder ranging from 10 to 245 kDa (Thermo Scientific, USA) was used as reference.

*Mass spectrometric analysis:* The mass spectrometric measurements were carried out on a Bruker Ultraflex MALDI ToF/ToF mass spectrometer operated in positive linear mode and equipped with a pulsed nitrogen UV laser (Bruker Daltonics, Hamburg, Germany). For removing the salts which might interfere with the MS analysis the

samples were subjected to ZipTip procedure using $C_{18}$ tips (Merck Millipore). The samples were co-crystallized with a-Cyano-4-hydroxycinnamic acid (HCCA) using the dried-droplet method. The samples and matrix were loaded onto a 384-spot target plate of the MALDI-ToF instrument and allowed to dry at RT.

## RESULTS

The current study uses Chamomile plant as raw starting material. Following extraction procedure, the total phenolic and protein content was assessed using colorimetric methods. This table presents the protein and phenol content (measured in µg/ml) of chamomile pollen extracts obtained through different extraction techniques (ultrasound - U, shaking - S) and using different buffers (NaOH, PBS). Notably, the highest protein and phenolic content were discerned in the ultrasound-treated sample with a basic solution, reaching 393.86 µg/ml for protein and 755.67 µg/ml for phenol content. In contrast, the S_PBS (Shaking with PBS) extract exhibited the lowest phenol content at 441.67 µg/ml and 142.43 µg/ml for protein. To summarize, among the tested samples, the ultrasound-extracts demonstrated greater concentration compared to the conventional shaking method.

Table 1. Protein and phenol content (µg/ml) of pollen Chamomile extracts. The total phenolic content was expressed as µg gallic acid equivalent (GAE) /ml of extract

| Sample ID | Extraction Technique | Buffer | Protein (µg/ml) | Phenol (µg GAE/ml) |
|---|---|---|---|---|
| **U_NaOH** | Ultrasound | NaOH | 393.86 | 755.67 |
| **U_PBS** | Ultrasound | PBS | 315.29 | 610.33 |
| **S_NaOH** | Shaking | NaOH | 382.43 | 449.00 |
| **S_PBS** | Shaking | PBS | 142.43 | 441.67 |

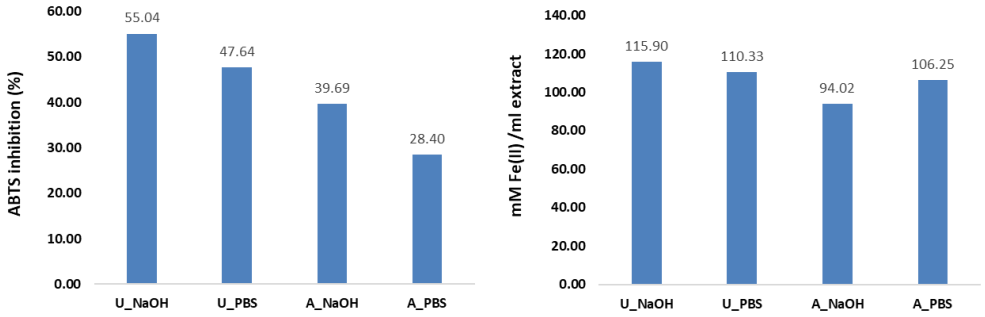



Figure 1. The antioxidant activity of different chamomile extracts expressed as: percentages of inhibition in ABTS assay (left) and mM Fe(II) equivalents per ml extract in FRAP assay (right)

The antioxidant activity of Chamomile extracts was assessed utilizing two distinct methods: the ABTS reducing power method and the FRAP assay. The outcomes depicted in Figure 1 reveal that among the investigated samples, the U_NaOH extract exhibited the highest % scavenging activity at 55.04%, closely followed by U_PBS at 47.64%. A consistent pattern was observed using the FRAP method, where the U_NaOH sample demonstrated the highest value of 115.9 mM $Fe^{2+}$ equivalents/ml extract, with U_PBS following closely at 110.33 mM $Fe^{2+}$ equivalents/ml. In contrast,

the A_PBS extract displayed the lowest ABTS inhibition at 28.4%, while A_NaOH generated the smallest amount of $Fe^{2+}$ equivalents/ml at 94.02 mM.

Furthermore, various analytical methods, including SDS–PAGE electrophoresis and mass spectrometry, were employed to analyze the protein content of the extracted fractions. Initially, the molecular weight of proteins was examined using SDS-PAGE. As depicted in Figure 2, all samples exhibited smeared bands, posing challenges in identifying the mass range of the proteins. Notably, the extract obtained with ultrasound displayed a more pronounced response in the region of small molecular masses, whereas samples obtained with shaking method showed less intense bands.

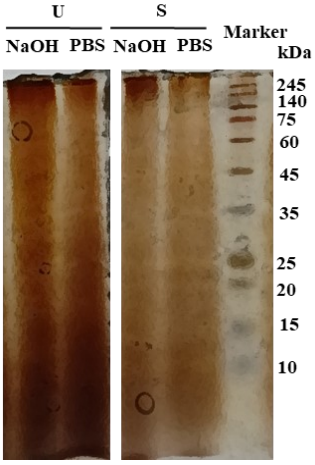



Figure 2. SDS-PAGE electrophoresis of chamomile protein content.

Mass spectrometry is an efficient and reliable techniques for protein analysis with regard to precise molecular mass assignment. MALDI-TOF MS allows intact protein mass determination with ample accuracy. Figure 3 presents the mass spectra for chamomile extracts using different buffers (NaOH, PBS) and allows us an immediate comparison of sample pattern. As observed, except the signals lower than 600 m/z that belong to matrix, both samples generated peaks in the 650-670 m/z range. In contrast, the extract obtained in a phosphate buffer medium, besides the common signal at 650 m/z, exhibited an additional response at 666 m/z. This indicates that the alkaline environment is conducive to extracting a greater variety of peptide compounds, a finding corroborated by the Bradford method.

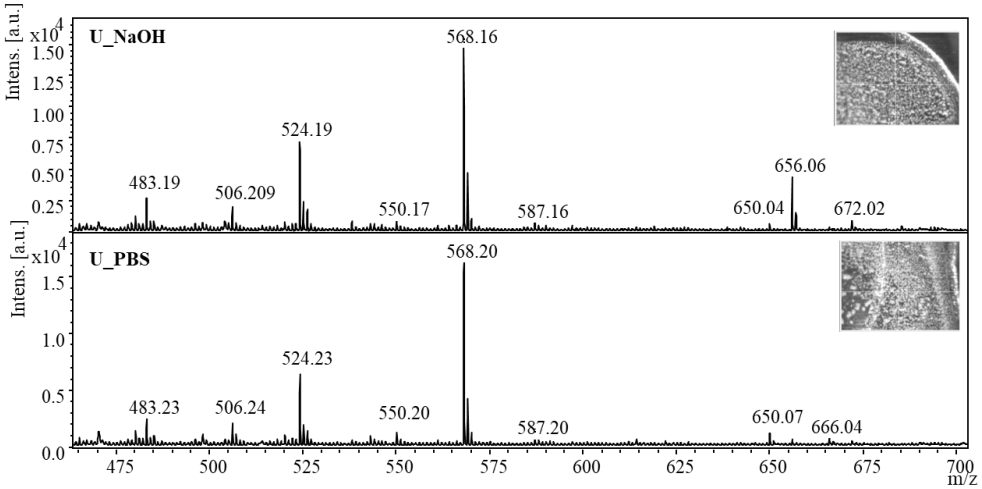



Figure 3. MALDI-TOF mass spectra of chamomile extracts. The crystal morphology of samples prepared for MS analysis (inserted figures).

Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR–ATR) stands as a widely employed analytical technique crucial for structural characterization. Figure 2 illustrates the FT-IR spectra of acetone-precipitated chamomile extracts, recorded within the range of 4000-700 $cm^{-1}$. Absorption peaks in the amide I at 1652

cm$^{-1}$ were assigned to the alfa-helix, a conclusion not supported by the amide III band observed at 1230 cm$^{-1}$ characteristic for random coil. The region within 1200-900 cm$^{-1}$ predominantly featured polysaccharide peaks, with the peak at approximately 1073 cm$^{-1}$, associated with OH stretch, highlighting the presence of carbohydrates in the samples. Additionally, other signals in the FTIR spectra could be attributed either to the extraction medium or other compounds found in the extract sample.

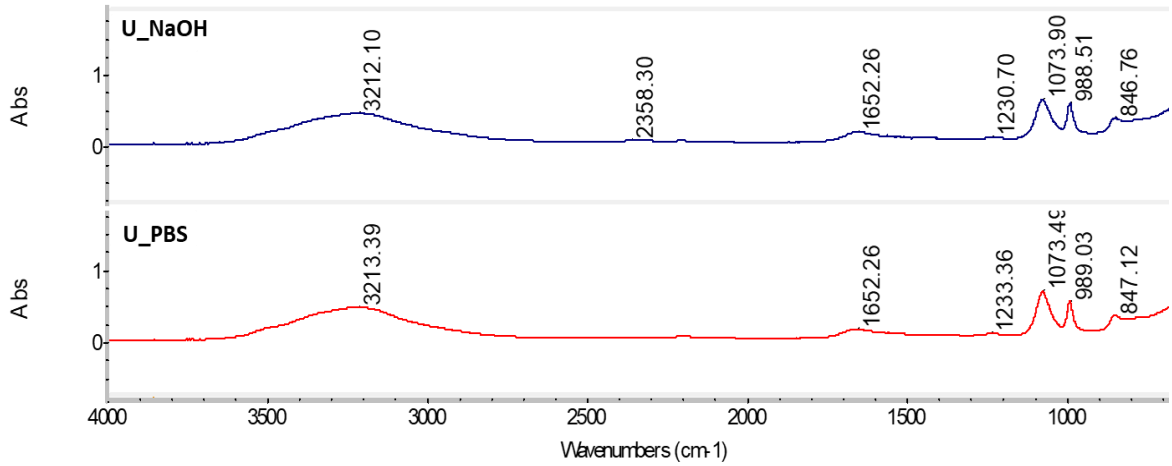



Figure 4. FT-IR spectra of ultrasound-extracted chamomile samples in NaOH solution and PBS buffer.

## DISCUSSION

In exploring the biological activity of natural products, antioxidant activity stands out as a fundamental aspect. This pervasive activity forms the foundation for various other biological effects, including antimicrobial and cardioprotective activities. Precursor compounds typically exhibit antioxidant activity through two primary mechanisms: preventing the oxidation process or neutralizing free radicals. Therefore, in evaluating the antioxidant capacity of natural products, it is advantageous to employ diverse tests that tap into various mechanisms of action. In this study, the antioxidant capacity of chamomile extract underwent examination using two different tests, providing a comprehensive understanding of its potential as an antioxidant source. Antiradical assays demonstrated the chamomile extract's substantial capability to neutralize ABTS free radicals. Furthermore, FRAP reduction assay affirmed the extract's high reduction ability. Extraction techniques and solvent nature can mediate the antioxidant and free radical scavenging capacity of a sample as observed by Žlabur [13] et al. while comparing the water and ethanol extracts of chamomile flowers obtained by conventional and ultrasound-assisted extraction. In our study, ultrasound treatment notably reduced the extraction time of bioactive compounds, concurrently elevating both the total phenolic and protein content, thereby enhancing the overall antioxidant capacity.

In general, an alkaline extraction pH within the range of 8.0-10.0 has been consistently chosen in various studies as the solvent for plant protein extraction [14]. Alkaline substances such as NaOH and KOH are commonly utilized to maintain a basic pH, resulting in a higher extraction yield compared to organic extraction methods. The basic pH conditions lead to the disruption of disulfide bonds in proteins, contributing to

improved protein recovery and yield [15]. The solubility of proteins increases with a rise in the solvent's pH, attributed to the ionization of acidic and neutral amino acids at higher pH levels. Therefore, the observation aligns with the results obtained in this study, where extracting proteins in an alkaline environment led to higher protein quantities.

## CONCLUSION

The global trend toward natural products for medicinal and health purposes underscores the significance of exploring the antioxidant potential of chamomile. Chamomile, a renowned medicinal plant, is a reservoir of diverse bioactive compounds, including antioxidant proteins. This study underscores the potential of ultrasound-assisted extraction for maximizing the antioxidant activity of chamomile. The extracts obtained through this method, especially with NaOH, exhibited superior antioxidant properties. The role of solvent type, particularly the alkaline environment, emerged as a critical factor influencing protein yields. The application of advanced analytical techniques, including gel electrophoresis, mass spectrometry, and FTIR spectroscopy, provided a nuanced understanding of the chamomile extracts. SDS-PAGE revealed distinct protein patterns, with ultrasound extracts displaying more intense bands. In conclusion, these findings contribute valuable insights for optimizing chamomile extraction processes, emphasizing the role of ultrasound in enhancing antioxidant potential. Further research is encouraged to refine extraction methods and increase the proportion of bioactive compounds in chamomile extracts.

## ACKNOWLEDGEMENTS

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# EVALUATION OF HIGH NUTRITIONAL BREAD OBTAINED FROM WHOLE WHEAT FLOUR, KONJAC AND PSYLLIUM BLENDS

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## ABSTRACT

The current orientation towards a healthy, calorie-free, high nutritional diet requires the identification and study of new ingredients with high nutritional properties, which introduced into the bread consumed daily, contribute to a suitable diet both for people with special diet needs and for healthy people. In this regard, the use of vegetal matrices such as konjac flour and psyllium bran in the bakery products represents a new idea that could be implemented in the food industry in order to diversify the range of low glycemic and high fiber bakery products.

The aim of the study is to investigate the effect of konjac flour and psyllium bran as sources of dietary fibre at different level of substitution of whole wheat flour (0%, 5%, 10%) on the physical-chemical composition, textural and sensorial properties of developed bread.

Results showed that the nutritional value of the studied bread samples increased significantly with increasing levels of konjac flour and psyllium bran. The mean sensory scores obtained showed that bread with 10% substitution KF5PB5 (5% konjac flour + 5% psyllium husk) had the highest taste, texture, crust and overall acceptability scores in all the attributes evaluated showing high acceptability from consumers.

This study aimed to obtain seven types of bakery products with high fibre content and gluten free: control bread – CB; 5% konjac flour – KF5; 10% konjac flour – KF10; 5% psyllium bran – PB5; 10% psyllium bran - PB10; 5% konjac flour and 5% psyllium bran – KF5PB5; 10% konjac flour and 10% psyllium bran – KF10PB10 and evaluate their bakery parameters, nutritional value and consumer acceptance.

**Keywords:** bread, blends, high quality, psyllium, konjac, consumer preferences.

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## INTRODUCTION

In recent decades, bread has been produced mainly from wheat flour. Consumers are becoming more interested in healthy nutrition, preferring diets that taste good, are reasonably priced and contain functional ingredients such as dietary fibre and antioxidants. In addition, the higher price of wheat flour has forced manufacturers to look for new formulas to make bread with higher dietary fibre content, higher quality, and cheaper. Consequently, incorporating konjac flour or psyllium husk into bread would enhance its nutritional and functional qualities [8].

Konjac is highly nutritious with over 45% Glucomannan, a soluble fiber which retains up to 200 times its weight in water, increasing its volume. Konjac contains also 12.7% crude protein, 16 different types of amino acids up to 7.8%, minerals such as calcium, phosphorus, iron, zinc, manganese, chromium and copper. More specifically, konjac flour has no calories, so it is not fattening, has a regulating role in lipid metabolism, laxative effect, anti-diabetic, anti-inflammatory, probiotic, antioxidant, etc. [12]. Glucomannan has an extraordinary ability to absorb liquids and change into a gel that makes you feel full and reduces appetite. This gel passes through the intestine very quickly, so excess food is no longer automatically converted into fat, but is eliminated naturally [13].

Psyllium (*Plantago ovata*) plant grows in most parts of the world [6] and its health benefits are widely recognized [1]. The soluble and insoluble dietary fiber of psyllium husk possesses very good gel-forming and high water absorption ability capacities [4].

Bran, obtained from Psyllium seeds, is composed of both soluble and insoluble fibres. The composition of psyllium includes: 75% xylose, 23% arabinose, traces of other sugars and about 35% of non-reducing residues [2].

This study aimed to obtain seven types of bakery products with high fibre content and gluten free: control bread – CB; 5% konjac flour – KF5; 10% konjac flour – KF10; 5% psyllium bran – PB5; 10% psyllium bran - PB10; 5% konjac flour and 5% psyllium bran – KF5PB5; 10% konjac flour and 10% psyllium bran – KF10PB10 and evaluate their bakery parameters, nutritional value and consumer acceptance. Studied samples prepared from whole wheat flour with different substitution proportion were sensorial evaluated for appearance/loaf shape, aroma, taste, texture, crust and overall acceptability using 9 point hedonic scale.

## MATERIALS AND METHODS

*2.1. The technological process to obtain konjac and psyllium bran bread*

The bread samples were obtained within the Micropilot station of University of Life Sciences "King Mihai" from Timisoara (ULST), the nutritional values was determinate in the Interdisciplinary Research Platform (ULST) and the bakery parameters were determined within Bakery and Milling Laboratory of Faculty of Food Engineering.

Konjac flour (KF) produced by FitFood, psyllium bran (PB) and whole wheat flour (WF) produced by Solaris was purchased from Enigma shop, Timisoara, Romania.

The bread with konjac flour and psyllium husk was obtained in 7 experimental variants: control bread CB; 5% konjac flour – KF5; 10% konjac flour – KF10; 5% psyllium bran – PB5; 10% psyllium bran - PB10; 5% konjac flour and 5% psyllium bran – KF5PB5; 10% konjac flour and 10% psyllium bran – KF10PB10, according to the manufacturing recipe presented in table 1.

**Table 1**. Recipe for bread with konjac flour and psyllium bran

| Samples | Ingredients | | | | | |
|---|---|---|---|---|---|---|
| | Konjac flour (g) | Psyllium bran (g) | Whole wheat flour (g) | Yeast (g) | Salt (g) | Water (mL) |
| CB | - | - | 500 | 6 | 7.5 | 250 |
| KF5 | 25 | - | 475 | 6 | 7.5 | 250 |
| KF10 | 50 | - | 450 | 6 | 7.5 | 300 |
| PB5 | - | 25 | 475 | 6 | 7.5 | 250 |
| PB10 | - | 50 | 450 | 6 | 7.5 | 300 |
| KF5PB5 | 25 | 25 | 450 | 6 | 7.5 | 300 |
| KF10PB10 | 50 | 50 | 400 | 6 | 7.5 | 350 |

The yeast suspension was obtained by mixing yeast in 50 g of warm water (30°C) and leave for 10 min for leavening. After yeast leavening the ingredients (whole wheat flour, komjac flourm psyllium bran, salt and the rest of the water) were introduced in a mixer and mixed for 5 minute at 80 rpm speed. The dough formed was mixed for 5 min at the second speed of 160 rpm. The obtained dough was placed in a covered plastic bowl for 1 h at a temperature of 24°C. Then, the dough was shaped and placed in a pan greased with oil, where it was left to rise for 30 min at a temperature of 35°C. Afterwards, the dough was baked simultaneously in a preheated electric oven at 230 °C for 24 min. The technological scheme for obtaining bread with high fiber content flour is presented in Figure 1.

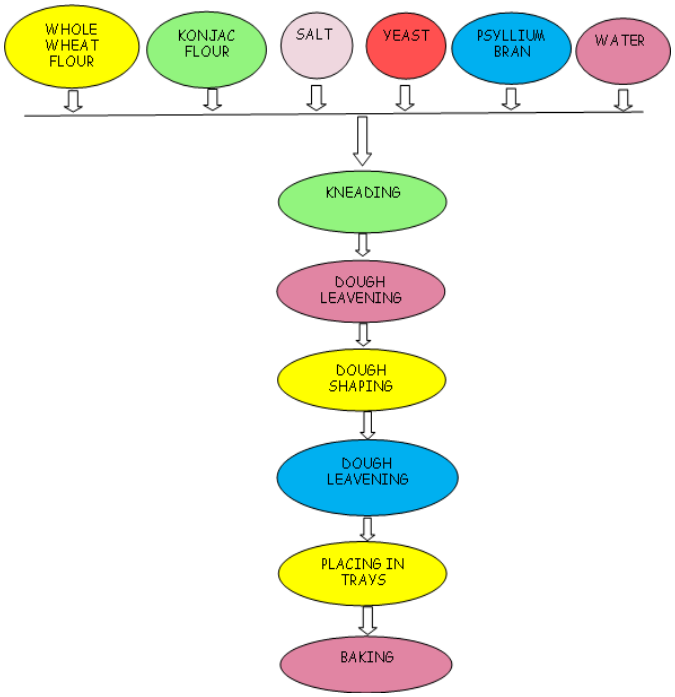



**Figure 1.** The technological scheme for obtaining bread with high fiber content flour

*2.2. Determination of nutritional characteristics of bread samples*

The nutritional value was determined according with ISO Methods: SR 91/2007 pct.10 (moisture); SR 91:2007 pct.14.4 (total lipid); SR EN ISO 8968-1:2014 (protein); and SR ISO 2171/2010 (mineral substances or ash) [5]. The carbohydrate content (%) was determined as the difference between 100 and the sum of the following fractions: proteins, lipids, ash and moisture.

### *2.3. Determination of bakery parameters*

For each bread sample, the following bakery parameters were used to assess quality: volume, porosity, core elasticity and height/diameter ratio. All analytical methods used were carried out according to Romanian Standard STAS 91/83 [10]
Briefly, the bread volume was determined with the FORNET device, it is based on measuring the volume of rapeseed displaced by the product analysed, and it is reported in terms of a percentage. The bread volume is calculated with the following formula:

$$Volume\ (V) = (V1/m) \times 100\ (cm^3/100\ g)$$

The determination of elasticity consists of pressing a piece of core in a cylinder form with parallel sides and a thickness of 60 mm for 1 min and measuring the return to the initial shape after stopping the pressing. The elasticity of the core is calculated with the formula:

$$Elasticity\ (E) = (B/A) \times 100$$

(A = height of the core cylinder before pressing;
B = height of the core cylinder after pressing and its return to the initial position.)
The determination of porosity is the pore volume found in 100 g of the core. The method is based on determining the specific mass of the pore-free core. The porosity is expressed in terms of volume percentages and is calculated with the formula:

$$Porosity = [V - m/\rho)/V] \times 100\ [\%\ vol]$$

(v= the volume of the core cylinder, in $cm^3$;
m = the weight of the core cylinder, in g;
ρ = density of the compact core, in $g/cm^3$.)
The product height and diameter are measured, and their ratio is calculated with the formula:

$$Height/diameter\ ratio = H/D$$

(H = maximum height of the product,
D = arithmetic mean of two perpendicular diameters, in centimeters.)
The values obtained are the arithmetic means of the two parallel analyses.

### *2.4. Determination of sensorial properties of bread samples*

Seven bread samples (CB, KF5, KF10, PB5, PB10, KF5PB5, KF10PB10) obtained from whole wheat flour, konjac flour and psyllium bran, were evaluated by a panel consisting in 35 assessors (15 males and 20 females), between the ages of 21 and 48, non-smokers, without known cases of food allergies. The samples were presented on cardboard plates and served in random order under normal lighting conditions at room temperature. The plates were encoded in double-digit characters. Panelists were asked to evaluate the sensory attributes of the bread samples, including appearance/loaf shape, aroma, taste, texture, crust and overall acceptability using 9 point hedonic scale. Panelists compared and assign the samples scores between 1 and 9 (1 = disliked extremely, 5 = neither liked nor disliked, 9 = liked extremely) [3].

### *2.5. Statistical Data Analysis*

Every calculation was done in triplicate, and the results were reported as the average value ± standard deviation (SD). The t-test with equal variance was used to compare the statistical differences between the samples' results ($p \leq 0.05$). The data was processed using Microsoft Excel 365 (Version 2208, Redmond, WA, USA) as the statistical tool.

## RESULTS AND DISCUSSION

### *3.1. Nutritional characteristics of studied bread samples*

The nutritional characteristics results (Table 2) shows that all samples enriched with konjac flour (KF5, KF10), psyllium bran (PB5, PB10) or both (KF5PB5, KF10PB10) are richer than CB in terms of the mineral substances, protein, lipid and water concentrations. KF is characterized by a significant protein intake at 10% addition (10.51%) compared to CB (7.67%), mineral content at 10% addition (1.95% - KF10) and 5% - 10% addition in combination with PB 5% and 10%, as follows: 1.92% - KF5PB5, 2.14% - KF10PB10), which recommends KF as a nutritive matrix in bakery industry. The moisture content increased from 34.20±0.86% (CB) to 35.33±0.88% (KF10PB10) being noted that the moisture content increase with the addition of konjac flour and psyllium bran in different percentages. Moisture is necessary for the keeping quality of bread and high moisture has negative effect on storage stability of bread, according to Mudau, M., et al., 2021 [9]. However, the higher water content of psyllium breads could facilitate the development of mold, which should be taken into account when marketing this type of bread.

**Table 2.** Nutritional characteristics for bread samples made with composite flours (CB, KF5, KF10, PB5, PB10, KF5PB5, KF10PB10

| Samples | Nutritional Characteristics | | | | |
|---|---|---|---|---|---|
| | Moisture (%) | Mineral Content (%) | Proteins (%) | Lipids (%) | Carbohydrates (g/100 g) |
| | | | Sample breads | | |
| CB | 34.20±0.86$^{a}$ | 1.08±0.03$^{a}$ | 7.67±0.19$^{a}$ | 5.02±0.13$^{a}$ | 52.03±1.30$^{d}$ |
| KF5 | 34.6±0.87$^{a,b}$ | 1.29± 0.03$^{b}$ | 9.62±0.24$^{d,e}$ | 5.32±0.13$^{b}$ | 49.17±1.23$^{c}$ |
| KF10 | 35.21±0.88$^{c,d}$ | 1.95±0.05$^{c}$ | 10.51±0.26$^{f}$ | 5.41±0.14$^{b,c}$ | 46.92±1.17$^{a}$ |
| PB5 | 34.73±0.87$^{b,c}$ | 1.25± 0.03$^{b}$ | 8.02±0.20$^{b}$ | 6.10±0.15$^{d}$ | 49.90±1.25$^{d}$ |
| PB10 | 35.28±0.88$^{d}$ | 1.88±0.05$^{c}$ | 8.86±0.22$^{c}$ | 6.40±0.16$^{e}$ | 47.58±1.19$^{b}$ |
| KF5PB5 | 35.18±0.88$^{c,d}$ | 1.92±0.05$^{c}$ | 9.24±0.23$^{d}$ | 5.62±0.14$^{c}$ | 48.04±1.20$^{b}$ |
| KF10PB10 | 35.33±0.88$^{j,d}$ | 2.14±0.03$^{d}$ | 9.77±0.24$^{e}$ | 6.25±0.16$^{d,e}$ | 46.51±1.16$^{a}$ |

$^{a-j}$ different letters between samples for the same parameter signify statistical differences according to t-test

The value obtained for mineral substances is slightly higher than that obtained by Franco M, et al., 2022 and Laignier, F., et al., 2022 [7] (1.56% - 1.80%). The mineral content in the studied bread samples, increased with the addition of kojac and psyllium (1.08±0.03%, 1.25±0.03%; 1.29±0.05%, 1.88±0.05%, KF5PB5 1.92±0.05%, 1.95±0.05%, and 2.14±0.03%, respectively, for CB, PB5, KF5, PB10 KF10 and KF10PB10). These results show that the partial substitution (up to 20%) of wheat with psyllium bran+konjac flour would increase the mineral content, the results being in agreement with similar studies Franco, M, et al., 2022 and Laignier, F., et al., 2022 [4;7].

In terms of protein content were registered higher values in the case of KF5 (9.62±0.24%) and KF10 (10.51±0.26%) compared with all other samples, being known that konjac contains 12.7% protein and 16 different types of amino acids, according to Sudhanshu B.S., Ramesh C.R., 2016 [12].

The lipid content was in the range of 5.02±0.13% (CB) and 6.40±0.16% (PB10), the results being in agreement with those highlighted by Franco, M, et al., 2022 and Laignier, F., et al., 2022 [4;7], which showed a lipid content below 0.1% (psyllium bread) and 8.13% (konjac bread) at the same proportions as current study.
The total carbohydrate content was determined by difference, subtracting from 100 the values found for moisture, protein content, lipids, ash. Carbohydrate content range between 46.51±1.16% (KF10PB10) and 52.03±1.30% (CB), in agreement with the results pointed out by Laignier, F., et al., 2022 [7] for konjac bread (37.21%) and Franco, M, et al., 2022 [4] for psyllium bread (31.95 % - 36.94% ).
Bread samples of konjac flour and psyllium bran proved to be excellent sources of protein and minerals, the determination showing a low carbohydrate content, and these samples can be recommended for people with special dietary needs and for those interested of a healthy life style.

*3.2. Bakery parameters of bread samples*
The samples of bread with psyllium bran (5% and 10%), konjac flour (5% and 10%) and mixture of konjac flour and psyllium bran were subjected to determination in terms of volume, porosity, elasticity and height/diameter (H/D) ratio. The results obtained are presented in Table 3

**Table 3.** Bread quality indicators for psyllium and konjac bread (CB, KF5, KF10, PB5, PB10, KF5PB5, KF10PB10

| Indicator | M.U | CB | KF5 | KF10 | PB5 | PB10 | KF5PB5 | KF10PB10 |
|---|---|---|---|---|---|---|---|---|
| Volume | cm3/100 g | 412±13.18$^{a}$ | 425±13.70$^{b,c}$ | 431±13.79$^{c}$ | 428±13.60$^{b}$ | 434±13.89$^{c}$ | 426±13.63$^{b}$ | 433±13.89$^{c}$ |
| Porosity | % | 73.1±2.39$^{a}$ | 74.7±2.39$^{b}$ | 75.40±2.41$^{c}$ | 75.48±2.42$^{c}$ | 76.46±2.45$^{d}$ | 75.72±2.42$^{c}$ | 74.2±2.37$^{b}$ |
| Elasticity | % | 92±2.98$^{a}$ | 93±2.98$^{a,b}$ | 95± 3.04$^{c,d}$ | 94±3.01$^{b,c}$ | 95±3.04$^{c,d}$ | 96±3.07$^{d}$ | 93±2.98$^{a,b}$ |
| H/D | - | 0.432±0.02$^{a}$ | 0.460±0.02$^{c}$ | 0.518±0.02$^{d}$ | 0.483±0.01$^{b}$ | 0.520±0.02$^{d}$ | 0.476±0.02$^{b,c}$ | 0.522±0.02$^{d}$ |

$^{a-d}$ different letters between samples signify statistical differences according to *t-test*

Analysis of the data obtained in table 3 indicates that sample PB10 recorded the highest values in terms of volume (434±13.89cm3/100g), H/D ratio (0.519±0.02), porosity (76.46±2.45%) and elasticity (96±3.07%). Psyllium bran would thus have an influence on the bakery quality indicators of the bread obtained.
The konjac flour had a lower influence on the volume of the final product obtained, the values registered for KF5 and KF10 framing between 425 ±13.70 cm3/100g and 431 ±13.79 cm3/100g. Regarding the H/D ratio, samples with different proportions of komjac flour recorded 0.460±0.02 and 0.518±0.02 for KF5 and KF10 respectively. The samples with mixture of konjac flour and psyllium bran (5% and 10%), registered a H/D ratio of 0.476±0.02 (KF5PB5) and 0.522±0.02 for KF10PB10, respectively.
Romanian Standard 91/83 states that bread is deemed appropriate in volume and shape for values of the H/D ratio between 0.4 and 0.6; bread is domed for values above 0.6, and flattened for values below 0.40. We can therefore conclude that the bread samples studied has the proper volume and shape. The bread samples under study not only had the right shape and size, but they also had a porosity ranging from 73.1±2.39% (CB) to 76.46±2.45% (PB10). The elasticity of the studied bread samples range between 92±2.98% (CB) to 96±3.07% (KF5PB5).
*3.3. Sensory evaluation of studied bread samples*

Consumer's acceptability of the studies bread samples was carried out using sensory evaluation by a panel of 35 assessors (15 male and 20 female) using 9-point hedonic scale. Figures 2 indicate the mean scores for the sensory attributes (appearance/loaf shape, aroma, taste, texture, crust and overall acceptability). In terms of appearance the panellists ranked the bread samples in the following order: KF5>KF5PB5> PB5>CB>KF10>PB10>KF10PB10 (figure 2).

The most highly appreciated was the KF5PB5 sample, which obtained mean scores of 8.571 for taste, 8.629 for texture and crust and 8.686 for overall acceptability. Sensory analysis is very important in the product development process as a quality indicator because it measures the impact of products on consumers.

In terms of taste the samples registered good scores in the following order: KF5PB5> KF5> PB5> KF10>CB>PB10>KF10PB10 (figure 2).

Among all studied samples, KF10PB10 sample was less appreciated compared with other samples, in terms of all sensory attributes analysed (figure 2), the most appreciated was KF5PB5 sample which obtained the highest average in the consumers' evaluation. Similar results were obtained by Laignier, F., et al., 2022 [7], who reported a high acceptance of consumers for product with up to 12,5% konjac flour.

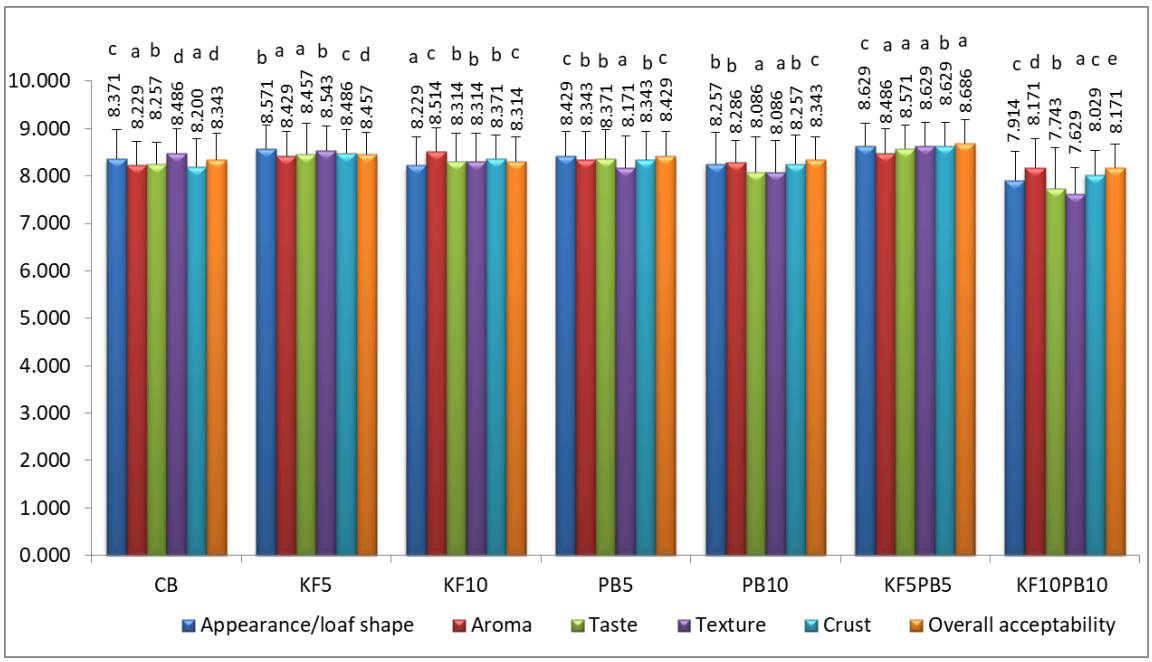



Figure 2. Mean scores (n=35) of sensory attributes (appearance/loaf shape, aroma, taste, texture, crust and overall acceptability) for bread samples (CB, KF5, KF10, PB5, PB10, KF5PB5, KF10PB10). Results are expressed as the mean ± standard deviation, indicated by error bars. *a-e letters indicates significant differences ($p < 0.05$) between samples assessed by *t*-test

## CONCLUSION

By substituting of whole wheat flour with psyllium bran and konjac flour, it is possible to obtain breads with a larger volume and a specific shape and porosity, similar to those of classic breads, but more nutritious. Increasing the amount of psyllium bran and konjac flour increases their fibre, protein and mineral content and reduces their calorie content. Incorporating up to 10% of psyllium bran and konjac flour can facilitate increased consumption and improve their nutritional characteristics.

Following the studies carried out in this paper, we can recommend the bread with konjac (5% and 10%) and the bread with a mixture of psyllium bran and konjac flour (5% each), due to the highest scores obtained in what concerns the taste and general acceptability, in the evaluation of the consumers, but also in terms of the nutritional and baking characteristics.

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# EVALUATION OF THE CHARACTERISTICS OF OLEOGELS BASED ON CARNAUBA WAX AND THE ROLE OF THEIR ADDITION IN THE WHEAT DOUGH

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## ABSTRACT

Different oleogels were obtained from olive oil (OL), grape seed oil (GO), sunflower oil (SO), walnut oil (WO) and hemp oil (HO) using carnauba wax as a gelling at different concentrations of 5%, 7%, and 9% (w/w). The physical characteristics of the oleogels obtained, such as oil binding capacity (OBC), peroxide index (IP) and color, were determined. From a structural point of view, the oleogels were further investigated by Fourier transform infrared spectroscopy (FT-IR). It was found that a higher concentration of oleogelator resulted in a denser structure of the oleogels, which provided a stronger oil retention capacity in the structure. The use of oleogels with carnauba wax in wheat flour dough was analyzed from a rheological point of view using a rheofermentometer device. The Rheofermentometer parameters namely the total $CO_2$ volume production (VT, mL), maximum height of gaseous production (H'm, mm), volume of the gas retained in the dough at the end of the test (VR, mL) and retention coefficient (CR, %) were determined. According to our data, all the oleogel additions contributed to the formation of stable dough. Generally, compared to the control sample (prepared only from wheat flour) the dough rheological properties during fermentation have been improved by oleogels addition in wheat flour.

**Keywords:** oleogel, rheological properties, dough fermentation, FT-IR analysis, physical-chemical characteristics

## INTRODUCTION

Shortening is used in bakery products such as cookies, cake/muffins, bread, cookie fillings, etc. The composition of its which has trans and saturated fatty acids increase blood cholesterol and the risk of atherosclerosis but also of other pathologies such as cardiovascular disease, obesity, cancer, diabetes [1]. In bakery products shortening has an important role to cover starch granules and gluten to prevent their adherence to each other. It is also responsible for dough plasticity, air entrapment during creaming, air bubbles stabilization during mixing, gluten lubrication. In addition, shortening offers

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softer textures with a finer porosity, delicate flavor, lubricating and moistness effect in the mouth [2]. Increasing health concerns about shortening uses in bakery products makes food producers to develop alternative fats with low content of trans and saturated fatty acids [3]. In the last years, oleogelation has gained attention and may be an alternative to unhealthy solid fats used nowadays in food products. Oleogelation means a liquid oil entrapment in an organogel network using oleogelator agents. This process modifies the liquid structure of oils into a solid one without production of trans-fatty acids [2]. Different vegetable oils may be used for oleogels production. For oleogelation process different oleogelators may be used such as plant waxes, phospholipids, fatty alcohols, sorbitan esters, mono- and diacylglycerols, phytosterols, ethylcellulose, e.g. [4]. From those, the use of plant waxes as oleogelators is of a high interest due to their gelling ability, abundance and low cost. Carnauba wax is obtained from the leaves of the Brazilian palm tree, *Copernicia cerifera C. Martius*. Is one of the hardest waxes and compared to other natural waxes it has one of the highest melting points (80–85$^0$C) which means that its present good oil-binding capacity for mineral oils and ester oils [2, 5]. The carnauba wax composition includes wax esters (38–40%, saturated), p-hydroxycinnamic aliphatic diesters (20–23%), hydroxy esters (12–14%), monohydric alcohols (10–12%) and p-methoxy cinnamic aliphatic diesters (5–7%) [6]. To obtain good quality oleogles for bakery products, the interaction between vegetable oil type and carnauba wax concentration levels are important factors. Different oil types may be used to obtain oleogels. Depend on its composition it may affect oleogelator gelling ability. For example, a higher amount of saturated fatty acid in oil composition may increase the oleogelator gelling ability [3]. For this purpose, several studies have been made on physical-chemical composition of oleogles with different types of oils [3,4, 5,7]. Our study wants to complete the previous studies made by using different types of oils such as olive, grape seed, sunflower, walnut and hemp seed oil using carnauba wax as oleogelator. To analyze the impact of carnauba wax concentration on oleogel properties different levels of it (5%, 7%, 9%) have been used for oleogel production. More, to evaluate the impact of oleogels obtained in bakery products process the dough rheological properties during fermentation obtained from a refined wheat flour and 5% oleogel addition have been evaluated using a rheofermentometer device.

## MATERIALS AND METHODS

### *Materials*

The wheat flour used in this were cultivated in 2022 in Romania and presented the fallowing characteristics: moisture content 10.9% (ICC 110/1) ash content 0.65% (ICC 104/1), protein content 11.5% (ICC 105/2), starch content 71.0% (AACC approved 76-13.01), wet gluten 22.7% (ICC 137/1), Zeleny value 35.3 mL (ICC 116/1). The vegetable oils used for oleogels production were cold pressed ones and were purchased from companies specializing in natural products. The following vegetable oils used in this study were the following: olive oil (OL), grape seed oil (GO), sunflower oil (S), walnut oil (WO) and hemp seed oil (HO). The gelling agent used was carnauba wax (CW) (Sigma-Aldrich, Hamburg, Germany).

### *Oleogels production*

For oleogels production, the vegetable oil and carnauba wax (at different addition levels of 5, 7 and 9%) were heated up to a maximum temperature of 80°C until the wax was completely dissolved. During heating, the suspension was agitated at a speed of 500 rpm using a multipoint magnetic stirrer (TA Instruments, New Castle, DE, USA). After

that, the mixture of oil and carnauba wax was maintained for tempering at room temperature after which they were transferred into glass tubes and kept at a temperature of $5^0$C for 5 days for stabilization.

***Physical-Chemical analysis***

*The colour characteristics*

The color characteristics namely the darkness/brightness (L*), shade of blue/yellow (b*), shade of red/green (a*) and absolute color difference ΔE were determined using the CIELab system (Konica-Minolta 200, Tokyo, Japan). The method used for analysis was previously described by Ropciuc et al. [3].

*Peroxide index evaluation*

The determination of the peroxide index was determined using the international standard method ISO 3960:2007 after the method previously described by Ropciuc et al. [3].

*The oil binding capacity*

The oil binding capacity was determined according to the method described by Ropciuc et al. [3]. For this purpose, 1.0 g of oleogel was centrifuged using a microcentrifuge (Hermle Z206A) for 15 minutes at 4000 rpm at 24°C.

***FT-IR analysis***

The Fourier-transform infrared spectroscopy FT-IR spectra on oleogels obtains was made using a Nicolet iS-20 spectrophotometer (Thermo Scientific, Karlsruhe, Dieselstraße, Germany) in attenuated total reflectance ATR mode, in the mid-infrared region of 4000–650 $cm^{-1}$, with a resolution of 4 $cm^{-1}$. The sample was placed on the ATR surface and their collection were made using OMNIC software (version 32, Thermo Scientific).

***Dough rheological properties during fermentation***

In order to evaluate oleogel behavior during wheat flour dough fermentation (the addition of 5% of each type of olegel was incorporated in wheat flour) a rheofermentometer device (Chopin Rheo, type F4, Villeneuve-La-Garenne Cedex, France) was used. The following parameters were determined according to AACC method 89-01.01: maximum level of gas production (H'm), total volume of CO2 production (VT), volume of gas retained in the dough at the end of the test (VR), and retention coefficient (CR).

***Statistical Analysis***

All data were expressed as mean ± standard deviation. The statistical significance of differences between samples was tested by using one way analysis of variance (ANOVA) with Tukey's test, to determine the significant differences at a 5% level. For this purpose, Statistical Package for Social Science statistical package (v.16, SPSS, Chicago, IL, USA) was used.

## REZULTS AND DISCUSSION

Physical-chemical composition of different types of oleogels based on carnauba wax is shown in Table 1. As may be seen, the type of oil had a significant influence on oleogels color parameters values. Oleogels with grape seed oil presented the highest degree of transparency in terms of lightness when compared to the other oleogel types. The L* for oleogel sample with GO and 9% of carnauba wax addition was the highest, namely 66.54. Generally, the increase level of carnauba wax addition for oleogel production increased a* value indicated that the natural yellowish color of carnauba wax had a significant impact on it. The yellowness (b*) of the oleogels samples varied

function of the oilseed used for oleogels preparation, probably due to the owing yellow color intensity of the oils which interfere with the yellowish color of carnauba wax used for oleogels preparation. A similar trend has also been noticed for total colour difference values. The peroxide index is one of the most important values for oleogel characteristic due to the fact that it is an indicator of primary lipid oxidation of the samples [8]. IP's were lower for the samples with the highest level of carnauba wax addition, which shows that oil oxidation decreased by the gelling of oils regardless of the amount of wax incorporated, this data being in agreement with those reported by others [9]. The lowest IP's values were obtained for the samples with sunflower and grape seed oil and 9% carnauba wax addition between which no significant difference ($p < 0.05$) was noticed. This decrease in IP may be due to the impact of the fatty acid composition from oil composition. It is possible that oils as GO and SO to contain a lower amount of unsaturated fatty acid compared to the other oils used which possess higher activation energy for forming fatty acid radicals, leading them less susceptible to oxidation. It is well known that GO and SO contain a low amount of ω3 alpha-linolenic acid (ALA) which have been reported that reduce stability against oxidation [10]. This data suggests that sunflower oil and in especially grape seed oil presents a superior ability to stabilize oil within the oleogel network in especially when high levels of carnauba wax were used. The oil binding capacity (OBC) is one of the most important properties of oleogels due to the fact that indicates the degree of liquid oil entrapment by the gelator, which was in our case carnauba wax. Because oleogels has a high amount of oil, they have to present high values of OBC to prevent oil leakage [7]. The OBC values of our samples varied between 68.21 and 99.82%. The lowest value was obtained for oleogel prepared with olive oil and 5% carnauba wax addition whereas the highest level was obtained for oleogel sample with walnut oil and 9% carnauba wax addition. As may be seen, for all oleogels samples the OBC values increased proportionally with the increase level of carnauba wax for their preparation. This indicates that wax concentration is directly related to OBC which are in agreement with data reported by others [9]. No significant differences ($p < 0.05$) were obtained for the oleogels samples prepared with the highest level of carnauba wax and GO, WO and HO addition. From the vegetable oils used for oleogels preparation the olive oil leaded to the lowest OBC values.

**Table 1.** Physical-chemical composition of different types of oleogels based on carnauba wax

| Sample | L* | a* | b* | ΔE | IP (mg/L) | OBC (%) |
|---|---|---|---|---|---|---|
| OL_5CW | $50.64 \pm 0.97^{cd}$ | $-5.68 \pm 0.20^{ce}$ | $34.59 \pm 0.95^{h}$ | $62.63 \pm 0.74^{i}$ | $1.31 \pm 0.05^{i}$ | $68.21 \pm 0.34^{a}$ |
| OL_7CW | $54.60 \pm 0.74^{fg}$ | $-6.24 \pm 0.29^{bc}$ | $31.43 \pm 0.30^{g}$ | $57.35 \pm 0.22^{fg}$ | $0.86 \pm 0.02^{f}$ | $83.49 \pm 1.20^{b}$ |
| OL_9CW | $59.34 \pm 0.47^{h}$ | $-6.49 \pm 0.24^{b}$ | $38.03 \pm 0.41^{i}$ | $58.05 \pm 0.17^{g}$ | $0.48 \pm 0.00^{e}$ | $97.55 \pm 0.15^{f}$ |
| GO_5CW | $53.06 \pm 0.25^{ef}$ | $-5.67 \pm 0.20^{ce}$ | $19.08 \pm 0.13^{a}$ | $54.48 \pm 0.27^{d}$ | $0.44 \pm 0.01^{bc}$ | $95.55 \pm 0.24^{e}$ |
| GO_7CW | $62.21 \pm 0.40^{i}$ | $-6.75 \pm 0.19^{ab}$ | $24.63 \pm 0.25^{c}$ | $51.24 \pm 0.13^{b}$ | $0.55 \pm 0.01^{d}$ | $97.70 \pm 0.20^{fg}$ |
| GO_9CW | $66.54 \pm 0.21^{j}$ | $-7.07 \pm 0.14^{a}$ | $28.31 \pm 0.59^{e}$ | $48.56 \pm 0.23a$ | $0.27 \pm 0.00^{a}$ | $99.66 \pm 0.08^{i}$ |
| WO_5CW | $51.63 \pm 0.96^{de}$ | $-2.63 \pm 0.87^{j}$ | $28.50 \pm 0.72^{e}$ | $59.97 \pm 0.14^{h}$ | $0.85 \pm 0.01^{f}$ | $95.09 \pm 0.22^{e}$ |
| WO_7CW | $49.45 \pm 0.82^{bc}$ | $-3.10 \pm 0.13^{j}$ | $26.99 \pm 0.22^{d}$ | $57.04 \pm 0.31^{e}$ | $0.78 \pm 0.01^{e}$ | $97.48 \pm 0.80^{f}$ |
| WO_9CW | $49.24 \pm 0.87^{abc}$ | $-3.99 \pm 0.21^{i}$ | $22.02 \pm 0.13^{b}$ | $58.21 \pm 0.14^{g}$ | $0.42 \pm 0.00^{b}$ | $99.82 \pm 0.14^{i}$ |
| HO_5CW | $47.73 \pm 0.16^{a}$ | $-5.04 \pm 0.17^{fg}$ | $28.28 \pm 0.14^{e}$ | $62.23 \pm 0.10^{i}$ | $0.94 \pm 0.01^{gh}$ | $90.65 \pm 0.22^{c}$ |
| HO_7CW | $48.03 \pm 0.29^{ab}$ | $-4.41 \pm 0.16^{hi}$ | $23.16 \pm 0.08^{b}$ | $60.05 \pm 0.18^{h}$ | $0.91 \pm 0.00^{g}$ | $92.70 \pm 0.59^{d}$ |
| HO_9CW | $52.91 \pm 0.20^{e}$ | $-6.41 \pm 0.16^{b}$ | $26.83 \pm 0.09^{d}$ | $57.44 \pm 0.16^{fg}$ | $0.98 \pm 0.00^{h}$ | $99.77 \pm 0.10^{i}$ |
| SO_5CW | $49.73 \pm 0.15^{c}$ | $-4.72 \pm 0.13^{gh}$ | $19.67 \pm 0.09^{a}$ | $57.01 \pm 0.13^{e}$ | $0.85 \pm 0.00^{f}$ | $90.02 \pm 0.11^{c}$ |
| SO_7CW | $55.77 \pm 0.10^{g}$ | $-5.62 \pm 0.14^{ef}$ | $26.83 \pm 0.09^{d}$ | $54.81 \pm 0.16^{d}$ | $0.60 \pm 0.00^{d}$ | $95.85 \pm 0.07^{e}$ |
| SO_9CW | $59.96 \pm 0.24^{h}$ | $-5.41 \pm 0.15^{ef}$ | $30.24 \pm 0.19^{f}$ | $53.16 \pm 0.19^{c}$ | $0.29 \pm 0.00^{a}$ | $98.99 \pm 0.14^{gi}$ |

OL – oleogel with olive oil, GO – oleogel with grape seed oil, WO – oleogel with walnut oil, HO – oleogel with hemp seed oil, SO – oleogel with sunflower oil, L* – lightness (L* = 0 is black and L* = 100 is white), a* (-a = green and +a = red), b* (-b = blue and

+b = yellow), ΔE - total color difference, IP-peroxide index, OBC-oil binding capacity; means in the same column with different letters for each oleogel type are significantly different ($p < 0.05$).

The FTIR spectra of different types of oleogels based on carnauba wax are shown in Figure 1. As may be seen, the FTIR spectra of oleogels have a similar trend. The oleogel samples presented specific peaks at different areas around 720, 1180, 1460, 1750, 2850, 2900, and 3000 $cm^{-1}$, respectively. The various peaks are high (1180, 2850, 2900 $cm^{-1}$) or medium (720, 1460, 3000 $cm^{-1}$), which represent the intramolecular and intermolecular hydrogen bonds, respectively. This data indicates the presence of C=O and C-H groups in the oleogels. The peak from 720 $cm^{-1}$ may be due to the cycloalkane bending of alkyl groups from oleogel compositions. The peak of 1180 $cm^{-1}$ corresponds to the C-O-O, C-O, and C-O-H groups from the oleogels structure [3]. The absorption peak due to C-H stretching appeared at 1460 $cm^{-1}$, which indicates the presence of C-H in $CH_3$ and $CH_2$ groups, respectively. The peak at 1750 $cm^{-1}$ represents the stretching vibration of C=O, which is an indication of the presence of ester bonds [11]. The peaks at 2850 and 2900 $cm^{-1}$ corresponded to the symmetric and asymmetric stretching vibration of the $CH_2$ group which may be characteristic to waxes being previously reported to different types of white beeswax oleogels [3]. The peak at 3000 $cm^{-1}$ is due to the C-H stretching vibration of the cis-double bonds (=CH) of oleogels [3,11]. Different studies have been reported that oleogels obtained with carnauba wax produces strong intermolecular hydrogen bonding between the wax and the oil [11,12]. Therefore, FTIR spectra did not shown any significant variations regarding peak position of oleogels samples even if they were obtained using different vegetable oils. This may be due to the fact that oleogels obtained with carnauba wax were formed based on molecular self-assembly or building blocks, which are stabilized by noncovalent interactions such as short-range Van der Waals attractive interactions (dispersion forces), hydrogen bonding and π–π stacking [11].

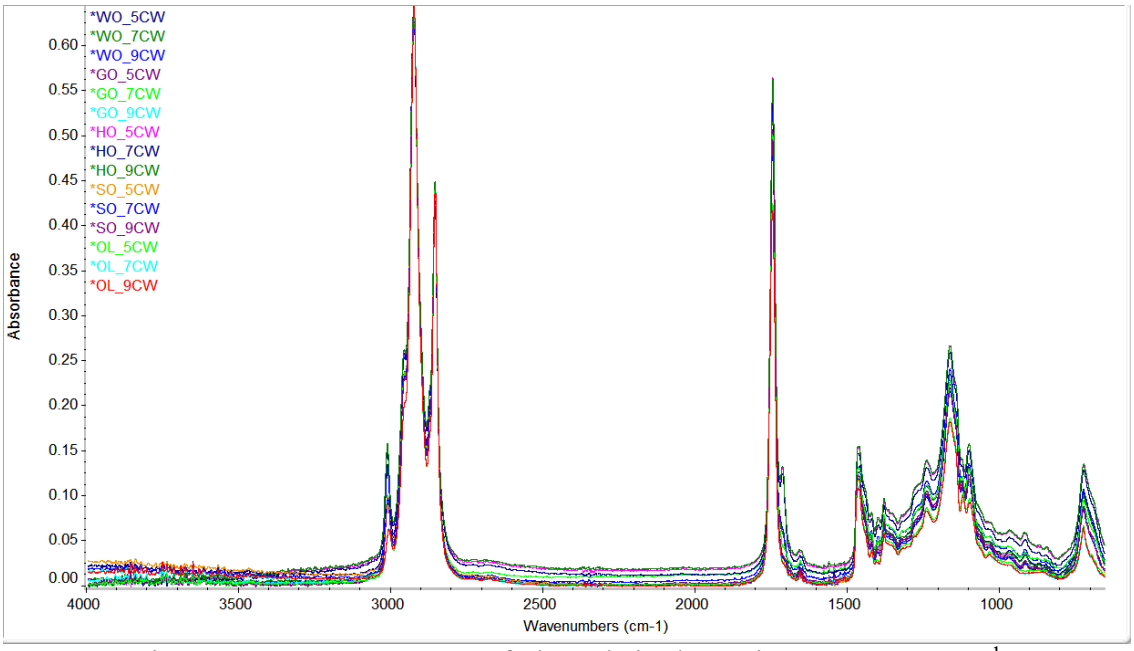



Fig. 1. ATR-FT-IR spectra of oleogels in the region 4000-650 $cm^{-1}$

The dough rheological properties obtained from white wheat flour and 5% addition of different oleogels types are shown in Table 2. Compared to the control sample (dough obtained only using wheat flour) all dough samples with different types of oleogels

incorporated in them presented significant ($p < 0.05$) higher levels of maximum height of gaseous production (H'm) except OL_9CW sample which did not present a significant difference ($p < 0.05$) compared to the WF sample. Regarding the total volume of $CO_2$ produced during fermentation (VT) and volume of the gas retained in the dough at the end of the test (VR) the highest values were obtained for the dough sample with oleogel prepared from olive oil and 5% carnauba wax addition whereas the lowest ones for dough samples with oleogel prepared from grape seed oil and 5% carnauba wax addition. It may be seen that VT and VR values increased for dough samples in which carnauba wax is in a high amount for oleogels obtained with grape seed oil, walnut oil and hemp seed oil and decreased when carnauba wax is in a low amount for oleogels obtained with grape seed oil and sunflower oil. During dough fermentation, bubble expansion increases the surface area and adsorption of lipid crystals. Crystalline lipids from oleogels with higher melting points that remain solid at the end of fermentation are most effective for gas retention. However, when too much lipids in a solid form are present in dough, its increase capacity during fermentation is inhibited [13]. More, addition of oleogels containing solid lipid in an adequate amount may strengthens dough and, therefore, improves gas retention. Compared to the control sample, the retention coefficient presented higher values for dough samples obtained with oleogels from grape seed oil, walnut oil and oleogels with high amount of carnauba wax from olive oil and sunflower oil. It is well known that in bread making fats improves gas retention and therefore increase it volume. According to Jung et al. [14] oleogel with solid-like properties are very effective in increasing loaf volume of bread which means that it may improve CR value and also H'm and VT. Taking into account that CR is the ratio between the VR and VT values, it may be concluded that in some cases where CR presented lower values compared to the control sample the dough's capacity to retain gases is lower than the quantity of gases formed during the dough fermentation process [15].

**Table 2.** Rheological properties during fermentation of the dough samples from wheat flour with 5% oleogel addition of different types based on carnauba wax

| Dough sample | H'm (mm) | VT (mL) | VR (mL) | CR (%) |
|---|---|---|---|---|
| WF | $52.73 \pm 0.57^{a}$ | $1171.33 \pm 2.51^{f}$ | $1044.33 \pm 4.50^{e}$ | $89.33 \pm 0.01^{de}$ |
| OL_5CW | $57.43 \pm 0.15^{d}$ | $1263.00 \pm 2.00^{i}$ | $1096.33 \pm 1.52^{g}$ | $87.33 \pm 0.01^{b}$ |
| OL_7CW | $54.80 \pm 0.10^{b}$ | $1203.66 \pm 3.51^{g}$ | $1046.33 \pm 4.04^{e}$ | $87.66 \pm 0.00^{b}$ |
| OL_9CW | $52.93 \pm 0.25^{a}$ | $1131.00 \pm 4.00^{e}$ | $1024.66 \pm 2.51^{d}$ | $91.00 \pm 0.01^{f}$ |
| GO_5CW | $55.06 \pm 0.25^{b}$ | $986.00 \pm 3.00^{a}$ | $941.33 \pm 3.51^{a}$ | $95.33 \pm 0.00^{hi}$ |
| GO_7CW | $57.33 \pm 0.15^{d}$ | $1023.66 \pm 3.51^{b}$ | $982.66 \pm 4.50^{b}$ | $96.66 \pm 0.01^{i}$ |
| GO_9CW | $59.43 \pm 0.25^{f}$ | $1274.66 \pm 3.51^{j}$ | $1030.33 \pm 2.51^{d}$ | $93.33 \pm 0.00^{a}$ |
| WO_5CW | $58.46 \pm 0.25^{e}$ | $1116.33 \pm 3.05^{d}$ | $1042.66 \pm 3.51^{e}$ | $93.33 \pm 0.00^{g}$ |
| WO_7CW | $57.23 \pm 0.15^{d}$ | $1124.00 \pm 3.00^{de}$ | $1027.66 \pm 2.08^{d}$ | $91.00 \pm 0.02^{f}$ |
| WO_9CW | $56.10 \pm 0.20^{c}$ | $1132.33 \pm 4.04^{e}$ | $1012.66 \pm 3.51^{c}$ | $89.33 \pm 0.01^{e}$ |
| HO_5CW | $63.00 \pm 0.20^{h}$ | $1239.00 \pm 3.00^{h}$ | $1090.00 \pm 2.00^{g}$ | $87.33 \pm 0.00^{c}$ |
| HO_7CW | $63.36 \pm 0.15^{h}$ | $1257.00 \pm 2.00^{i}$ | $1127.33 \pm 2.51^{h}$ | $89.00 \pm 0.01^{e}$ |
| HO_9CW | $63.06 \pm 0.15^{h}$ | $1239.00 \pm 2.00^{h}$ | $1093.66 \pm 3.51^{g}$ | $88.66 \pm 0.00^{cd}$ |
| SO_5CW | $60.86 \pm 0.21^{g}$ | $1211.66 \pm 3.51^{g}$ | $1079.33 \pm 2.51^{f}$ | $89.33 \pm 0.00^{de}$ |
| SO_7CW | $57.30 \pm 0.20^{d}$ | $1175.66 \pm 3.51^{f}$ | $1021.00 \pm 3.00^{cd}$ | $86.33 \pm 0.01^{b}$ |
| SO_9CW | $54.50 \pm 0.20^{b}$ | $1034.33 \pm 3.05^{c}$ | $978.33 \pm 4.04^{f}$ | $94.33 \pm 0.01^{h}$ |

Dough samples from: WF-wheat flour, WF with 5% of : OL – oleogel with olive oil, GO – oleogel with grape seed oil, WO – oleogel with walnut oil, HO – oleogel with hemp seed oil, SO – oleogel with sunflower oil, H'm: maximum height of gaseous production; VT: total volume of CO2 produced during fermentation; VR: volume of the gas retained in the dough at the end of the test; CR: retention coefficient; means in the same column with different letters for each oleogel type are significantly different ($p < 0.05$).

## CONCLUSION

Carnauba wax oleogels were successfully produced using different types of vegetable oils. This may be effective to reduce saturated fatty acids from food products and to increase the unsaturated ones (in especially essential ones) with more health benefits for human consumption. The color parameters darkness/brightness (L*), shade of blue/yellow (b*), shade of red/green (a*) and absolute color difference ΔE which are essential for their use in food products are significant influenced by carnauba wax amount and vegetable oil type used for their production. The grape seed oil used leaded to the highest L* values, whereas the increase level of carnauba wax for oleogels production to hire a* values. The oleogels obtained presented good oil binding capacity in especially when high levels of carnauba wax were used to produce them. The peroxide indexes are in concordance with the OBC data, meaning that a reduced release of oil from the oleogels corresponds to a lower probability of oxidation. However, their values are related to the oil composition from the oleogels structure. FTIR spectra indicated that oils conducted to the formation in oleogels of the intermolecular hydrogen bonds and crystalline polymorphs. The use of carnauba wax oleogels in dough recipe contributed to an improvement of dough rheological properties during fermentation which recommend their use in bread making. Our results provided the potential utilization of carnauba wax as a successful gelling agent for the preparation of oleogels and established a basis for selection of a vegetable oil phase based for their production in order to be used in bakery products.

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# EVALUATION OF THE FATTY ACID PROFILE OF SOME VEGETABLE OILS OF ROMANIAN ORIGIN

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## ABSTRACT

Waste from the food industry is of considerable value and is a valuable raw material, containing proteins, minerals, carbohydrates, lipids, etc. Large quantities of fruit kernels are currently wasted every year from processing plants all over the world, including Romania. This not only means the loss of a potentially valuable resource, but also creates serious problems regarding their storage and logistics in terms of environmental pollution. In order to value such by-products, the aim of this work was to study and evaluate 7 samples of vegetable oils obtained under laboratory conditions, as well as their saturated and unsaturated fatty acid profile. The oils were extracted from plant material of Romanian origin: apricot, plum, sour cherry kernels, apple seed and linseed, almond and walnuts. GC-MS gas chromatographic method was used to determine the fatty acid composition. The dominant fatty acids were the unsaturated ones, i.e. oleic acid (apricot kernel oil,) in the range 51.72- 61.74 % and linoleic acid (apple seed and walnut oil) in the range 57.38 -59.46 %, while linolenic acid (53.15%) predominated mainly in linseed oil. Saturated fatty acids (palmitic and stearic) were also identified in all 7 oil samples, but in lower amounts (palmitic in the range 6.71 - 17.97% and stearic acid in the range 0.40-9.15%).

With a high fat content and unsaturated fatty acid composition, these kernel and seed oils can be used in the food industry.

**Keywords:** vegetable oil, fatty acid, GC-MS, fruit kernels

## INTRODUCTION

Large quantities of fruit kernels are currently thrown away from processing plants every year, all over the world, and of course in Romania as well. This not only means turning a potentially valuable resource into a by-product, but also creates serious storage and preservation problems. Both the oil and the core from these fruit kernels can be used for

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economic valorization. Significant amounts of oil, edible protein and fibrous material can be provided by pits obtained from processing plums, cherries, apricots, but also from seeds obtained from apple processing.

Currently, there is no system for systematic collection and use of this potential raw material; thus, a valuable product with great industrial potential remains undervalued. Some kernels are difficult to collect due to the direct consumption of fresh fruit by consumers, but most of the fruit is used in food processing plants. For example, plum, cherry and apricot stones can be obtained as a by-product from many firms or food companies that process these fruits, are wasted polluting the environment or are available at very low cost prices.

Plums (*Prunus* spp.) are among the most commonly processed fruits, with global crop yield reaching 11.5 million tons in 2013 [1]. During processing into dry fruits, jams and jellies tons of fruit kernels (agro-industrial by-products) are created. As global plum production and the amount of agro-industrial by-products continue to increase [1], the selective use of fruit kernels will benefit the environment and the industry. Among natural biologically active compounds, lipids have a special practical importance. These belong to the compounds widespread in the plant kingdom. Some of them are essential components of the plant cell, which indicates their important physiological role [2]. Plums belong to the genus *Prunus* with several species, moreover great diversity of cultivars developed and cultivated in different countries can be found; all of which may significantly affect the oil yield and fatty acid composition in the plant material [1] .

Apricot *Prunus armeniaca* L., a plant of the family *Rosaceae*, well known for its delicious fruits, is widely distributed in most countries of the world. Fruit apricot stands out for its high nutritional value and is a valuable raw material for the manufacturing industry. The core is a good source of protein, essential amino acids, carbohydrates and lipids in the human diet. Apricot kernel oil is a significant source of saturated and unsaturated fatty acids, including palmitic, stearic, myristic, oleic, palmitoleic, linoleic and linolenic acid [3], [4]. It also contains phytosterols: campesterol, stigmasterol, and sitosterol, polyphenols, carotenoids, α-, $\gamma$ - and δ-tocopherol, other vitamins: vitamin C, thiamin, riboflavin and niacin and minerals: Na, K, Ca, P, Mg, Zn, Fe and Mn [3].

Plum and apricot kernels are a high-value by-product of the fruit manufacturing industry that can be extracted for oil. This oil, in addition to the lipid fraction, contains many different bioactive compounds such as β-carotene and tocopherols. β-carotene, known as provitamin A, plays an important role in wound healing, increases the body's resistance to toxins and prevents and fights cancer [5].

Sour cherry (*Prunus Cerasus* L.) is a popular fruit belonging to the family of *Rosacea*, subfamily *Prunoideae*. Sour cherry is widely used across North America, Europe, and Asia. This byproduct of sour cherry processing is a good source of phenolic and antioxidant compounds, fat, protein, and dietary fiber. One of the main components of the sour cherry kernel is oil (17–36%), which is a rich source of polyunsaturated and monounsaturated fatty acids [6].

The current fact is that the considerable interest is increasingly noticeable regarding the possibility of finding new sources of food that will meet the health requirements and nutritional needs of world population. Such resources are limited and more attention should be resources recovered from existing waste, such as apple seed oils [7]. The

seeds recovered from agro-food industries it is a source of nutrients and bioactive compounds. From this point of view, there has been an increasing interest lately in obtaining vegetable oils from seeds for their content in phytochemicals with potential use in pharmacological, cosmetic and food applications [8].

Nuts represent diverse plant families, although they have special common characteristics such as high oil content compared to other oilseed species. Almonds stand out in this category (*Prunus dulcis*, family *Rosaceae)*, constituting an appreciable source of nut oil [9]. The almonds have numerous nutritional benefits as well as industrial and medicinal importance as they contain good quality oil, protein, fiber, mineral and bioactive compounds [10]. The almond group is composed of two species, namely, *Prunus dulcis* (sweet almonds) and *Prunus amara* (bitter almond). Almond oil is extracted mainly from sweet almonds, which contain around 50% oil [9]. As for nuts, they are generally used as dried nuts, and also added to several food formulations for its nutty flavor. Walnut is a rich source of oil, minerals and tocopherols. It has high oil content that can vary from 59 to 72% depending on variety and location [11]. Walnut oil is a valuable product in terms of its wide use in food and health. Walnut oil is naturally rich in polyunsaturated fatty acids (PUFA), mainly linoleic and linolenic acids and is therefore low in monounsaturated fatty acids (MUFA), represented by oleic acid and saturated fatty acids [12].

## MATERIALS AND METODHS

*Plant material*

The plant material of Romanian origin, subject to extraction in the present study, namely the plum, apricot, cherries kernels, apple seeds and linseeds, the almonds and walnuts, were purchased from local producers.

To obtain oil samples by solvent extraction, ground fruit kernels and seeds were extracted with petroleum ether (Merck, 40-60°C) in a Soxhlet apparatus and the remaining solvent was removed by distillation.

*Fatty acids composition*

A 2 µL volume of each sample was injected in a HP6890 Series Gas Chromatograph coupled with a Hewlett Packard 5973 Mass Selective Detector. The gas chromatograph was equipped with a split-splitless injector and a Factor Four TM Capillary Column HP-5ms fused silica column of 5% phenyl-methyl polysiloxane, 30m*0.25 mm, film thickness 0.25 µm. The GC oven was set to a temperature range of 100 to $300^0$C with 60C/min, and a solvent delay of 7 min. The injector temperature was maintained at $230^0$C. The carrier gas of the sample was helium at 1.0 mL/min and the sample was injected in the split less mode. The MS conditions were the followings: ionization energy, 70 eV; electronic impact ion source temperature, $200^0$C; quadrupole temperature, $100^0$C; scan rate 1.6 scan/s; mass, 40-500 amu. For the identification of the compounds the mass spectra of the samples were compared with those of the NIST/EPA/NIH Mass Spectral Library 2.0 [13].

## RESULTS AND DISCUSSIONS

*Fatty acid composition*

The fatty acid composition of the seven type oils extracted from fruit kernels and seeds are presented in Table 1.

**Table 1**. Fatty acid composition of seven types kernel and seed oils (%)

| **Fatty Acids** | Carbon chain | AKO | PKO | SKO | ASO | LO | AO | WO |
|---|---|---|---|---|---|---|---|---|
| palmitic | C16:0 | 8.11± 0.10 | 11.38 ±0.04 | 17.97 ±0.02 | 9.88 ±0.07 | 6.71 ±0.02 | 9.35 ±0.07 | 10.41± 0.09 |
| stearic | C18:0 | 3.12 ±0.07 | 9.15± 0.09 | 0.53± 0.08 | 0.40± 0.09 | 4.35± 0.08 | 3.03± 0.08 | nd |
| oleic | C18:1 | 61.74± 0.06 | 51.72± 0.08 | 48.36± 0.09 | 31.63± 0.08 | 18.42± 0.14 | 57.40± 0.07 | 16.46± 0.06 |
| linoleic | C18:2 | 27.00± 0.04 | 27.62± 0.06 | 32.24± 0.04 | 57.38± 0.10 | 17.34± 0.06 | 30.20± 0.06 | 59.46± 0.02 |
| linolenic | C18:3 | nd | 0.10± 0.03 | 0.88± 0.07 | 0.68± 0.06 | 53.15± 0.08 | nd | 13.64± 0.04 |
| ΣSFA | | 11.23± 0.17 | 20.53± 0.13 | 18.50± 0.10 | 10.28± 0.16 | 11.06± 0.10 | 12.38± 0.15 | 10.41± 0.09 |
| ΣUFA | | 88.74± 0.10 | 79.44± 0.17 | 81.48± 0.20 | 89.69± 0.24 | 88.91± 0.28 | 87.60± 0.13 | 89.56± 0.12 |
| ΣMUFA | | 61.74± 0.06 | 51.72± 0.08 | 48.36± 0.09 | 31.63± 0.08 | 18.42± 0.14 | 57.40± 0.07 | 16.46± 0.06 |
| ΣPUFA | | 27.00± 0.04 | 27.72± 0.09 | 33.12± 0.11 | 58.06± 0.16 | 70.49± 0.14 | 30.20± 0.06 | 73.10± 0.06 |

The results obtained represent the average of triplicates, expressed as percentages with estimates of the standard deviation. AKO: apricot kernel oils, PKO: plum kernel oils, SKO: sour cherry oils, ASO: apple seed oils, LO: linseed oils, AO: almond oils, WO: walnut oils, nd: not detectable, SFA: saturated fatty acids. UFA: unsaturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids.

The relatively low content of saturated fatty acids (such as palmitic acid and stearic acid) with amounts between 10.28% and 20.53% is of nutritional interest. In this sense, walnut oil was highlighted where stearic acid was undetectable and apple seed oil with an insignificant amount (0.40%), respectively cherry seed oil (0.53%). Also, linseed oil stood out with the lowest amount of palmitic acid (6.71%). The high content of unsaturated fatty acids in the oils, such as oleic (16.46–61.74%) and linoleic acid (17.34–59.46%), may be a potential use in the food industry in the future.

The comparative study between the values of fatty acids obtained in this paper and their values from literature data is highlighted in table 2.

**Table 2.** Fatty acid composition (% mol) of the 7 types of kernel and seed oils described in the literature

| AKO | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 | References |
|---|---|---|---|---|---|---|
| | 3.17 | 1.40 | 70.70 | 22.41 | 0.90 | Stryjecka et al, 2019 [3] |
| | 4.74±0.27<br>4.91±0.12 | 1.28±0.04<br>1.24±0.03 | 65.71±1.64<br>62.31±1.24 | 28.16±1.66<br>28.61±0.94 | - | Makrygiannis et al., 2023 [4] |
| PKO | 5.20±0.7 | 1.5±0.4 | 60.8±7.8 | 31.4±7.4 | 0.1±0.1 | Górnaś et al., 2017, [1] |
| | 5.67-6.06 | 1.63-1.70 | 72,02-74,33 | 16.43-17.85 | - | Giligashvili et al., 2023, [2] |
| SKO | 5.06–7.38 | 7.43-15.76 | 25.25–45.30 | 35.50–46.06 | - | Górnaś et al., 2016, [14] |
| | 6.54± 0.12 | 2.03± 0.04 | 35.45 ± 0.24 | 42.34± 0.26 | 0.13± 0.02 | Kazempour-Samak et al., 2021, [6] |
| ASO | 8.07-9.18 | 1.75-2.30 | 27.02-36.57 | 50.34-60.01 | 0.25-0.40 | Bada et al., 2014, [7] |
| | 7.1-7.6 | 1.7-2.2 | 32.2-34.2 | 53.7-57 | 1.0-1.4 | Madrera et al., 2018, [8] |
| LO | 6.03-9.61 | 5.74-9.92 | 22.54-28.93 | 10.30-12.70 | 38.07-53.39 | Beema et al., 2023, [15] |
| AO | 4.7-15.8 | 0.3-2.5 | 50.4-81.2 | 6.21-37.1 | 0-11.1 | Fernandes et al., 2017, [9] |
| | 4.97-7.51 | 1.09-2.72 | 58.89-76.34 | 13.97-27.49 | 0-1.42 | Özcan et al., 2020, [10] |
| WO | 4.3-9.8 | 1.0-4.0 | 11.8-29.8 | 52.0-64.7 | 7.2-12.7 | Batun et al., 2017, [11] |
| | 4.01-5.41 | 2.41-3.10 | 18.40-33.56 | 51.39-63.12 | 6.52-11.69 | Yang et al., 2022, [12] |

## CONCLUSIONS

The purpose of this paper was to investigate fatty acid composition of 7 types of edible oils of Romanian origin, and to characterize fatty acid distribution pattern.

In conclusion, the plant material subjected to extraction in the present work contains a considerable amount of oil composed of many fatty acids, especially unsaturated fatty

acids. These data suggest that the apricot, plum, sour cherries kernels, apple seeds and linseeds, almond kernels and walnuts could constitute new sources of edible oils.

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# EVALUATION OF THE INFLUENCE OF GOJI FRUIT ADDITION ON THE NUTRITIONAL, BIOACTIVE AND SENSORY CHARACTERISTICS OF CHICKEN MEAT PATE

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## ABSTRACT

The main aim of the paper was to obtain a chicken meat pate in which different proportions of goji berries were added in order to increase its functionality and protect it against lipid oxidation. The biofunctional properties of goji berries were initially evaluated by determining total polyphenol content (TPC), total flavonoid content (TFC) and total antioxidant activity using the DPPH method. The values obtained allowed estimation of the amount of goji fruit that could successfully replace the synthetic antioxidant (sodium nitrite) commonly used in meat preparations. Three samples of chicken pate with different proportions of goji fruit and two samples of pate were obtained, one of which had sodium nitrite added and the other without any addition. The five pate samples were obtained following the same basic recipe and the same technological process. All five pate samples were analysed from a physical-chemical point of view by evaluating their proximate composition, i.e. moisture, ash, protein, fat, salt and carbohydrate content, and finally their nutritional value was calculated. The sensory analysis aimed to evaluate the taste, smell, appearance, consistency and general acceptability of the pate samples obtained. The values obtained for the five samples were compared to highlight the nutritional, oxidative stability and sensory benefits of goji berries.

**Keywords:** goji berries, proximate composition, chicken pate, oxidative stability, bioactive characteristics

## INTRODUCTION

Meat products deteriorate during processing and storage due to lipid oxidation. Lipid oxidation leads to progression of primary and secondary oxidation of products, deterioration of nutritional quality and changes in taste, which not only causes health hazards but can also lead to economic losses due to deterioration of product quality [1].

In recent years, due to the negative effects of synthetic antioxidants and the global trend to avoid or minimise the use of artificial (synthetic) food additives, the mechanism of action of antioxidants versus natural antioxidants has evolved considerably. Research on natural antioxidants has also increased in recent years, especially as these antioxidants

are found in all parts of plants, including grains, fruits, nuts, seeds, leaves, roots, pith and bark [2].

Recently, the meat industry has sought to replace synthetic antioxidants with antioxidants from natural sources because of the adverse health effects of certain synthetic antioxidants. Fruits, vegetables, by-products and other plant materials are excellent substitutes. The use of natural sources can cause colour changes in meat or meat products, and spices have been shown to affect the flavour profile of processed meat and poultry products [3].

The objectives set in this work were: to establish recipes for making chicken pate; to obtain chicken pate with different proportions of goji: chicken pate without added (negative control) (PCN), pate with added sodium nitrite (positive control 100 mg/kg) (PCP). chicken pate with 2.5 g/kg goji (P2.5), chicken pate with 5 g/kg goji (P5), chicken pate with 10 g/kg goji (P10); organoleptic, physico-chemical and sensory characterisation of the pâte obtained.

In order to highlight the nutritional benefits of goji berries, the two products were analyzed from a sensory and physical-chemical point of view in the physical-chemical analysis laboratory of the Interdisciplinary Research Platform, U.S.V. "Regele Mihai I" in Timisoara. From a functional point of view, the total polyphenol content, the total flavonoid content and the total antioxidant activity of goji berries were evaluated by DPPH method. Physico-chemically, water, ash, protein, fat, NaCl and freshness contents were monitored. From a sensory point of view, taste, smell, appearance, consistency and general acceptability were followed.

## MATERIALS AND METHODS

### Materials

Fresh raw materials were purchased from a supermarket (Timisoara, Timis County, Romania). Spices such as ground black pepper, bay leaf, nutmeg (Fuchs Condimente RO SRL, Romania) and salt (Salrom, Romania) were purchased from a local supermarket. The reagents used for the physical-chemical analyses were purchased from Sigma-Aldrich (St Louis, MO, USA) and Chimreactiv (Bucharest, Romania) and were of analytical purity.

### Phytochemical Profile of Goji Berry

*Preparation of Extracts*

From the dried goji sample, 1 g was weighed into a container with a lid, to which 10 ml of 70% (v/v) ethanol (Chimreactiv, Bucharest, Romania) was added. The mixture formed was shaken for 30 minutes using a magnetic stirrer (IDL, Freising, Germany), after which the extract obtained was filtered through Whatman N°1 filter paper. This extract was used for the determination of total polyphenol content (TPC), flavonoids (TFC) and total antioxidant activity (AA).

*Determination of Total Phenolic Content (TPC)*

The total polyphenol content (TPC) of goji berries was determined according to the Folin-Ciocalteu method described by Cocan et al. [4]. Values were expressed as mg gallic acid equivalent (GAE)/100 g dry matter (d.w.). Results were carried out in triplicate and the value was expressed as mean value ± standard deviation (SD).

*Determination of Total Flavonoid Content (TFC)*

The determination of total flavonoid content was carried out according to the method described by Hulea et al [5]. The sample was analysed in triplicate and the result expressed as mean value of mg QUE/100g + standard deviation (SD) in mg QUE/100 g.

*Determination of Antioxidant Activity by 1,1-diphenyl-2- picrylhydrazyl (DPPH)*

The determination of antioxidant activity by 1,1-diphenyl-2- picrylhydrazyl (DPPH) was carried ouă according to the method described by Ciulca et al. *[6].*

## Manufacture of Pate Formulas

The pate samples were prepared in the butchery laboratory of the University of Life Sciences "King Mihai I" from Timișoara, Romania. Five different pate sample, i.e. with 2.5% goji berry (P2.5), 5% goji berry (P5), 10% goji berry (P10), meat-only pate (PCN) and one sample of pate cu addition of sodium nitrite (PCN), were obtained according to the recipe shown in table 1.

Table 1. The recipes used to obtain pate formulas

| Sample | Chicken liver (g) | Chicken meat (g) | Chicken fat (g) | Salt (g) | Black pepper (g) | Onion flakes (g) | Nutmeg (g) | Sugar (g) | Goji berries (g) | Sodium nitrite (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| PCN | 430 | 500 | 70 | 12 | 1.4 | 50 | 1 | 2.4 | - | - |
| PCP | 430 | 500 | 70 | 12 | 1.4 | 50 | 1 | 2.4 | - | 0.1 |
| P2.5 | 430 | 500 | 70 | 12 | 1.4 | 50 | 1 | 2.4 | 2.5 | - |
| P5 | 430 | 500 | 70 | 12 | 1.4 | 50 | 1 | 2.4 | 5 | - |
| P10 | 430 | 500 | 70 | 12 | 1.4 | 50 | 1 | 2.4 | 10 | - |

The liver was washed, cleaned, and sliced and kept in cold water for about 2 hours. The meat was cut into pieces of approx. 50 g and cooked approx. 15 minutes. The fat was used raw and as fresh as possible. All components were minced on a volf (Maxima Holland MMM 12-229, Mijdrecht, The Netherlands) through a 3 mm sieve. They were then processed in a cuter (La felsinea 10M, Piazzola sul Brenta PD, Italy) adding salt, sugar, ground spices and the juice from the meat oparification (10% of the quantity of the composition), resulting in an aliphatic paste. The resulting paste was placed in glass jars which were hermetically sealed. The jars of pate were sterilised at 72°C for 30-40 minutes and then cooled in running water, with ice added in summer. The cooled product was labelled and stored in a refrigerator at +4°C until analysis.

## Proximate Composition of Pate Samples

In order to evaluate the proximate analysis of the pate samples, the content of water (according to SR ISO 1442:2010), ash , total protein (according to SR ISO 936:2009), total fat (according to SR ISO 1443:2008) and salt (according to SR ISO 91-2007) was determined. The carbohydrate content was calculated according to equation 1.

Carbohydrate (%) =100 - (lipid (%) + protein (%) + ash (%) + moisture (%)) (1)

Finally, the energy value of the pate formulas was calculated using equation 2.

Energy value (kcal/100 g)=[fat (%)×9]+[protein (%)×4]+[carbohydrate(%)×4] (2)

### Oxidative Stability Assessment

*Determination of Peroxide Value (PV)*

The iodometric method was used to determine the PV of sausage formulas, expressed as meq $O_2$/kg fat [4].

*Determination of Thiobarbituric Acid (TBA)*

The TBA test was carried out according to the method described by Cadariu et al [7]. Three determinations were made from each sample, their mean value ± standard deviation (SD) was reported and the results were expressed in μM MDA/g sample.

### Sensory analysis

The pate samples were evaluated by 28 trained evaluators (14 males and 14 females, aged between 18 and 23 years) at the Faculty of Food Engineering (Timisoara, Romania). During the sensory analysis, natural light (daylight) and appropriate temperature (room temperature) were provided. Pate samples were prepared on slices of bread 5 minutes before evaluation. The characteristics analysed by each assessor were: colour, appearance, smell, taste, texture and general acceptability. Thus, a nine-point hedonic scale was used for each characteristic as follows: very much like (5); moderately like (4); neither like nor dislike (3); slightly dislike (2); moderately dislike (1); extremely dislike (0).

### Statistical Data Analysis

Results were expressed as the mean of three determinations ± standard deviation (SD). Statistical differences ($p < 0.05$) between samples were performed by t-test using Microsoft Excel 365 (Version 2208, Redmond, WA, USA).

## RESULT AND DISCUSSION

### Phytochemical Profile of Goji Berry

The total polyphenol content (TPC), flavonoid content (TFC) and total antioxidant activity (DPPH) of the goji berries used to make the samples are shown in Table 2.

Tabelul 2. Total phenolic, total flavonoids and antioxidant activity of goji berry

| Sample | TPC mg GAE/g dw) | TFC (mg QUE/g dw) | DPPH (μM TE/g dw) |
|---|---|---|---|
| **Goji fruits** | 21.44 | 4.28 | 77.83 |

For the evaluation of the phytochemical profile a content of 21.44 mg GAE/g dw, 4.28 mg QUE/g dw and 77.83 μM TE/g dw were recorded. The recorded values are in agreement with the values reported in the literature as follows, Mocan et al [8] reported for goji berries studied at different stages of maturity a TPC content ranging from 3.31 - 23.49 mg GAE/g dw and 2.58 - 11.03 (mg QUE/g dw). Vulić et al. [9] reported a TPC content for the analysed goji berries between 255.87 - 281.91 mg GAE/100 g f.w. Zhang et al. [10] reported for different varieties of goji berries a TPC content between 26.90 - 73.40 mg GAE/g. TFC content between 36.10-54.70 mg QUE/g and DPPH content between 35.88 - 85.46 μM TE/g dw.

**Proximate analysis of the pate samples**

The proximate composition provides valuable information on the characteristics of the raw materials used and allows calculation of the nutritional value of the finished products [11]. Table 3 shows the proximate composition of the pate samples.

The water content values recorded for the pate samples ranged from 58.79-60.96% (table 3). From the analysis of the obtained values, it can be seen that as the goji content increases, the water content in the pate samples also increases.

**Table 3.** Proximate composition of the pate samples

| Sample | Chemical Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Moisture (g/100 g)** | **Protein (g/100 g)** | **Lipids (g/100 g)** | **Ash (g/100 g)** | **Chloride content (g/100 g)** | **Carbohy-drates (g/100 g)** | **Energy Value (kcal/100 g)** |
| PCN | 58.81±1.54 $^{a}$ | 15.03±0.39 $^{a}$ | 22.24±0.61 $^{a}$ | 0.65±0.02 $^{a}$ | 1.56±0.05 $^{a}$ | 1.71 | 267.12 |
| PCP | 58.79±1.57 $^{a}$ | 15.18±0.40 $^{a}$ | 21.76±0.60 $^{a}$ | 0.64±0.02 $^{a}$ | 1.57±0.05$^{, a}$ | 2.06 | 264.80 |
| P2.5 | 59.58±1.72 $^{a,b}$ | 17.47±0.48 $^{b,c}$ | 18.46±0.55 $^{b}$ | 0.75±0.04 $^{b}$ | 1.59±0.06 $^{a}$ | 2.15 | 244.62 |
| P5 | 60.18±1.74 $^{b,c}$ | 18.28±0.52 $^{c,d}$ | 16.84±0.49 $^{c}$ | 0.84±0.05 $^{b}$ | 1.58±0.05 $^{a}$ | 2.28 | 233.80 |
| P10 | 60.96±1.81 $^{c}$ | 19.75±0.55 $^{d}$ | 14.36±0.47 $^{d}$ | 0.99±0.05 $^{c}$ | 1.59±0.06 $^{a}$ | 2.35 | 217.64 |

$^{a-d}$ different letters between samples signify statistical differences acording to t-test

In the pate samples studied, a protein content of 15.03-19.75% was recorded (table 3). It can also be observed that the protein percentage increases with the increase of the proportion of goji fruit in the samples analysed, which can be considered that this product may have particular nutritional benefits.

The fat content of the pate samples analysed is shown in table 3. Thus, following extraction using the Soxhlett extractor the values recorded for the pateu samples analysed ranged from 14.36 - 22.24 %, with the highest fat content recorded for the PCN sample and the lowest value recorded for P10. From the analysis of the experimental data it can be seen that the fat percentage decreases with increasing goji content, this value being below the maximum allowed limit of 30%.

Table 3 shows the mineral content of the samples analysed. From the analysis of the experimental data, it can be seen that the range of values recorded for the pateu samples was between 0.64 - 0.99%, with the mineral content increasing with increasing goji content.

The NaCl content (%) of the samples analysed ranged from 1.56-1.59% (table 3), the maximum limit of 2% set by Order 210/2006 on the conditions of acceptability of cooked meat products was not exceeded.

Thus, the carbohydrate content of the studied pate samples was in the range of 1.71 - 2.35% (Table 3), the lowest value being recorded for PCP (1.71%) and the highest value for chicken pate with added 10% P10 goji (2.35%).

The calculated energy value (Conf. EC Regulation 1169/2011) for the pate samples studied was in the range 217.64-267.12 Kcal/100 g (Table 3). From the analysis of the obtained data it can be concluded that as the percentage of goji in the product increases there is a decrease in the energy value. Thus, the highest energy value was recorded in the chicken pate sample without added goji (PCP) and the lowest value in the pate sample with 10% added goji (P10).

The values recorded are below the maximum allowable limit of 74%, which is in line with literature data showing a water content in patties between 63.92 - 70.73% [12]. In another study Nielsen et al. [13] reported a water content for pate samples between 65.5 - 66.2%, 1.91 - 1.93 % ash, 17.6 - 18.5% fat and 13.2 - 13.4 % protein. Similar proximate composition values were recorded by Munekata et al. [14] in their study in which they introduced beer residue extract, chestnut leave extract, and peanut skin extract into the recipe as follows: 50.99 - 51.38 g/100g moisture, 51.64 - 57.78 g/100 d.w. fat and 29.25 30.04 g/100g d.w. protein. Also in this case there was an increase in moisture and protein content and a decrease in fat content, similar to the results recorded in our study. Smarzyński et al. [15] reported for pate formulas that were supplemented with cricket powder a content of 43.54 - 44.96 % water, 15.97 - 21.01% protein, 27.46 - 31.11% fat, 1.8 -2.17% ash, 1.59 - 11.13% carbohydrates and 355.54 - 370.39 kcal/100g energy value.

**Oxidative Stability Assessment**

*Determination of Peroxide Value (PV) and Thiobarbituric Acid (TBA)*

The peroxide value (PV) provides information on the initial stage of fat oxidation and must be below 10 mEq $O_2$/kg for the product to be below the rancidity threshold.

Figure 1 shows the effect of supplementation of goji berry pate formulas on the peroxide value compared to the control sample (PCN) and the sodium nitrite-added pate sample (PCP).

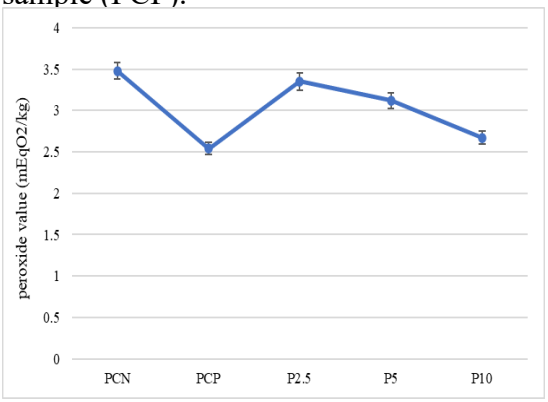



Figure 1. Effect of supplementation of goji fruit pate formulas on peroxide value (PV)

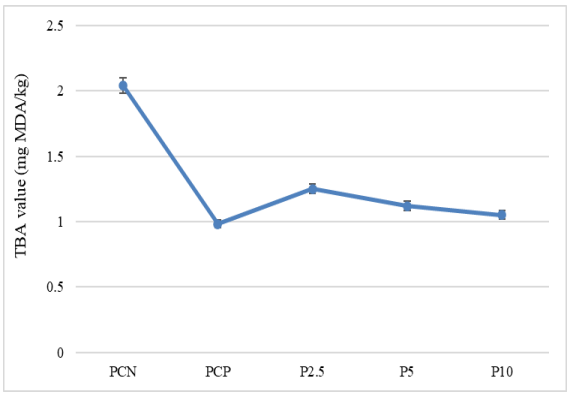



Figure 2. Effect of supplementing goji fruit pate formulas on TBA value

PV was significantly higher in the control sample (PCN) (3.48) compared to the added sodium nitrite (PCP) samples, respectively compared to the goji berry added pate samples (P2.5 - 3.35 mEq $O_2$/kg, P5 3.12 mEq$O_2$/kg and P10 - 2.67 mEq$O_2$/kg). Thus as the proportion of goji fruit increased the PV value decreased, in the case of P10 the value recorded (2.67 mEq $O_2$/kg) being comparable to the value recorded for PCP 2.54 mEq $O_2$/kg). This can be attributed to the high content of antioxidant compounds in goji berries. Similar results have been reported in the literature, i.e. Santos et al. [16] reported PV values ranging from 0.20 - 3.30 mEq $O_2$/kg. A similar effect due to the addition of products rich in bioactive compounds was also reported in the study published by Cedeño-Pinos et al. [17] in which they added different proportions of rosemary in the composition of the pate, with PV decreasing as the proportion of rosemary increased, values ranging between 3.82 - 5.37 meq $O_2$/kg.

Figure 2 shows the TBA values after the addition of goji berry pate formulas compared to the control sample and the pate sample with added sodium nitrite. Significantly higher MDA values were recorded for the control sample (PCN - 2.04 mg MDA/kg) compared to the sample with added sodium nitrite (PCP - 0.98 mg MDA/kg) and the samples with added goji berries (P2.5 1.25 mg MDA/kg, P5 - 1.12 mg MDA/kg and P10 - 1.05 mg MDA/kg). In the case of the samples with added goji berries it can be observed that the MDA values decrease with increasing proportion of added goji berries. In the case of P10 the recorded value (1.05 mg MDA/kg) is close and comparable to the TBA value recorded for the PCP sample (0.98 mg MDA/kg), which means that 10% goji fruit can successfully replace the synthetic antioxidant.
Santos et al. [16] reported TBA values for chicken pate ranging from 0.4-0.95 mg MDA/kg. A similar effect due to the addition of products rich in bioactive compounds was also reported in the study published by Cedeño-Pinos et al. [17] in which they added different proportions of rosemary to the pate composition, the PV decreased as the proportion of rosemary increased, with values ranging from 0.79-0.86 mg MDA/kg.

**Evaluation of Sensory Analysis**

The sensory analysis looked at the influence of the addition of goji in chicken pate on sensory characteristics. The results of the sensory analyses of the five samples are shown in figure 3. The total values recorded for the pate samples (figure 3) were within the range 3 - 5. For all five characteristics assessed (appearance, colour, texture, taste, flavour and gen eral acceptability) the highest scores were recorded for the P10 sample, followed by P5, P2.5 and the lowest scores were recorded for PCN and PCP.
In conclusion from a sensory point of view goji fruit in moderate proportions improves the characteristics of the product in which it is added.

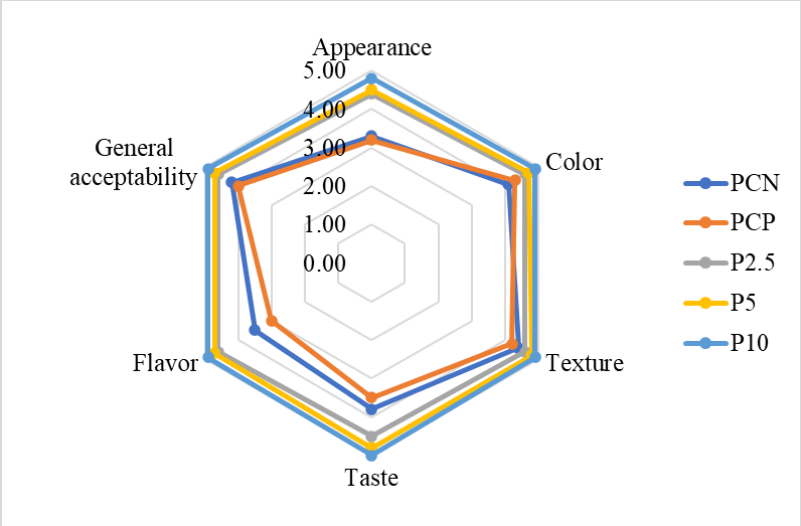



Figure 3. Sensory analysis of the analyzed sausage samples

## CONCLUSION

This study aimed to investigate the use of goji berries as a replacement for sodium nitrite in new pate formulations. Initially the biofunctional properties (TPC, TFC and AA) of goji berries were evaluated and based on the results obtained the proportions of goji berries that can replace sodium nitrite and provide oxidative stability to the products were determined. Proximate analysis concluded that the use of goji berries

leads to improved nutritional characteristics and oxidative stability. According to the results of the sensory analysis, the overall acceptability of goji fruit pate formulations was similar to that of pate made exclusively from meat. The sensory evaluation showed that the addition of goji fruit in the pate formulas was well accepted in terms of external appearance, cross-sectional appearance, consistency, taste and flavour. In conclusion, goji berries can be considered a good ingredient to replace sodium nitrite in pate formulas. The conclusion of this study suggests that replacing sodium nitrite with goji berries in pate formulas is promising.

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# EVALUATION OF THE OILS STABILITY OBTAINED BY CULTIVATING SOME SUNFLOWER HYBRIDS HIGH OLEIC HIGH STEARIC ( HOHS) IN THE WESTERN REGION OF ROMANIA

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## ABSTRACT

The oil obtained from linoleic sunflower hybrids grown mainly by Romanian farmers shows a low oxidation stability. In the period 2021-2023, high oleic and high oleic high stearic sunflower hybrids were cultivated experimentally in the western part of Romania. A good climatic and pedological adaptability was observed both in the intensive conventional culture and in the biologic one. The fatty acid profile showed the same characteristics. The oil obtained from linoleic sunflower hybrids grown mainly by Romanian farmers shows a low oxidation stability. Oils were extracted from the obtained products by different methods: cold pressing, hot extraction, microwave extraction. The fatty acid profile of the oils was determined by gas chromatography. The oxidation stability testing of the oils was done with the Oxitest aparatus, under conditions of accelerated oxidation at different temperatures, from the values of the induction periods until the appearance of the oxidation products, determining the shelf life of the oils.

**Keywords:** sunflower hybrids, oxidative oil stability, shelf life

## INTRODUCTION

The use of oils with high oxidation stability in agro-food processing ensures a longer life for food and thus food waste can be reduced. Sunflower oil obtained from linoleic hybrids contains a high proportion of polyunsaturated fatty acids, beneficial for the health, but with low oxidation stability.

Sunflower hybrids with a high content of oleic acid and stearic acid have a reduced proportion of polyunsaturated fatty acids, which determines a high oxidation stability[4, 5]. The study focused on the analysis of the oil obtained from Alta Sun 452 (HO)- and Nutrisun (HOHS)- hybrids cultivated experimentally in the western region of Romania 46°6′47″N 20°47′5″E in conventional and biological cultures.

## MATERIALS AND METHODS

The profile of fatty acids in the oil obtained by different methods: cold pressing, Soxhlet extraction and microwave extraction was analyzed by gas chromatography, performed with Shimadzu QP 2010 Plus apparatus (Columbia, SC, USA), with RT2560 column (100 m × 0.25

mm × 0.20 μm), from the Interdisciplinary Research Platform of University of Life Sciences "King Michael I" from Timisoara.

The oil samples were derivatized with methanolic solution of potassium hydroxide 2 M. Briefly, 0.01mL of oil sample 0.4 mL of methanolic solution of potassium hydroxide 2 M and 4 ml hexan were added and vortexed 5 minute. After 10 minute was added 0.5 g of potassium acid sulfate, and were centrifuged at 3000 rpm for 15 min. The FAMEs from the hexane fraction (1 μL) were used for GC-MS analysis. The flow rate of the carrier gas (Helium) was 1 $mL \cdot min^{-1}$, and the splitting ratio was 1:50. The column temperature (100°C) was maintained for 5 min, and then a gradient at 3°C/min until 250°C was maintained for 10 min. The injection port temperature was 250°C, and the temperature of the ion source and the GC-MS interface was 210°C and 255°C. Fatty acids were identified based on the NIST 05 spectrum library. The fatty acid composition was expressed using the peak area normalization method by relating the peak area corresponding to a given compound to the total area of all peaks. All the analyses were conducted in three replicates.

The evaluation of the oxidation stability of the oils was carried out by the accelerated oxidation method at 6 barr pressure of $O_2$ and different temperatures 90, 85, 80ºC, in triplicates, determining the induction period IP at which the oxidation products appear. 10 g of oil samples were use to determine de induction period with the OXITEST apparatus from Velp, Italy. Based on the IP, the shelf life of the oils was estimated.

## TABLES AND GRAPHS

Table 1 Profile of fatty acids in seed and oil from Altasun 452(HO) and Nutrisun (HOHS) in conventional and biological culture

| FAMEs | HO CV Seed % | HO BIO Seed % | HOHS BIO Seed % | HOHS CV seed % | HO CV oil % | HO BIO oil % | HOHS BIO oil % | HOHS CV oil % |
|---|---|---|---|---|---|---|---|---|
| C16:0 | 4.67 | 4.66 | 5.66 | 5.69 | 3.99 | 3.99 | 5.14 | 5.14 |
| C18:0 | 2.60 | 2.60 | 11.19 | 11.23 | 2.44 | 2.44 | 14.29 | 14.40 |
| C18:1 | 90.53 | 90.42 | 80.41 | 80.54 | 89.73 | 89.86 | 76.61 | 76.66 |
| C18:2, 9,12 | 1.47 | 1.47 | 1.53 | 1.42 | 2.96 | 2.83 | 2.77 | 2.77 |
| C20:0 | 0.73 | 0.85 | 1.21 | 1.12 | 0.88 | 0.88 | 1.19 | 1.03 |
| SFA | 8.00 | 8.11 | 18.06 | 18.04 | 7.31 | 7.31 | 20.62 | 20.57 |
| MUFA | 90.53 | 90.42 | 80.41 | 80.54 | 89.73 | 89.86 | 76.61 | 76.66 |
| PUFA | 1.47 | 1.47 | 1.53 | 1.42 | 2.96 | 2.83 | 2.77 | 2.77 |

SFA – saturated fatty acids

MUFA – monounsaturated fatty acids

PUFA – polyunsaturated fatty acids

The oleic acid content in the analyzed samples was between 76.61-80.54 for the Nutrisun hybrid, respectively 89.73-90.53 in the case of the Alta Sun ultra high oleic hybrid. The ratio SFA/MUFA was 0.08 for HO and 0.22-0.27 for HOHS. PUFA has a low level, but for oil stability has a positive impact.

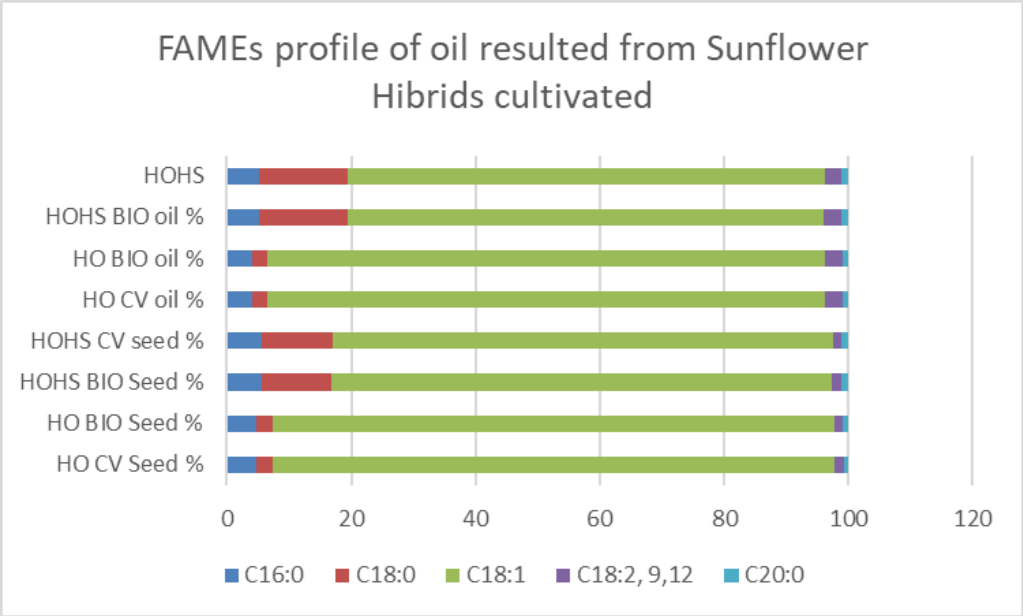



Figure 1 FAMEs content in oils obtain from Nutrisun and Altasun 452 hybrids

The harvest obtained shows the same profile of fatty acids as the seeded hybrids, with an oleic acid content of over 89% in the case of the Alta Sun hybrid, respectively of 76% oleic acid and 11% stearic acid in the case of the Nutrisun hybrid. There were no differences between the biological and the conventional cultures from a qualitative point of view.

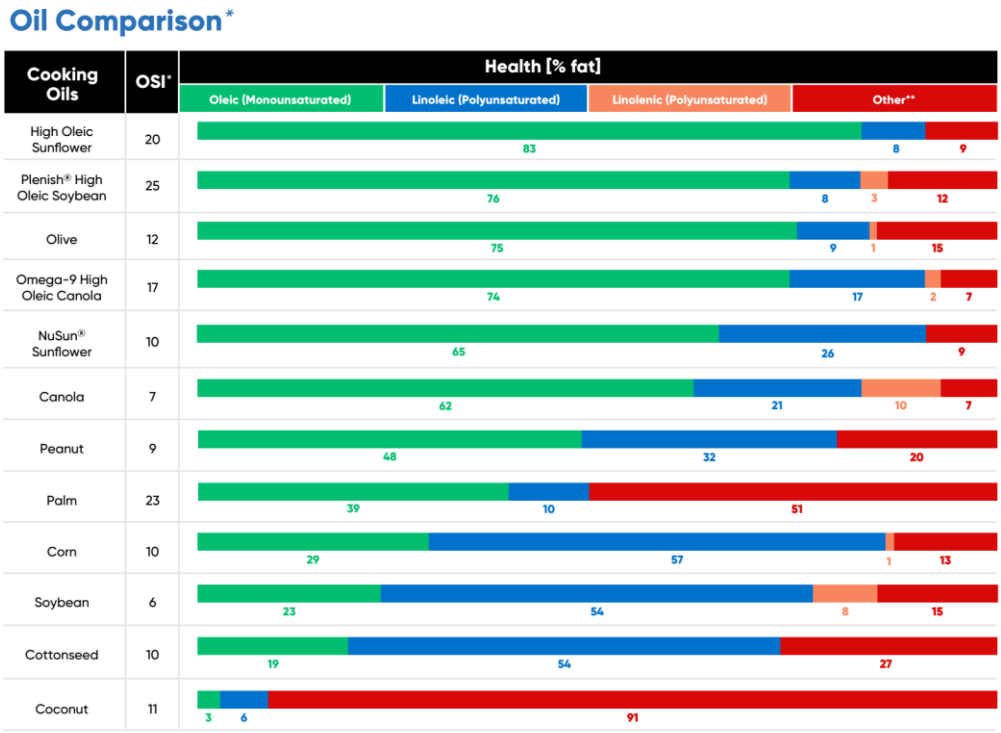



Figure 2 Oil comparison fatty acids content and stability [6]

The high proportion of oleic acid in cooking oils is directly proportional to their stability, the oxidation stability index OSI of high oleic oils being 20 compared to that of olives with 17.

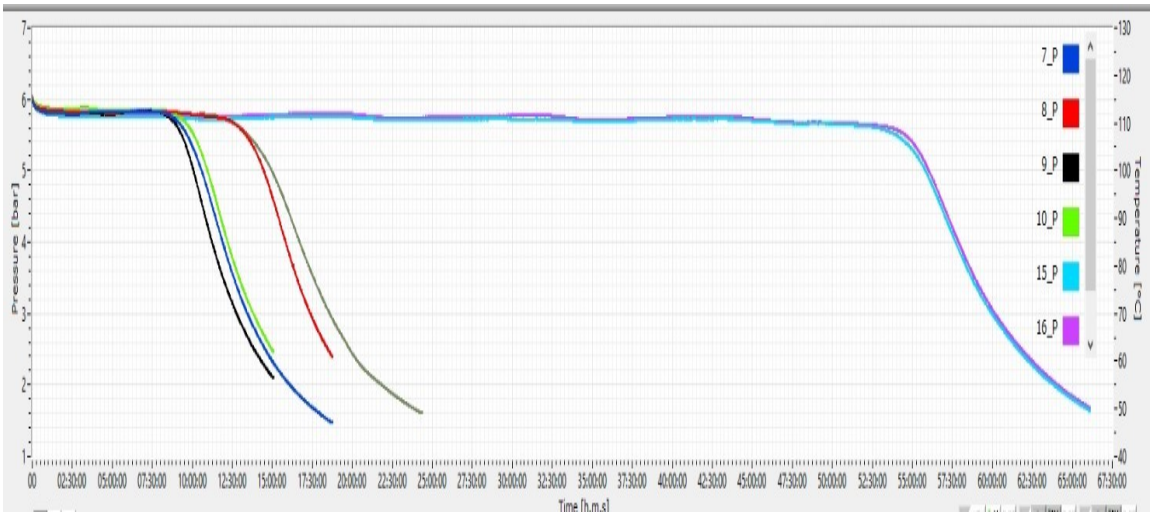



Figure 3 Induction periods of Nutrisun oil (HOHS) at 80, 85 and 90ºC

Induction period IP determinated with OXITEST is similar with oxidative stability index OSI determinated with RANCIMAT apparatus,. RANCIMAT and OXITEST instruments achieved the same comparative results following diferent procedures.

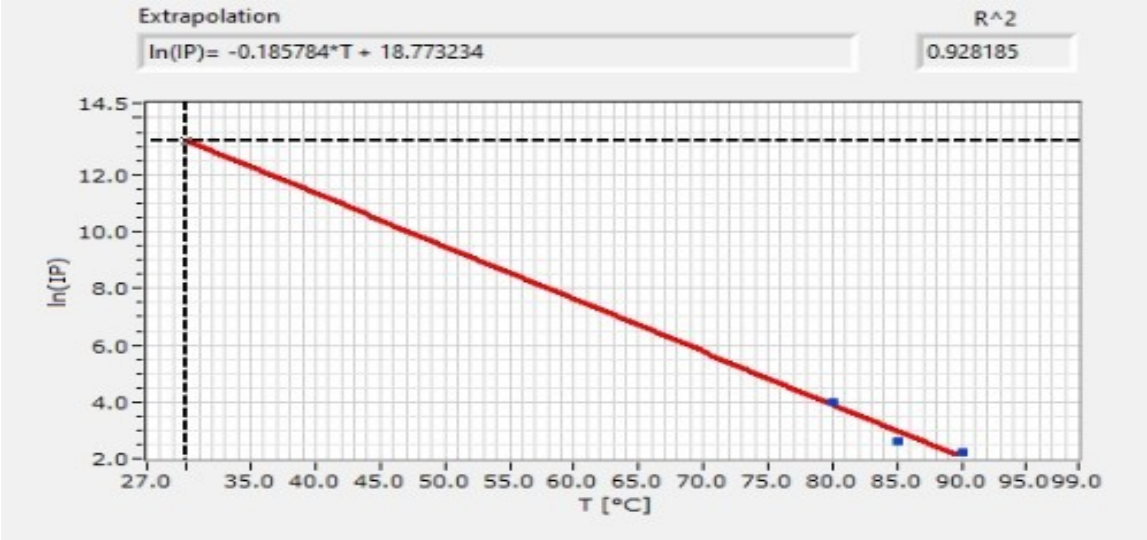



Figure 4 Estimation of shelf life by extrapolation ln(IP)=f(T)

The values of the induction period determined at the temperatures of 90, 85, 80ºC were extrapolated, allowing the estimation of the shelf life of oils or products with fat content. In the case of the analyzed Nutrisun oil, its value was 1208 days at25ºC.

**RESULT**

The qualitative composition of the oils resulting from the sunflower hybrids determined by gas-chromatographic analysis highlighted a good adaptability of the cultivated hybrids, the differences between the seeded material and the obtained harvest being insignificant.

The oil with a high content of stearic acid and oleic acid whose oxidation stability was determined with the Oxitest device presented an induction period until the appearance of oxidation products of 9.29 hours at 90ºC, 13.5 at 85 ºC, and 54.43 at 80 ºC, in time that the oil

with high oleic acid content had an induction period of 72.35 to 90 °C. These values were correlated with acidity and peroxide indices.

## DISCUSSION

The high-oleic sunflower oil was the most stable oil with the highest IP value, while the high stearic high-oleic oil also showed better oxidative stability than the traditional sunflower oil. The considerably better oxidative stability of the HO and HOHS sunflower oil was attributed to its low level of unsaturation. Bootello and Dunford also reported lower IP values for traditional sunflower oil compared to high-oleic oil [1-3]. The use of oils with a high content of oleic acid and stearic acid in thermally processed food products will increase their shelf life.

## CONCLUSION

The obtained results showed a good adaptability to the pedoclimatic conditions of the cultivated hybrids. Qualitatively, the obtained harvests correspond to the compositional characteristics of the seeded material. The profile of fatty acids as methyl esters was similar regardless of the type of cultivation, conventional or biological, 5% palmitic acid, 14.5% stearic acid, 76.6% oleic acid and 2.8% linoleic acid.

HOHS sunflower oil presented induction periods 3 times longer than linoleic sunflower oil and a saturated fatty acid/unsaturated fatty acid ratio similar to olive oil.

We encourage Romanian farmers to cultivate these hybrids, because HSHO sunflower oils can be fractionated to produce fats with high levels of solids and different fusion profiles that could be used in a wide range of food formulations, thus becoming a promising, healthy and sustainable alternative source of solid fat with high stability.

## ACKNOWLEDGEMENTS

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# EXTRACTION OF ANTIOXIDANTS FROM *MORINGA OLEIFERA* LEAVES, STABILITY OF THE RESULTING ANTIOXIDANTS, AND USE OF THE REMAINING RESIDUE AS LIVESTOCK FEED

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## ABSTRACT

*Moringa oleifera,* commonly known as horse radish tree or drumstick tree is a perennial multipurpose tropical tree. It can be used as medicine, cosmetic and functional food, among others. The benefits of using the leaves are ascribed to the presence of a number of antioxidants. Nowadays it is widely cultivated and naturalized in many countries. Simple methods to extract antioxidants from Moringa leaf are lacking, which were established, stability of antioxidants was assessed, and residue left after extraction was explored for use as animal feed, with the aim to scale-up the process to industrial scale. A natural source antioxidant-rich fraction prepared in this study could have applications in protecting against free radical mediated damage, to enhance shelf-life of products, and to prevent and/or cure diseases. Antioxidants are known to have anti-diabetic, anti-inflammatory, anti-aging, cardio-and neuro-protective, intestinal microbiota regulatory effects, among others.

The optimized procedure consisted of ultrasonication or extraction in a shaking water bath for 40 minutes at 30°C in 45% aq. ethanol at 1:10 (w/v) plant:solvent ratio. A high extraction yield by weight of 31-33% was obtained. The preparation was rich in antioxidants. On dry weight (DW) basis, total phenolics were from 19.6 to 33.5 mg catechin equivalents/g, FRAP (Ferric Reducing Antioxidant Power) from 83.3 to 140.7 nmol $FeSO_4$ equivalents/g and DPPH (2,2-diphenyl-1-picrylhydrazyl) activity from 8.9 to 18.2 mg Trolox equivalents/g. The HPLC fingerprinting showed the presence of more than a dozen compounds, and the UV spectra were typical for flavonoids and phenolic acids, containing high amounts of chlorogenic acid and rutin. The antioxidants in a liquid state or after freezedrying were stable for over 3 months at room temperature, 4-6°C or -18°C. The residue left after extraction had 42% crude protein and the essential amino acids contents were as high as that in soymeal. The residue can replace soymeal in dairy and beef cattle diets at 1:1 level by weight, and in fish diet at 30% replacement of fishmeal protein. This can decrease cost of animal feeding by 20-35%, giving higher profit to farmers and industry. Also, this residue is likely to provide health benefit to the animals being rich in catechin and kaempferol.

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The method developed is low-cost and easy to scale up, especially in a developing country settings. Good stability of the antioxidant preparation makes its attractive for use in cosmetic, pharmaceutical and food industries. It can also be a replacer of synthetic antioxidants in food and feed applications including ethoxyquin (used in fish feed), banned in many countries including the EU. The moringa antioxidant-rich fraction obtained in this study that corresponded to 1 g each of ascorbic acid, ethoxyquin or butylated hydroxytoluene (BHT) (all synthetic antioxidants) was 3.64 g, 4.3 g and 2.1 g, respectively. This antioxidant potential of moringa is very high, among other known oxidants isolated from plant parts. The study is an important step towards industrial scale development of isolating antioxidant-rich fraction and of incorporating the residue in animal diets, with zero waste – a vital requisite for circular economy.

**Keywords:** Moringa oleifera, antioxidants, stability, moringa leaf residue, protein quality, animal feed

## INTRODUCTION

*Moringa oleifera* Lam. (Moringaceae) commonly called as horse radish tree is widely cultivated and naturalized in the tropics. In recent years, *M. oleifera* has drawn increasing interest and is considered as a multipurpose plant that can be used in various ways as medicine or/and functional food, especially for the treatment of inflammations and infectious diseases, as well as cardiovascular, gastrointestinal, haematological and hepatorenal disorders [1]. The active compounds in leaves include vitamins, minerals, amino acids, phenolics, and flavonoids [2]. Recently, an untargeted metabolomic analysis of *M. oleifera* leaves showed more than 260 individual polyphenols [3]. Due to this rich inventory of bioactive substances moringa parts appear to have promising applications in food and pharmaceutical industries [4].

Moringa leaves are also of great interest in animal production. Their feeding enhanced livestock productivity [5]. The leaves may be used in animal diets to improve quality of meat products and eggs, and leaf extracts may serve to enhance the oxidative stability of meat products [6].

Some reports on extraction of active compounds from moringa are available, wherein different extraction techniques were applied, including traditional as well as unconventional approaches [7] [8] [9]; however, a simple optimized approach that can be used with a minimal set of laboratory equipment and is easily scalable, is lacking.

After extraction of antioxidants, a substantial amount of residue is left. In order to have zero waste from the extraction process, we also focused on potential use of the residue as animal feed. So, the aims of this research were to:

a) find a resource saving and simple process to obtain high yields of antioxidants from moringa leaf powder; b) test the oxidative stability of the obtained extracts under different storage conditions; c) compare the antioxidation potential of the moringa preparation with those of known synthetic antioxidants; d) assess variability in the levels of antioxidants in different batches of moringa leaves; and e) explore the possibility of using the residue left after extraction of antioxidants as animal feed.

## MATERIALS AND METHODS

### Chemicals

Standards used in the antioxidant assays were: catechine (Roth 6200.1), trolox (3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic acid, Sigma-Aldrich), BHT (butylated hydroxytoluene, Sigma-Aldrich PHR 117), ethoxyquin (Sigma-Aldrich 31519), ascorbic acid (Sigma A5960) and Ferrous sulfate heptahydrate, Merck 1.03965). All other reagents used ethanol, methanol, Folin-Ciocalteu reagent (2N, Merck), sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine), ferric chloride hexahydrate and aluminium chloride hexahydrate were of analytical grade.

### Plant material

Dried leaf powder from *M. oleifera* grown in Kenya was obtained from Kilifi, Moringa Estates Ltd, Malindi, Kenya. A bulk sample of moringa leaves, originated from Chambuko, Kenya was used to develop the optimized method. In addition, 6 leaf samples harvested in different seasons and from two different places were evaluated. After harvesting, the leaf samples were washed with water, dried under shade using air flow, and ground to pass through 0.1 mm screen.

### Extraction

The extractions were performed using an ultrasonic bath (Bandelin, Sonorex RK 156H) or a shaking water bath (GFL Gesellschaft für Labortechnik, Burgwedel, Germany). For extraction of antioxidants, optimization of ethanol concentration, extraction time and plant powder to solvent ratio (w/v) was done. After extraction the filtered solution was evaluated for antioxidants.

### Optimization using ultrasonication

The plant powder (0.5 g) was incubated in 6 ml solvent (30%, 45%, 60%, 70% 80% and 96% aqueous ethanol) in a closed test tube and sonicated for different lengths of time (20, 40 and 60 min) at 30°C. The optimized conditions at which maximum antioxidant potential was obtained were 45% aqueous ethanol and 40 min extraction time.

### Optimization using shaking water bath

The plant powder (2 g) was weighed in an Erlenmeyer flask and incubated with 20 ml solvent, applying a shaking frequency of 130 revolution per min (rpm). Different extraction times (initially 1-3 hours (h) and thereafter 20-60 min) and temperatures (30-50 °C) were tested. The solvent used was 45% aqueous ethanol. The optimized conditions obtained were 45% aqueous ethanol, 40 min extraction time and 30°C.

### Yield determination of the extract by weight

The yield of extractable compounds was determined using both an ultrasonic water bath and shaking water bath using respective optimized conditions. To this purpose, two or four g moringa leaf powder were incubated for 40 min at 30°C in 20 ml 45% aqueous ethanol. Plant powder to solvent ratios of 1:5 and 1:10 (w:v) were evaluated. The shaking frequency of the water bath was 130 rpm. After filtration, 5 ml of the resulting extract was spread on a 9 cm diameter Petri dish and dried on a heated plate (30°C) under the laboratory hood. After further drying overnight in a dry chamber, the obtained dry extract

was weighed. The yield as % was calculated according to the formula: weight(dried extract)*100/weight(plant). It was determined twice and in duplicate each time.

Preparation of extracts for stability evaluation

The stability of extracted antioxidants (using shaking water bath) in a liquid and a dry state was performed. The extract was prepared from the leaf powder bulk sample and the antioxidation potential was evaluated for three months. Leaf powder (50 g) was extracted in 500 ml 45% aqueous ethanol in the shaking water bath (30°C for 40 min at 130 rpm). From the resulting extract, after filtration, three portions of 5 ml each were taken and stored at room temperature (22-25°C), in cold (4-6°C) and freezer (-18°C). From the remaining extract, about 300 ml, the volume was reduced with a rotatory evaporator and then was freeze dried. The resulting dry extract was portioned and stored under the conditions mentioned above.

Extraction with acid hydrolysis

To obtain the flavonoid aglycones, moringa leaf sample was extracted in a solvent (ethanol:water:HCl in 7: 2:1; v/v) in a ratio of 1:10 (w/v) by refluxing at 90-95°C for 1 h. Thereafter, it was filtered and diluted 1:10 (v/v) with water before the HPLC analysis.

Batch to batch variation in antioxidation potential

The optimized extraction conditions were applied to 7 moringa leaf samples collected between 2017 and 2020 in Kenya.

Polyphenols and antioxidative activities

The measurements of polyphenols and antioxidative activity rely on colorimetric reactions and were adapted for measurement using an iMark microplate reader (BioRad, Hercules, CA, USA). On a microplate, 4 replicates of each sample or standard were carried out as described by Chizzola et al [10].

Total Phenolics: Phenolic compounds react in alkaline conditions with the Folin-Ciocalteu reagent to give a blue color. In microplate wells, a 10 µL extract was added to 100 µL distilled water, followed by addition of 5 µL Folin-Ciocalteu reagent, 10 µL $Na_2CO_3$ (35 % in distilled water.) and again 125 µL of distilled water. After storing for 1 h in the dark, the absorbance was measured at 750 nm. To calibrate color formation, increasing concentrations (ranging from 1.5 to 6.0 µg of catechin in the assay volume) were taken. The extracts were diluted 1:20 with ethanol before the assay. The total phenolics value was presented as milligrams catechin equivalent per g of dried sample (mg CE/g DW).

Total flavonoids: The assay is based on the reaction of flavonoids with $NaNO_2$ and $AlCl_3$ that produces pink-colored complexes. In this assay, a total sample volume of 140 µL was used. For most samples a partition of 20 µL of the extract and 120 µL of distilled water was appropriate. To this portion, 15 µL $NaNO_2$ (2.5 % in distilled water.) followed by 15 µL aluminium chloride (10 % $AlCl_3.6\ H_2O$ in distilled water were added. After 5 min an aliquot of 50 µL of 1 N NaOH was added and the absorbance of the mixture in the plate was measured after 5 min at 490 nm. The calibration curve was constructed with increasing concentrations of catechin (2.5–12.5 µg in the assay volume. Extract dilution was 1:10 with ethanol. The total flavonoids value was presented as milligrams catechin equivalent per g of dried sample (mg CE / g DW).

DPPH-Assay: The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical). In this assay, pink colour of DPPH is decolorized by the reaction with antioxidant compounds. In the microplate wells, to an increasing portion of the sample (5–25 µL) made up to 100 µL with methanol, an aliquot of 100 µL of the DPPH reagent (0.015% in methanol) was added. It was kept in the dark for 30 min. Increasing volumes (0–8 µL) of trolox (0.62 mg/ml in ethanol) made up to 100 µL with methanol instead of the samples were used to construct a calibration curve. A preparation consisting of 50 µL trolox, 50 µL distilled water and 100 µL of the DPPH reagent, wherein the DPPH was completely decolourized was taken as blank and subtracted from all measurements. The decolouration was measured at 490 nm. The extracts used in the assay were diluted 1:10 with ethanol. The resulting DPPH radical scavenging activity was presented as milligrams trolox equivalent per g of dried sample (mg Trolox/g DW).

Ferric Reducing Antioxidant Power (FRAP) Assay: The principle relies on the ability of antioxidants to reduce ferric ($Fe^{+++}$) ions. The resulting ferrous ions ($Fe^{++}$) form a deep blue complex TPTZ. The reaction was carried out by mixing in the microplate wells 10 µL sample, 15 µL methanol and 180 µL working reagent. After 5 min, the color formation was measured at 595 nm. The working reagent consisted of 25 ml acetate buffer (300 mmol/L) pH 3.6, 2.5 ml of 10 mmol/L TPTZ in 40 mmol/L HCl and 2.5 ml $FeCl_3$ solution (20 mmol/L). A calibration curve was constructed using increasing amounts of $FeSO_4.7H_2O$, from 1.25 to 6.25 µg/well, instead of the samples. Sample dilution before the assay was 1:20 with ethanol. The reducing activity was presented as nmol ferrous ions per g of dried sample (nMol Fe/g DW).

Comparison of antioxidant standards

As the calculation of antioxidant activity relies on the calibration of the color formation of a standard in the respective assay, the activities of trolox, catechin, BHT, ascorbic acid and ethoxyquin were compared. Using the slopes of the calibration curves obtained with the different reference compounds, the respective conversion factors were calculated. These conversion factors were used to arrive at the equivalence of the antioxidants in the 45% aqueous ethanol extract from moringa leaves as compared to trolox, catechin, BHT, ascorbic acid and ethoxyquin, on weight-by-weight basis.

Phenolic fingerprinting using HPLC

The phenolic fingerprint of the moringa extracts weas performed using a Shimadzu HPLC system consisting of a LC-20ADXR pump, a SCL-40 controller, a SIL-20ADXR autosampler, a CTO-20AC column oven operated at 25 °C, and a SPD-M20A photodiode array detector. The separation was done on a Waters Symmetry 300TM C18, 5.0 µm particle size, 4.6 x 250 mm column. The mobile phase used was acetonitrile (solvent A) and 1% acetic acid in water (solvent B). From 0 to 15 min, the analysis was started using an isocratic solution: 15% solvent A and 85% solvent B, thereafter solvent A was increased to 35% to minute-25, to 55% to minute-30 and finally to 60% to minute-35. The flow rate was 1.0 ml/min and the injection volume 20 µl.

Composition of moringa residue

Crude protein, lipid, neutral detergent fibre (NDF) and ash content were determined in accordance with the standard methods of AOAC. The amino acid composition was determined using an amino acid analyser after hydrolysing the samples with 6 M HCl at

100°C for 22 h. The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The contents of different amino acids were presented in g/16 g of nitrogen and were compared with the FAO/WHO reference and soybean proteins [11].

## RESULTS

Optimization of extraction conditions using ultrasonication

Using sonication, the effect of aqueous ethanol concentrations on extraction of antioxidants is presented in Table 1A. All three assays show the highest activities with the lowest ethanol concentrations (45%), indicating a good solubility of the antioxidant compounds in this solvent. In the HPLC analysis, on summation of the areas of the main peaks (Figure 1, left) the highest contents of phenolic compounds were also found in the extracts made with 45% aqueous ethanol. The time of extraction in the above set of analyses was 40 min.

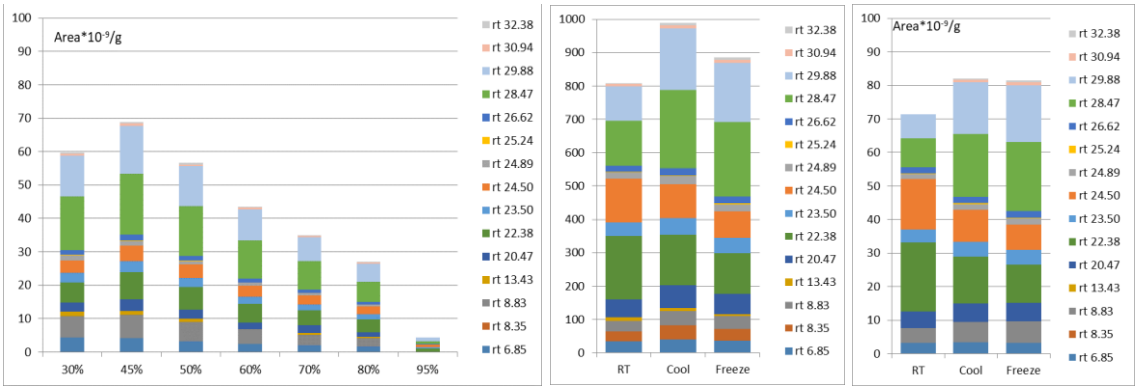



**Figure 1**: Pattern of major compounds according to retention time (rt) in HPLC peak areas at 354 nm. Extracts obtained with different ethanol concentrations (30% - 95%) by ultrasonication (Left), in dry extracts (Middle), and liquid extracts (Right) after three months storage, RT: room temperature 22-25°C, Cool: refrigerator (4-6°C), Freeze: freezer (-18°C)

Optimization of extraction conditions using shaking water bath

Further, shaking water bath was used to study the effect of extraction temperature and duration using 45% aqueous ethanol, which proved to be the most effective in the extraction procedure using ultrasonication (Tables 1B, C). A longer extraction duration up to 3 h did not enhance the antioxidation activities of the extracts. Similarly, a higher extraction temperature had only a marginal effect in all the assays, while in the DPPH assay a slightly higher activity was observed. With the aim to reduce time of extraction, shorter extraction durations, ranging from 20-60 min were evaluated (Table 1C). Only using the FRAP assay the activity at 60 min appeared somewhat lower compared to the shorter extraction times. Overall, the extraction times of 20 min and 40 min gave comparable results; however, a 40 min extraction was easier to handle the whole operation in our hands. This latter procedure had a better reproducibility as evidenced by the lower standard deviations in the FRAP and DPPH assays. In comparison to longer extraction times (1-3 h, Table 1B) total phenolic levels were slightly lower. But the slightly higher yield of antioxidants at higher extraction time is not worth the time and

energy spent. Based on the results and in the view to save resources, the procedure chosen was: solvent 45% aqueous ethanol, extraction temperature 30°C, extraction duration 40 min and a plant powder to solvent ratio of 1:10 (w/v).

**Table 1**: Extraction of antioxidants from *Moringa oleifera* leaf powder

| | | Total Phenolics | | FRAP | | DPPH | |
|---|---|---|---|---|---|---|---|
| | Mean SD | Mean | SD | Mean | SD | Mean | SD |
| **A: Influence of different ethanol concentrations in water** | | | | | | | |
| Ethanol | | | | | | | |
| 30% | | 20.1 | 0.1 | 130.2 | 10.5 | 20.3 | 0.2 |
| 45% | | 22.9 | 0.2 | 139.9 | 12.7 | 22.3 | 0.3 |
| 60% | | 23.4 | 0.4 | 133.0 | 7.4 | 21.6 | 2.0 |
| 70% | | 22.3 | 0.2 | 102.2 | 4.1 | 14.3 | 0.4 |
| 80% | | 16.3 | 2.2 | 42.9 | 10.5 | 6.6 | 1.2 |
| 96% | | 10.2 | 0.7 | 31.3 | 2.4 | 2.2 | 0.5 |
| **B: Influence of extraction time at different temperatures** | | | | | | | |
| 30 °C | 1 h | 18.9 | 0.5 | 108.4 | 2.5 | 23.8 | 0.1 |
| | 2 h | 19.3 | 0.4 | 98.7 | 2.9 | 23.2 | 0.1 |
| | 3h | 19.5 | 0.2 | 101.6 | 2.4 | 22.1 | 0.4 |
| 40 °C | 1 h | 18.2 | 1.2 | 107.0 | 3.2 | 24.1 | 0.5 |
| | 2 h | 18.6 | 0.5 | 102.8 | 4.0 | 22.7 | 0.6 |
| | 3 h | 18.4 | 1.1 | 102.7 | 1.4 | 24.9 | 0.6 |
| 50 °C | 1 h | 19.2 | 0.8 | 108.3 | 2.9 | 24.8 | 0.4 |
| | 2 h | 19.8 | 0.4 | 104.6 | 3.0 | 24.8 | 0.5 |
| | 3 h | 19.2 | 0.6 | 100.2 | 1.9 | 26.8 | 0.1 |
| **C: Influence of short extraction time** | | | | | | | |
| 20 min | | 17.9 | 0.7 | 106.1 | 4.5 | 25.9 | 1.1 |
| 40 min | | 16.4 | 1.1 | 104.7 | 2.5 | 26.6 | 0.8 |
| 60 min | | 17.7 | 0.3 | 93.7 | 5.3 | 27.0 | 0.3 |

| **D: influence of plant/solvent ration in water bath extraction or sonication** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Yield % | | Total Phenolics | | FRAP | | DPPH | |
| Water bath | | | | | | | | |
| 1:10 | 33.2 | 0.4 | 20.0 | 1.9 | 105.2 | 6.3 | 15.5 | 1.0 |
| 1:5 | 31.3 | 0.2 | 21.7 | 0.8 | 101.9 | 6.6 | 12.0 | 0.0 |
| Sonication | | | | | | | | |
| 1:10 | 34.0 | 0.7 | 19.0 | 1.3 | 102.9 | 5.8 | 16.5 | 0.2 |
| 1:5 | 28.2 | 0.6 | 19.5 | 1.0 | 100.7 | 3.3 | 12.1 | 0.0 |

A: 40 min of sonication (150 Watt) at 1:10 ratio of leaf to solvent (w/v)
B, C: water bath extraction with 45% aqueous ethanol and 1:10 w/v of leaf to solvent
D: extraction using 45% aqueous ethanol for 40 min
Yield: % of original leaf weight. Total Phenolics: catechine equivalents/g. FRAP: nMol Fe/g. DPPH: Trolox equivalents/g. Mean and standard deviation (SD) with n =3

Extraction yields

Under optimized condition using ultrasonication and shaking water bath, extraction yields were compared using different plant powder to extractant ratios. The extraction yield was around 30% (Table 4) indicating a high level of antioxidant-rich soluble compounds in the leaf powder. A 1:10 plant to solvent ratio /w/v) gave slightly higher yield and antioxidant activity and was also easier to handle. With the higher amount of plant powder and lower volume of aqueous ethanol e.g. with 1:5 ratio (w/v), the extraction medium got sticky and dense and it was difficult to obtain substantial volumes of the extract. Water bath extraction gave results comparable to the sonication, therefore water bath extraction was preferred as this procedure is simpler. Using the optimized condition, one kg of moringa leaf power would give 300 g of antioxidant-rich extract for various industrial applications.

Batch to batch variation

*Moringa oleifera* leaf powder samples harvested at different times of the year in Kenya were analysed for antioxidation potential (Table 5). Total phenolics ranged from 19.6 to 33.5 mg catechin equivalents/g, FRAP assay from 83.3 to 140.7 nmol $FeSO_4$ equivalents/g and DPPH activity from 8.9 to 18.2 mg Trolox equivalents/g. So, the highest content of active compounds and activity was 1.7 higher than the lowest using total phenolics and FRAP assays, and 2.3 higher using total flavonoid and DPPH assays. The two Mijomboni samples (Oct 2018 and Dec 2020), clearly had different antioxidant levels. The pooled sample used in the current study (Mijomboni) for optimization of extraction conditions was the one from October 2018 (Table 2). The highest activity was recorded in the sample from December 2020. Phenolic and flavonoid content and activities depend on the collecting time of the leaves.

**Table 2:** Antioxidants in different *Moringa oleifera* leaf powder samples, extract for 40 min in the shaking water bath at 30°C

| Growing site in Kenya | Harvest date | Total Phenolics | FRAP nmol Fe/g | DPPH mg trolox equivalent/g | Total Flavonoids |
|---|---|---|---|---|---|
| Chambuko | July2017 | 25.1 - 28.4 | 78.1 - 88.4 | 14.8 - 22.1 | 8.7 - 9.0 |
| Chambuko | April 2018 | 26.0 - 26.5 | 93.5 - 101.9 | 26.3 - 28.4 | 10.6 - 13.2 |
| Chambuko | May 2018 | 24.9 - 25.2 | 94.5 - 102.3 | 30.1 - 35.8 | 12.5 - 13.6 |
| Chambuko | June 2018 | 24.1 - 24.4 | 85.8 - 89.7 | 24.1 - 30.2 | 7.8 - 7.9 |
| Mijomboni-1 | October 2018 | 19.5 - 19.7 | 79.9 - 90.2 | 18.3 - 25.3 | 7.8 - 10.4 |
| Mijomboni-2 | October 2018 | 25.7 - 27.4 | 104.9 - 110.1 | 29.4 - 39.4 | 11.4 - 13.3 |
| Mijomboni | December 2020 | 32.7 - 34.4 | 135.9 - 145.4 | 36.2 - 48.0 | 15.8 - 20.7 |

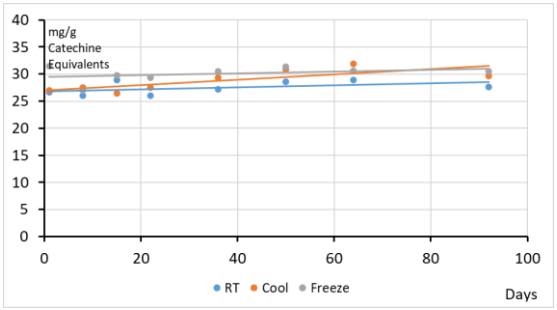
individual values of two measurements in duplicate were performed. Total Phenolics and Total Flavonoids in mg catechnine equivalents/g

Shelf-life of the extract

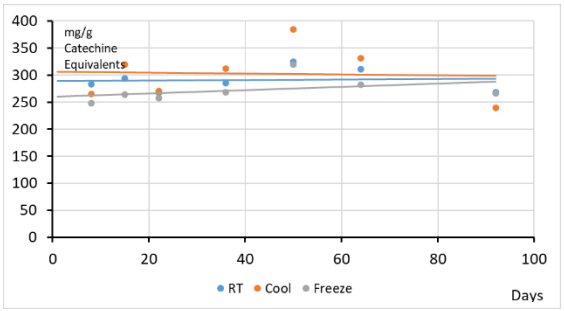
For practical applications, the stability of plant extracts is of great importance. Therefore, total phenolics, total flavonoids and antioxidant activity were monitored in the extract (obtained using optimized conditions) in a liquid state and after freeze-drying (Figure 1 middle and right, Figure 2). In the FRAP assay the measured levels remained constant (Figures 2E and 2F). Differences between storage conditions were small: room temperature storage showed a slight tendency towards lower activities with passage of

time. In the dried state the DPPH assay activity tended to decrease with time (Figure 2H). The reason for this is not clear because in the liquid state, no change in the DPPH activity was observed. Overall, the liquid extract and the dried powder showed a good stability up to three months (Figure 1, middle and right).

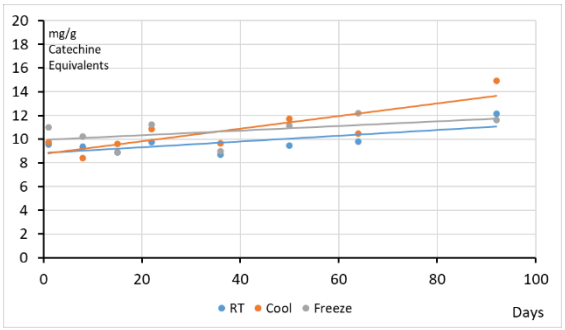
A: Liquid extracts, Total Phenolics



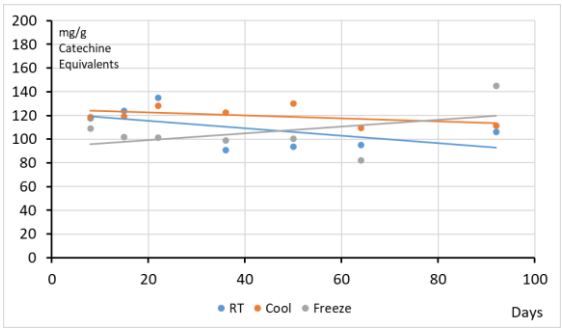

B: Freeze-dried, Total Phenolics



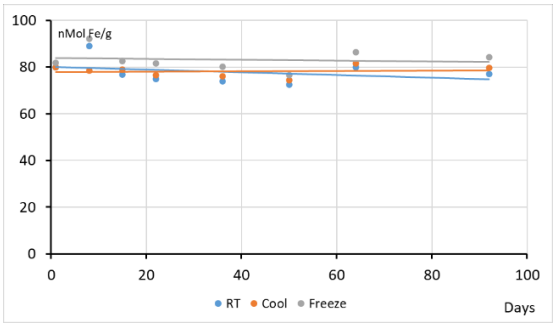

C Liquid extracts, Total Flavonoids



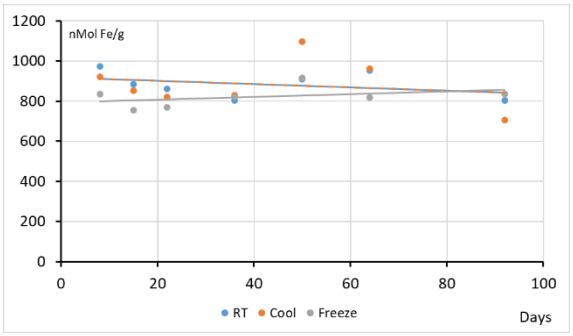

D: Freeze-dried, Total Flavonoids



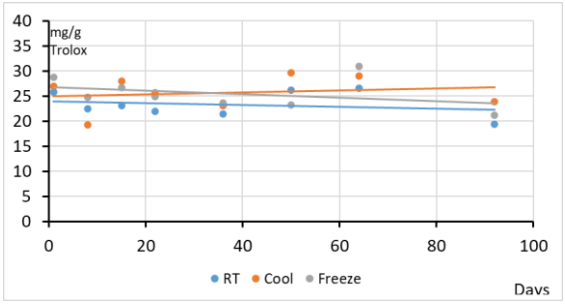

E: Liquid extracts, FRAP Assay




F: Freeze-dried, FRAP Assay




G: Liquid extracts, DPPH Assay




H: Freeze-dried, DPPH Assay

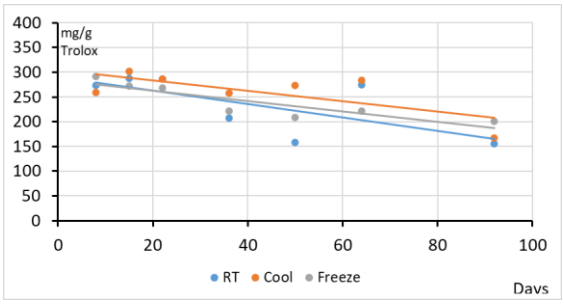



**Figure 2**: Stability of extract in liquid and freeze-dried states up to 3 months stored under different conditions. RT: room temperature 22-25 °C, Cold: refrigerator (4-6 °C), Frozen: Freezer (-18 °C)

Fingerprinting of the extracts

To get a better information on the composition of the extracts, fingerprinting using an HPLC was performed. A typical HPLC chromatogram is shown in Figure 3, left side, presenting more than a dozen compounds. The UV spectra were typical for flavonoids and phenolic acids. By comparison with standards, chlorogenic acid and rutin could be identified. Catechin, which is documented for moringa [12] could not be found. Additionally, an extraction including an acid hydrolysis was done. The resulting chromatogram (Figure 3, right side) shows two major peaks that could be assigned to quercetin and kaempferol, suggesting that the original leaves contain a large amount of glycosides of these two flavonoid aglycon. Quercetin and kaempferol, however, appeared to be very low or absent in the aqueous ethanol extracts. These flavonoids would be present in the residue left after extraction.

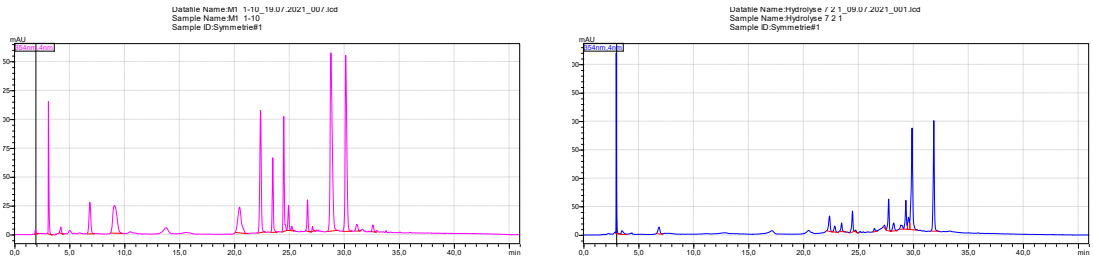


**Figure 3**: HPLC chromatograms of *Moringa oleifera* recorded at 354 nm
RT 9.2: chlorogenic acid, RT 20.5: rutin, RT 29.9: quercetin, RT 31.9: kaempferol
Left: leaf powder extract, right hydrolized leaf powder extract

Comparison of the extract against other reducing compounds

Finally, different reference compounds for antioxidant assays have been compared by weight (Table 3). A conversion factor greater than 1 indicates that more of the considered reference compound would be needed to obtain the same effect. So, in the total phenolics assay, BHT, ascorbic acid and ethoxyquin were less sensitive than catechin. Butylated hydroxytoluene and ascorbic acid did not react in total flavonoid determination because they are not flavonoid, and ethoxyquin was again less sensitive than catechin. In the DPPH assay, BHT and ascorbic acid were less active but ethoxyquin was more active than trolox. In the FRAP assay, BHT, ascorbic acid and ethoxyquin showed a higher activity than $FeSO_4.7H_2O$. An example is given for one sample in Table 4, showing active compound contents and antioxidative activity in relation to different reference compounds, by weight.

**Table 3**: Factors to convert antioxidant standards

| | |
|---|---|
| Total Phenolics | BHT = 1,573 x CAT; ASC = 1.1161 x CAT; ETH = 1.227 x CAT |
| Total Flavonoids | ETH = 6.36 x CAT; BHT, ASC no color formation |
| FRAP | BHT = 0.873 x FE; ASC = 0.270 x FE; ETH = 0.143 x FE |
| DPPH | BHT = 1.414 x TRO; ASC = 1.017 x TRO; ETH = 0.721 x TRO |

BHT: BHT equivalents, CAT: catechnine equivalents, ASC: ascorbic acid equivalents, ETH: ethoxyquin equivalents, TRO: trolox equivalents, FE: $FeSO_4.7H_2O$ equivalents, all in mg/g and FE in nmol/g

**Table 4**: Comparison of total phenolics, total flavonoids and antioxidant activities of moringa leaf sample using different reference compounds. (All values in mg/g leaf DM except for FRAP in nmol Fe/g)

| | Total Phenolics | Total Flavonoids | DPPH | FRAP |
|---|---|---|---|---|
| Catechine Equivalents | **27.1** | **12.4** | | |
| Trolox-equivalents | | | **27.4** | |
| $FeSO_4.7H_2O$-equivalents | | | | **108.5** |
| Ascorbic acid -equivalents | 31.5 | | 27.9 | 29.2 |
| Ethoxyquin-equivalents | 33.3 | 78.9 | 19.8 | 15.5 |
| BHT-equivalents | 42.6 | | 38.7 | 94.7 |

Measured values in bold, values in normal fonts calculated according to Table 3

Composition of the moringa residue and its amino acid content

Table 5 shows chemical composition of moringa residues and other commonly used protein-rich feed resources. The crude protein content of moringa residue is high (42.4%). It is also a rich source of minerals as is evident from the ash content. The essential amino acid composition of the moringa meal including of sulphur containing amino acids and lysine is high (Table 6).

**Table 5:** Proximate composition of moringa residue and its comparison with other protein-rich feed ingredients

| Feed resource | Crude protein (% DM) | Crude fat (% DM) | Crude fibre (% DM) | Ash (% DM) |
|---|---|---|---|---|
| Moringa residue | 42.4 | 7.2 | 21.0 | 7.1 |
| Soymeal | 47.0 | 1.7 | 4.4 | 7.2 |
| Fishmeal | 62.0 | 6.5 | 13.6 | 8 |
| Rapeseed meal | 38.1 | 2.4 | 14.3 | 7.6 |
| Sunflower meal | 32.4 | 2.2 | 27.9 | 7.1 |

**Table 6**: Amino acid composition (g/16 g nitrogen) of Moringa residue versus soymeal, fishmeal and FAO reference dietary protein requirement values

| Amino acids | Moringa leaf residue | Soymeal [13] | Fishmeal [13] | FAO† [14] | Moringa leaves [15] |
|---|---|---|---|---|---|
| Essential | | | | | |
| Methionine | 1.92 | 1.32 | 2.7 | 2.50‡ | 1.98 |
| Cystine | 1.09 | 1.38 | 0.8 | | 1.35 |
| Valine | 7.18 | 4.5 | 4.9 | 3.5 | 5.68 |
| Isoleucine | 4.83 | 4.16 | 4.2 | 2.8 | 4.50 |
| Leucine | 9.36 | 7.58 | 7.2 | 6.6 | 8.70 |
| Phenylalanine | 6.10 | 5.16 | 3.9 | 6.30§ | 6.18 |
| Tyrosine | 3.79 | 3.35 | 3.1 | | 3.87 |
| Histidine | 2.30 | 3.06 | 2.4 | 1.9 | 2.99 |

| | | | | | |
|---|---|---|---|---|---|
| Lysine | 5.94 | 6.18 | 7.5 | 5.8 | 5.60 |
| Threonine | 4.78 | 3.78 | 4.1 | 3.4 | 4.66 |
| Tryptophan | 2.13 | 1.36 | 1.0 | 1.1 | 2.10 |
| *Total* | *49.42* | *41.83* | *41.80* | *33.9* | *47.61* |
| Non essential | | | | | |
| Serine | 4.65 | 5.18 | 3.9 | - | 4.12 |
| Arginine | 6.07 | 7.64 | 6.2 | - | 6.23 |
| Glutamic acid | 9.39 | 19.92 | 12.6 | - | 10.22 |
| Aspartic acid | 9.33 | 14.14 | 9.1 | - | 8.83 |
| Proline | 4.68 | 5.99 | 4.2 | - | 5.43 |
| Glycine | 5.57 | 4.52 | 6.4 | - | 5.47 |
| Alanine | 6.17 | 4.54 | 6.3 | - | 7.32 |

† Reference for a 2-5 year-old child; ‡ Methionine + cystine; § Phenylalanine + tyrosine

## DISCUSSION

Antioxidant-rich extract

*M. oleifera* leaves are rich in phenolic acids and flavonoids that display potent antioxidant properties. The leaves of *M. oleifera* contain gallic acid, chlorogenic, ellagic, caffeic and syringic acids [8] [16] and flavonoids including myricetin [12] or various derivatives of kaempferol and quercetin [12] [17] [18], isoquercetin [2] and myricetin [19].

Ultrasound assisted extraction of phytochemicals is widely used to obtain active compounds from various plants. This technique facilitates the diffusion of phytochemicals from plant matrix into the solvent, is easy to handle and is less energy demanding when compared to other methods. In addition, it minimizes process times and can be carried out at low temperatures, which is an advantage for the extraction of thermo-labile compounds. Ultrasound extraction was superior to Soxhlet extraction [20]. In this study we have taken ultrasonification process as a ‘gold standard’ method, to arrive at the same level of antioxidants in the extract using shaking water bath, which is easier to conduct and up-scale for potential industrial applications.

Alcohol water mixtures give extracts high in antioxidative activities in comparison to other solvents tested [18] [21]. Ethanol was preferred to methanol because of its lower toxicity. Optimized conditions using shaking water bath (130 rpm), extraction in 45% aqueous ethanol for 40 min at 30°C using moringa leaves to solvent ratio of 1:10 (w/v) are simple and can be carried out in a laboratory equipped with basic facilities. Here, a method operating at low temperatures was feasible, while other authors found optimal extraction at higher temperatures and higher solvent use; some examples being sonication with 70% ethanol, 30:1 solvent-sample ratio, 50°C extraction temperature, and 42 min extraction time12, while Karamudin et al. [8] procedure uses hot water extraction at 82°C for 48 minutes and Naeem et al. [7] suggested use of 90°C, 50% methanol and 60 min extraction time.

The yield obtained of the antioxidant-rich powder (30%) was also high. In a laboratory equipped with one shaking water bath of medium size, approx. 16 litres of the extract (from 1.6 kg moringa leaves) can be prepared in a day (8-h shift). This volume can be reduced to approx. 8 litres in a day using 2 rotary evaporators of 1 litre capacity. After freeze drying using a medium size lyophilizer, around 500 g of the antioxidant-rich

lyophilized powder can be prepared in approx. 24 h. These calculations are approximate but show a high potential to up-scale the process.

The antioxidants obtained from Moringa leaves varied from batch to batch. The variation (about 2-fold) could be attributed to different stages of leaf/plant maturity and environmental conditions including soil [22] and altitude [23], although moringa leaf samples were collected from trees grown in the same area. Furthermore, the polyphenol content of moringa were influenced by availability of nutrients as nitrogen [24]. Also, a seasonal influence on antioxidative activities of moringa extracts has been reported [25]. Besides, active compounds of moringa depend on variety or accessions studied [1] [21]. This offers opportunity to further enhance the antioxidant levels in the extracts obtained in this study. The shelf-life of the products obtained, both in the extract and powder form is good. These can be stored for at least 3 months without any substantial loss of activity. Likewise, after 6 months of storage at 25°C, bioactive contents and activity in moringa extracts decreased only slightly. Higher temperatures and humidity, however, have a negative impact on the activity [26].

Oxidative stress is an important contributor to numerous pathological conditions such as cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases [27]. Several mechanisms in the body exist to protect the cellular molecules against reactive oxygen species induced damage. However, the innate defence may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are needed to maintain an adequate level of antioxidants to protect the cells against oxidative radicals. Synthetic antioxidants such as BHT and butylated hydroxyl anisole (BHA) are very effective and are used for industrial processing, but they may cause side effects. BHT is used in a wide range of cosmetic formulations and this could cause adverse effects on skin at high levels [28]. A natural source, the antioxidant-rich moringa extract prepared in this study could have applications in protecting against free radical mediated damage, to enhance shelf-life of products including cosmetics, and to prevent and/or cure diseases. Antioxidants are known to have anti-diabetic, anti-inflammatory, anti-aging, cardio-and neuro-protective, intestinal microbiota regulatory effects, among others [29].

Another synthetic compound, ethoxyquin has, for decades, been widely used as an antioxidant in the feed sector. However, In June 2017, the EU commission suspended the authorization of ethoxyquin for all animal species and categories. The moringa extract could also be an attractive alternative to ethoxyquin.

The colorimetric assays for polyphenols and antioxidant activity require reference compounds to calibrate the colour formation in order to express the results in mg, µg, mmol or µmol as equivalent of the reference standard chosen. A great variety of such standards can be used ranging from widely occurring phenolic acids or flavonoids, or synthetic antioxidants applied in food industry (e.g. BHT). Therefore, it is difficult to compare the results obtained from different authors even when they use the same assays. To overcome these difficulties the colour formation in the assays was measured with increasing amounts of catechin, trolox, BHT, ethoxyquin and ascorbic acid. The relative slopes of the resulting calibration graphs as conversion factors (Table 3) allows to calculate the content or activity measured for a given reference compound to another. For instance, a total phenolics content of 27.1 catechin equivalents corresponds to 33.3 ethoxyquin equivalents (Table 4) as multiplied by the conversion factor 1.227. Using

these values, the amounts of moringa dry extract that corresponded to 1 g each of ascorbic acid, ethoxyquin or BHT was 3.64 g, 4.3 g and 2.1 g, respectively. In other words, in an application if 1 g of BHT is used then 2.1 g of the moringa extract would need to be used, and in place of 1 g of ethoxyquin 4.3 g of the moringa extract would be needed to obtain the same antioxidation potential.

Moringa residue as animal feed

The crude protein (CP) content 42.2 g/kg DM of the residue is comparable with that reported (43.5%) earlier [30] in the ethanol-extracted moringa leaves. This crude protein content in the residue is comparable with that in soymeal and is higher than in rape seed meal and sunflower meal (Table 8). The CP content of the moringa residue is considerably higher than the mean CP content of forage legumes (17%) and grasses (11.5%) [31].

Moringa residue has excellent amino acid composition. The level of sulphur-containing amino acids (methionine + cystine) in the moringa residue is higher than those in soymeal and the FAO Reference protein. Lysine level is comparable with those in soymeal and the FAO Reference protein. On the other hand, contents of both lysine and sulphur-containing amino acids are lower than that in fishmeal. However, total essential amino acid level in the moringa residue is highest amongst all these good quality feed resources. The amino acid composition of the moringa residue is comparable with those of moringa leaves. Our previous studies have shown that protein digestibility of moringa leaves (>90%) is comparable with that of soymeal [30] [15], and we do not expect that the protein digestibilty of the moringa residue will be much different. These data suggest that the moringa residue is a good source of protein for animal diets. The protein from the conventional protein rich feeds could be replaced up to 100% by the moringa residues in ruminant diets, while that in monogastric diets the level of incorporation would be lower due to the higher fibre content in the moringa residue (neutral detergent fibre value of 21%). Also, lower enteric methane is expected on inclusion of moringa residue in ruminant diets [32]. For poultry, pig and fish, supplementation of a low level of synthetic lysine in the diets containing the moringa residue might be advantageous, especially at their early stage of growth. High levels of quercetin and kaempferol present in the moringa residue are also likely to be beneficial for the animal production and product quality [29].

## CONCLUSION

In conclusion, a simple method to obtain antioxidant-rich moringa preparation, in liquid and powder form, with high yield, good shelf-life and high capability to scale-up has been developed, for food, cosmetic and pharmaceutical applications. The optimized procedure consisted of ultrasonication or extraction in a shaking water bath (130 rpm) for 40 minutes at 30°C in 45% aq. ethanol at 1:10 (w/v) plant:solvent ratio. A high extraction yield by weight of 31-33% was obtained. As this extraction procedure is not exhaustive, the residue left after extraction of antioxidants is still rich in protein and flavonoids for incorporation in animal and fish diets. The process is low-cost, relies on simple technologies and does not leave any waste and is based on the principle of the circular economy.

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# FOOD ATTRIBUTES OF FORTIFIED FOODS: AN ANALYSIS OF CONSUMPTION BEHAVIOR

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## ABSTRACT

The issue of food security, in the face of threats as resource scarcity, climate change, population growth, pollution etc confronts the agri-food systems all over the world with the challenge of pursuing resilience and sustainability. Western populations do not currently experience food shortages, but others do not have enough food available and some others suffer from hunger. Besides, a topic gaining much attention is the malnutrition condition of the global population. International research present fortification as a novel approach to promote public health in the fight against severe nutritional deficiencies (vitamin A, iron, and zinc), particularly in developing Nations, in the context of micronutrient malnutrition.
Consumers' acceptance of fortified products are receiving more and more attention from the institutional, scientific and industrial sphere.
The present survey aims at investigating the level of consumer's knowledge regarding fortified products, the factors influencing their purchasing decisions, their food value systems in terms of sensory evaluation and economic valuation compared to conventional ones and the traits influencing the higher willingness to pay for these foods..
to identify The investigation on the factors that may influence the propensity and frequency of fortified products' consumption bases on a survey sample. Specifically, following the administration of an ad hoc questionnaire, the methodology comprehend statistical analyses: the Principal Component Analysis (PCA) that synthetized the food values and the consumption motivations comprised in the questionnaire and a non-hierarchical Cluster Analysis to categorize fortified products consumers.
The investigation conducted identified the Italian customer profile of fortified food product.

**Keywords:** Fortified foods, Foos Innovations, Food Technology, Consumption.

## INTRODUCTION

The issue of food security, in the face of threats as resource scarcity, climate change, population growth, pollution etc. confronts agri-food systems in all over the world with the challenges of pursuing resilience and sustainability. Western populations do not

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currently experience food shortages, but others do not have enough food available and some others suffer from hunger.
Alongside food security, however, there is another issue that can affect all populations and therefore also the Western one who have the availability of the necessary amount of food to ensure a satisfactory daily intake [1]. This is *hidden hunger*, i.e. micronutrient malnutrition due to the lack of critical micronutrients that also occurs when people get enough calories. It is about a disorder that affected between 691 and 783 million people in 2022 worldwide [2].
In this contest, a resilient cost effective, feasible and environmentally sustainable strategy is Fortification [3].
In particular, the process of food fortification happens either through natural processes, agronomic practices, conventional plant breeding, or modern biotechnology [4], [5]. Since fortification involves technological processes, fortified foods definitely belong to the group of products made with innovative techniques. A fortified food is a product with new added nutrient bioavailability components that may or may not be natively present in the food and so it helps in supporting daily nutritional needs and provide public health benefits [6]. Several studies have been carried out to identify and measure the bioavailability, and efficacy and efficiency of biofortification of basic crops. Studies have shown the increasing bioavailability of nutrients and that they can be assimilated at levels sufficient to improve the status of trace elements. For instance, biofortified iron pearl millet improves the nutritional status of children [4].
However, a lack of interest by consumers for biofortified products [7] the success of all these efforts made by the agri-food system to enrich food of micronutrients depends on their acceptance and consumption. may represents a barrier accepting and consuming fortified products. Understanding nutrition and be aware of the fight against disease and malnutrition trough biofortified products could constitute a motivation to increase the consumption willingness.
The measure of consumer's acceptance of biofortified products happens in terms of sensory evaluation and economic valuation compared to conventional ones [8] and it is receiving more and more attention from the institutional, scientific, and industrial world.
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The present survey aims at investigating the level of consumer knowledge regarding fortified products, the factors influencing their purchasing decisions, their food value systems andthe traits influencing the potential higher willingness to pay for these foods.
At this scope, an online questionnaire was arranged and distributed to a convenience sample of Italian consumers. The execution of a Principal Component Analysis (PCA) helped in identifying the consumers' preferences of fortified products, and to summarise and highlight the main socioeconomic variables and product attributes influencing fortified consumption. Successively, a cluster analysis supported the identification of homogeneous consumer groups. The results of this research contribute in exploring consumer's behaviour toward fortified products and therefore itcould be useful to industry and government.

## MATERIALS AND METHODS

The general objective of this work is to identify which factors may influence the propensity and frequency of fortified products' consumption on the basis of a survey sample. At this scope, it resulted necessary to identify potential drivers and to explore

the consumers' attitudes and lifestyles. In particular, the methodology adopted to meet this goal combines the administration of an ad hoc questionnaire and an exploratory factor analysis.
The following research questions steer at answering the research objectives set:
**RQ1.** Which food attributes are related to the consumption of fortified products?
**RQ2.** Do consumers' subjective and objective knowledges influence the propensity to consume fortified products?
**RQ3.** Do socio-demographic variables have an effect on the propensity and frequency of consumption of fortified products?
The questionnaire includes both qualitative and quantitative information.
The qualitative information were detected by formulating the questions in the form of semantic differential, that is, through the application of a Likert scale where 1 represents strongly disagree and 5 strongly agree, with different coded options and the motivations underlying the consumption of fortified food products.
The quantitative information were (socio-demographic, frequency of consumption, etc.), expressed on an ordinal scale.
The questionnaire comprehends three sections The first concerning the identification of general information on fortified products consumption characters (the degree of knowledge, the models and frequency of consumption, the types of products, etc.); the second addressing to the identification of the particular characteristics related to consumer preferences for such products (places of purchase); the third relating to the socio-economic characteristics of consumers interviewed (gender, age, level of education, etc.).
Data on consumers of fortified products in Italy were gathered online via Google Moduli from July to November 2023 through a random sampling method. Respondents were informed of the privacy and anonymity of their answers.
The Principal Component Analysis (PCA) was conducted to identify the particular characteristics of the consumption of fortified products, while summarizing and limiting the loss of information. In fact, through the development of the main component analysis the original variables are grouped into orthogonal factor dimensions. The components selected were those assuming an eigenvalue greater than 1 [9]. The final components were rotated using Varimax rotation to obtain clearer definitions and interpretations of the factorial dimensions [10].
The PCA's formula is the following:

$$Y_i = W_{i1}X_1 + W_{i2}X_2 + \ldots + W_{ip}X_p \quad (1)$$

where $X_1$, $X_2$, ... $X_p$ are the P standardized original variables and $W_{i1}$, $W_{i2}$, ... $W_{ip}$ are the values of the weights associated to each of them.
The validity of the statistical model was tested trough the KMO's test while the Bartlett's test assessed the adequacy of the data to the assumptions of the specified model, through the following expression:

$$KMO = \sum_{i}^{p}\sum_{j\neq i}^{p} r^2\, ij \Big/ \sum_{i}^{p}\sum_{j\neq i}^{p} r^2\ ij + \sum_{i}^{p}\sum_{j\neq i}^{p} c^2\, ij \quad (2)$$

The KMO test takes values 0 and 1. Usually, values below 0.50 are no considered [11] since low values indicates the inadequacy of the analysis in fact [12], who categorized the factor loadings as 0.30 = minimal, 0.40 = important and 0.50 =significant.

The Bartlett's test allows to verify the hypothesis that the correlation matrix coincides with the identity matrix. The increase in the Bartlett's test value determines the decrease of the corresponding p-value, which tests the lack of linear correlation; low test values indicate that the hypothesis can not be excluded and that the use of the factor model cannot be considered appropriate.

Finally, the cluster analysis allow the identification of homogeneous groups among consumers, by using the k-mean method on the factor scores obtained by PCA. The k-mean method is a non-hierarchical classification method wich allows building different clusters through an iterative process by minimizing the Euclidean distances between centroids [13]. The Cluster Analysis helped to determine whether sensory and extrinsic attributes differed significantly.

Once the clusters were obtained, to verify the effective differentiation based on socio-demographic characteristics, inferential statistics were carried out. Specifically, the test chosen was chi-square; in fact, this method can be used for frequency analysis because it allows for verification of the association or independence of two categorical variables [14].

## RESULT AND DISCUSSION

The representation of the results of this survey was organized according to the following grouping:

1. Socio-demographics characteristics
2. Descriptive statistics synthesis
3. Exploratory factor analysis (EFA) results
4. Cluster analysis results

1. The socio-demographics data (Table1) showed that most of the participants belonged to the Generation X category (37.6%), that female were more numerous (58.2%), that the 64.7% had a degree and the 78.2 % were employed. The majority of sample declared to have a monthly net income of 15,001-30,000€ (32.4%) or 30.001-50.000€ (36.5%). The hometowns mainly inhabited by the sample were large towns (45.9%).

**Table 1. Socio-economic characteristics of the sample.**

| *Gender* | *Frequency* | *Percentage* | *Employment* | *Frequency* | *Percentage* |
|---|---|---|---|---|---|
| Male | 70 | *41.2* | Employed | 133 | *78.2* |
| Female | 99 | *58.2* | Unemployed | 2 | *1.2* |
| Other | 1 | *0.6* | Retired | 15 | *8.8* |
| | | | Student | 20 | *11.8* |
| *People generation* | | | | | |
| Generation Z | 28 | *16.5* | *Town size* | | |
| Millennials | 42 | *24.7* | Small town [below 50,000 citizens] | 33 | *19.4* |
| Generation X | 64 | *37.6* | Medium town [50,000-100,000 citizens] | 17 | *10.0* |
| Baby boomers | 34 | *20.0* | Large town [100,000-500,000 citizens] | 78 | *45.9* |
| Silent | 2 | *1.2* | Metropolis [over 500,000 citizens] | 42 | *24.7* |
| *Education* | | | *Monthly net income* | | |
| Primary and Middle school | 2 | *1.2* | Below 15,000€ | 19 | *11.2* |
| High school | 23 | *13.5* | 15,001-30,000€ | 55 | *32.4* |
| Degree | 110 | *64.7* | 30,001-50,000€ | 62 | *36.5* |
| Master / PhD | 35 | *20.6* | Over 50,000€ | 34 | *20.0* |

2. The descriptive statistics synthesis aggregated the variables (Table 2) that later formed the basis of Principal Component Analysis and therefore it preceded it. The

Table displayed the mean and standard deviation and the relative range going between 1 and 5. The majority of the value attributes and consumption motivations had value higher of 3.

**Table 2. Descriptive statistics synthesis of the selected variables.**

| Variable | Mean | Std. Dev. | Min. | Max |
|---|---|---|---|---|
| Value attribute | | | | |
| *Naturalness* | 3.400 | 1.194 | 1 | 5 |
| *Taste* | 3.071 | 1.180 | 1 | 5 |
| *Price* | 2.994 | 1.179 | 1 | 5 |
| *Food safety* | 3.659 | 1.212 | 1 | 5 |
| *Practicality of use* | 2.988 | 1.109 | 1 | 5 |
| *Nutritional properties* | 3.594 | 1.144 | 1 | 5 |
| *Tradition* | 3.053 | 1.218 | 1 | 5 |
| *Origin* | 3.365 | 1.160 | 1 | 5 |
| *Equity* | 3.124 | 1.183 | 1 | 5 |
| *Appearance* | 2.818 | 1.108 | 1 | 5 |
| *Environmental impact* | 3.524 | 1.183 | 1 | 5 |
| Consumption motivation | | | | |
| *I consider it beneficial to eat fortified foods* | 2.888 | 1.117 | 1 | 5 |
| *Regular intake of fortified foods can prevent illnesses caused by unhealthy diets* | 3.041 | 1.163 | 1 | 5 |
| *For fortified foods I am prepared to give up at least some of their taste qualities* | 2.376 | 1.054 | 1 | 5 |
| *I am willing to pay more for fortified foods, it is a question of quality* | 2.529 | 1.131 | 1 | 5 |

3. The Principal Component Analysis (PCA) performed on food values and consumption motivations reduced their number considering only the significant one. The results identified four main latent constructs.

The PCA model extracted three main components and explains about 69.2% of the total variance and its validation was confirmed by the values of the KMO test (0.892) and the Bartlett test (0.000) (Table 3).

The first component explained 32.6% of the total variance. It aggregated only food attributes *Naturalness* (0.712), *Food safety* (0.775), *Nutritional properties* (0.721*), Tradition* (0.700), *Origin* (0.864), *Equity* (0.820) and *Environmental impact* (0.818) all having positive relationship and for this reason the name chosen was “Value drivers”.

The second component, instead, grouped only and all the consumption motivations and, being related to health elements, it took the name “Health factors”. It explained19.4 % of the total variance.

All consumption motivations had a positive correlation and the single factor loading were the following: *I consider it beneficial to eat fortified foods* (0.735); *Regular intake of fortified foods can prevent illnesses caused by unhealthy diets* (0.710); *For fortified foods I am prepared to give up at least some of their taste qualities* (0.826); *I am willing to pay more for fortified foods, it is a question of quality* (0.845).

The third component synthetized the 17.2 % of the total variance and comprehended all food values. It resulted of relevance to notice that the latter were those not included in the first one: *Taste* (0.790), *Price* (0.665), *Practicality of use* (0.545), *Appearance* (0.718). In relation to the variables belonging to “Factor 3” the name chosen was “Aestetic and practice factors”.

**Table 3. Rotated component matrix of variables (*).**

| Variables | *Factor 1* Value drivers | *Factor 2* Health drivers | *Factor 3* Aestetic and practice drivers |
|---|---|---|---|
| Naturalness | 0.712 | | |
| Taste | | | 0.790 |
| Price | | | 0.665 |
| Food safety | 0.775 | | |
| Practicality of use | | | 0.545 |
| Nutritional properties | 0.721 | | |
| Tradition | 0.700 | | |
| Origin | 0.864 | | |
| Equity | 0.820 | | |
| Appearance | | | 0.718 |
| Environmental impact | 0.818 | | |
| I consider it beneficial to eat fortified foods | | 0.735 | |
| Regular intake of fortified foods can prevent illnesses caused by unhealthy diets | | 0.710 | |
| For fortified foods I am prepared to give up at least some of their taste qualities | | 0.826 | |
| I am willing to pay more for fortified foods, it is a question of quality | | 0.845 | |
| Total Variance | *32.6* | *19.4* | *17.2* |
| KMO test | 0.892 | | |
| Bartlett's test of sphericity | 1687.406 | | |
| Sign. | 0.000 | | |

(*) Factor loadings less than 0.50 have not been printed.

4. Cluster analysis results. After having synthetized the food values and the consumption motivations through the PCA, three factors resulted. Starting from these three factors, it was possible to perform a Cluster Analysis (CA) from which 4 homogeneous Clusters derived. The different factorial scores in the centroids obtained by the K-mean application method in each Clusters underlined different characterization among these 4 Clusters (Table 4).

**Table 4. Rotated component matrix of variables (*).**

| Factors | Clusters | | | | p-value |
|---|---|---|---|---|---|
| | 1.Prigs (no. = 26) | 2.Health enthusiasts (no. = 24) | 3.Aestetes and pragmatists (no. = 55) | 4.Sceptics (no. = 65) | |
| Value drivers | **1.329** | -0.024 | 0.353 | **-0.822** | .000 |
| Health drivers | -0.862 | **1.431** | 0.272 | -0.414 | .000 |
| Aestetic and practice drivers | -0.704 | -0.773 | **0.957** | -0.243 | .000 |
| Generazione Z - Zoomers | 11.54 | 8.33 | 36.36 | 4.62 | |
| Generazione Y - Millennials | 26.92 | 16.67 | 21.82 | 29.23 | |
| Generazione X - Transizione | 42.31 | 54.17 | 18.18 | 46.15 | .001 |
| Baby boomers | 19.23 | 16.67 | 21.82 | 20.00 | |
| Silent | 0.00 | 4.17 | 1.82 | 0.00 | |

The first Cluster represented the 15.3% of the sample and showed high interest on many value's attributes and for this reason it took the name "Prigs". Quality and sustainability could synthetize the nature of the attention of Cluster 1. In fact, the nutritional properties, the naturalness and the food safety are all quality attributes. Tradition and quality also refered to sustainability next to equity and environmental impact. The first group responded to quality in the sense of the risk assessment of the product, while the second group refered to quality as attributes that come after the positive evaluation of food safety [15] giving additional value to the final product.

The second Cluster constituted the 14.1%. It grouped customers who pay great consideration to healthful aspects and so they received the name of "Health enthusiastic".
Specifically, they revealed a high sensitivity to all safety motivations when choosing to consume fortified products, so much so that they were willing to pay more when they find these characteristics.
The third Cluster aggregated the 32.4%. They represented the "Aestetic and practice factors" since they responded high positively to the Taste, Price attributes, but they also seeked for Appearance characteristics and for the Practicality of the use of food products. Slight positive results emerged also for value drivers and health factors.
The fourth Cluster grouped the 38.2% and included "Sceptic" consumers as all the factorial scores had negative values. In fact, this group was not sensitive to value attributes nor did it showed relevant consumption motivations.
The relation between Cluster analysis results and socio-demographic data showed significance only for the age of the sample. For three Clusters 1, 2 and 4 in particular the "Generation X" prevailed (Table 4). These results are not surprising since generation x is the most numerous in the reference sample. Therefore, the clusters do not differ in relation to generation, but consumers within the same category showed different attitudes.

## CONCLUSION

Innovations represent a continuous and significant contribution to the modernisation and development of the agri-food system coming from the supply side, which, however, must meet the consent of the demand side to lead to market success. The present investigation aimed at identifying the Italian fortified product customer profile to ascertain its willingness to consume this specific category of food while identifying the food attributes and motivations behind its consumptions' choices.
This research applied a comprehensive methodology including the PCA statistical analysis that synthetized the food values and the consumption motivations comprised in the ad hoc questionnaire shared with the sample, and a non-hierarchical cluster analysis to categorize fortified products consumers.
The characterizing elements of each of the four Clusters resulted shaped different costumers' categories. In fact, in the Prigs, Health Enthusiasts and Sceptics only one value showed positive result highlighting its mark while the other were negative. Only in the Aestetes and Pragmatics Cluster all value resulted positively significant, therefore aggregating common traits among the factor components.
Addressing special attention to the function of market information emerged as crucial in the intention of intensify fortified trade market, since awareness impact consumers' decisions to buy fortified foods. This product, being the result of innovation advancement, pointed out the concrete health benefits that its consumption can generate. Fortifying food represents an effective and accessible innovation method of securing the population with vitamins and micronutrients. In this contest, market information could fight the information asymmetry, notably characterizing the agri-food sector, and in the meantime could promote a change in customers' habits in a way that they could assume fortified products on a daily basis. Indeed, health-related marketing strategies could have a significant impact on market dynamics.

## ACKNOWLEDGEMENTS

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# FORECASTING WEEKLY COW MILK PRODUCTION USING A MULTIVARIATE TIME SERIES APPROACH

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## ABSTRACT

The continuous rise in global population necessitates the prediction of food resources, among which milk stands out as one of the staples. Dairy farms and processing plants need to know how much milk they will have available to manage their storage and processing capacities efficiently. Overestimating production can lead to wastage, while underestimating can lead to shortages. Knowing the expected milk quantity helps in planning the transportation, processing, and distribution of milk and milk products.

This study presents a cutting-edge method for forecasting weekly cow milk production using a multivariate time series approach. Leveraging collected data from IoT devices for environmental variables, genetic data from health diaries, and health metrics alongside traditional temporal data, this research aims to provide a comprehensive model for predicting milk yields. A multivariate time series model SARIMAX was trained, tested and evaluated, focusing on ensuring both accuracy and robustness. The findings demonstrate that by integrating multiple data streams, significant improvements in forecast precision can be achieved. Furthermore, this integrated approach provides insights into the key factors influencing weekly milk production, paving the way for informed strategies in dairy management. The proposed model showcases the potential for scalability across different dairy operations and regions, contributing to the global effort towards food security and sustainable agriculture practices.

**Keywords:** Weekly Milk Production, Multivariate time series forecasting, SARIMAX, endogenous and exogenous features

## INTRODUCTION

More than 264 million dairy cows are globally, collectively yielding nearly 600 million tonnes of milk annually [1]. Research indicates that when cows are primarily fed on locally grown grass around the farm, their milk production remains at a low-intensity level, with each cow typically producing around 3,500 litres per year. However, the results can be significantly more productive by adopting a different approach, where farmers cultivate corn and soy crops to feed their cows. In such cases, cows can produce more than 8,000 litres of milk per cow per year [2]. Sustainable development and successful farm management require convincing short-term and long-term milk production planning. Knowing the expected milk quantity helps in planning the

transportation, processing, and distribution of milk and milk products. Also, dairy farms can adjust their feeding and maintenance schedules based on the predicted milk yield. A clear forecast helps dairy businesses in budgeting, financial planning, and predicting revenues. Retailers and consumers expect a consistent supply. By forecasting production, the dairy industry can meet consumer expectations more effectively, ensuring that shelves are stocked, and products are fresh. Overproduction can lead to waste, which has environmental implications. Proper forecasting helps in aligning production with demand, reducing wastage, and its associated environmental footprint.

An overview of different models for predicting milk yield is presented in [3] where it is noticed that as herd size increase, the ability to predict future production using empirical curve forecasting models developed at lower milk production levels becomes problematic. The reason for this is due to increased milk production, which leads to greater differences between peaks and troughs.

Research is directed towards the search for different approaches to predicting milk quantity. Recently, other methods such as deep learning models have been proposed to predict milk production [4].

The amount of milk produced on a farm depends on many factors such as diet characteristics [5], rearing conditions, lactation cycles and external environment. This makes it difficult to build forecasting models and therefore cannot be successfully modelled as a univariate time series. Besides this, univariate forecasting methods have limited forecasting abilities when the data is missing or erroneous [6, 7]. On the other hand, too many variables in some multivariate prediction models without considering the application scenarios can lead to high computational complexity [8].

This paper aims to combine different types of data from both cows and macro-environmental factors to estimate weekly milk production. First, data were collected from a dairy cow farm and a selected set of variables. Various techniques were then performed to process this data. Further, seasonal autoregressive moving average with external input (SARIMAX) was established to correlate milk production with several exogenous variables. This can create a basis for better farm management in line with sustainable development trends.

## MATERIALS & METHODS

In this work a multivariate time series forecasting is proposed to predict weekly milk quantity in the farm using the past values of several variables. Following variables are used in the model:

*DateTime_x:* This is the timestamp. The ordering process of the observations and identifying the frequency of observations are based on it.

*Gender, Age, Lact., DIM:* Individual characteristics of the cow. They can influence milk production.

*Temp, Humidity, Cloud, Rain:* External environmental factors. They are important as they can have significant effects on milk production. Weather-related variables like this exhibit seasonality.

*Daily milk:* Daily milk quantity. This is the target variable.

The dataset contains 53036 records for 645 female cows. The number of cows varies during the observation period. Data were taken from different cows for this period. On average for the whole period there are records of 48 cows per day.

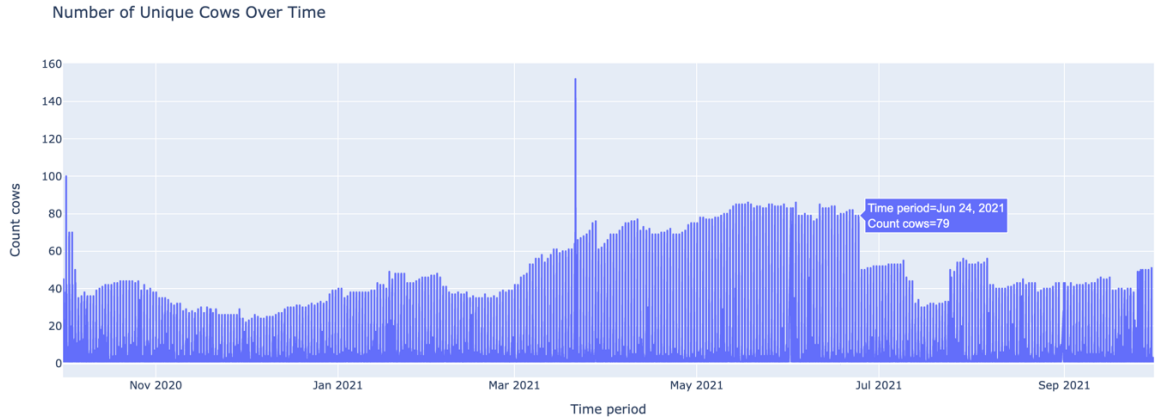



Figure 1: Records of number of cows per day for the entire period.

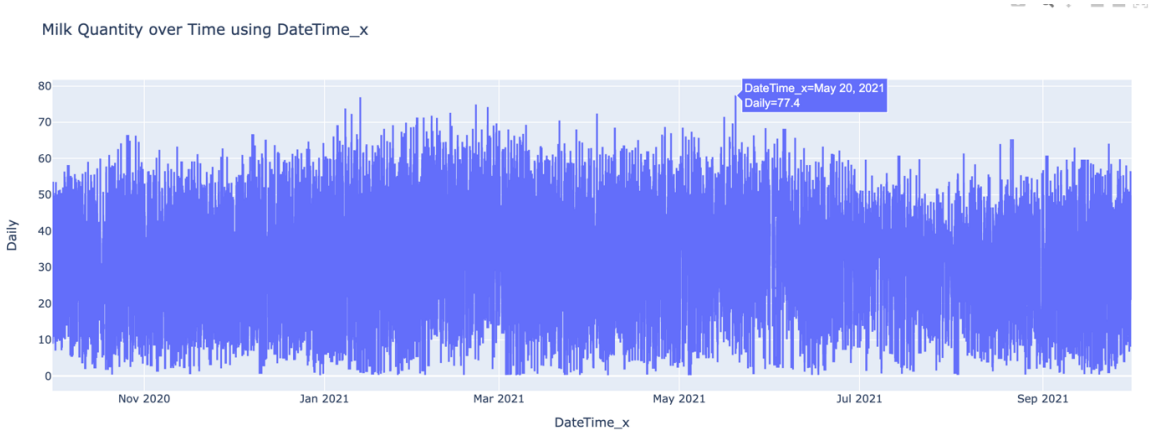



Figure 2: Average amount of milk per day per cow (raw data) in lb.

The workflow for multivariate time series forecasting of milk production sequentially applies different data processing techniques and methods [9] by following steps:

- Exploratory data analysis (EDA)
- Data processing –
    - Set correct data type.
    - Feature engineering – extract only female cows from Gender column.
    - Handling missing values. Missing values in *Lact., DIM, Daily.* The interpolate method is used to fill in missing values.
    - Outliers' management. The IQR method is used for outliers handling. The lower bound is set to 0.25, upper bound is set to 0.75, and threshold values is set to 1.5. The outliers and data distribution are shown on Fig. 3.
- Derive new features.

- Aggregate features by week. The number of rows has been reduced to 52, corresponding to 52 weeks of data. Three data frames are created.
- Merge data frames.
- Lag features. It refers to a feature that's derived from a previous value in the time series data. Looking back in time at previous data points.
- Modelling.

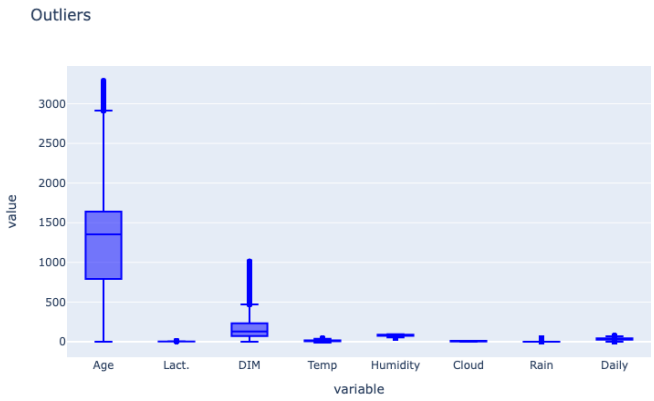



Figure 3: Data distribution.

**Derive new features.** Three new features were derived from the dataset, namely: Ratio of Rainy Days to Non-Rainy Days in a Week (Let's assume a day is considered "rainy" if the 'Rain' column has a value greater than 0), Metrics Capturing Significant Temperature Drops or Spikes (Let's consider a 5-degree change as significant) and Metrics Capturing Significant Humidity Drops or Spikes. A feature that indicates a season is created based on *Datetime* column.

**Rolling window.** A four-week rolling window for the average milk production is applied as a method to smooth out short-term fluctuations and better observe underlying trends in the data without obfuscating the weekly patterns. This can identify if there's a clear trend in milk production. Figure 4 shows weekly milk production over time, both raw and smoothed using a 4-week moving average.

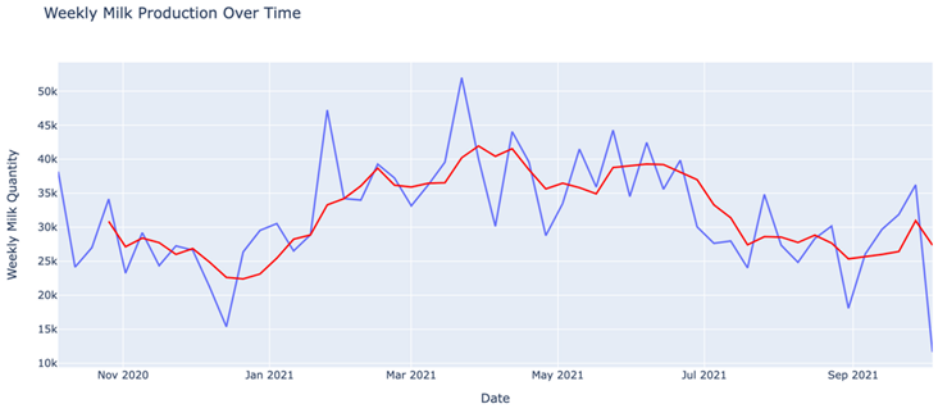



Figure 4: A four-week rolling window for the average milk production.

The red line, which is the 4-week rolling average, shows a smoother representation of milk production. From the figure, it seems there's a downward trend in milk production

starting from around May/June, which continues up to August/September. This could indicate seasonal factors affecting milk production during these months. The blue line, which represents the raw weekly milk data, shows significant fluctuation. This might suggest that there are certain weeks or months when milk production sees an uptick or a drop, potentially due to seasonal effects, changes in weather conditions, or other external factors.

In summary, the figure shows that while there are short-term fluctuations in weekly milk production, there might also be a broader seasonal trend influencing the output. This could be due to factors like changes in weather, operational changes, or cow-related factors like breeding cycles.

**Lag features.** Four new columns for weekly milk production are created that represent the values of the original feature from previous time points. It provides a mechanism for the model to consider historical data points when making a prediction.

**Split and normalize data.** The process of splitting the training and testing data is done in a ratio of 80:20. Then, scaling is done only on the training data. The Standard scaler is applied so the data have the properties of a standard normal distribution with $\mu=0$ and $\sigma=1$.

**Time-series data decomposition.** The Seasonal and Trend decomposition uses Loess to break down the series into its trend, seasonal, and residual components. This provides clearer insights into the type and strength of seasonality in the data. The results are presented in Figure 5.

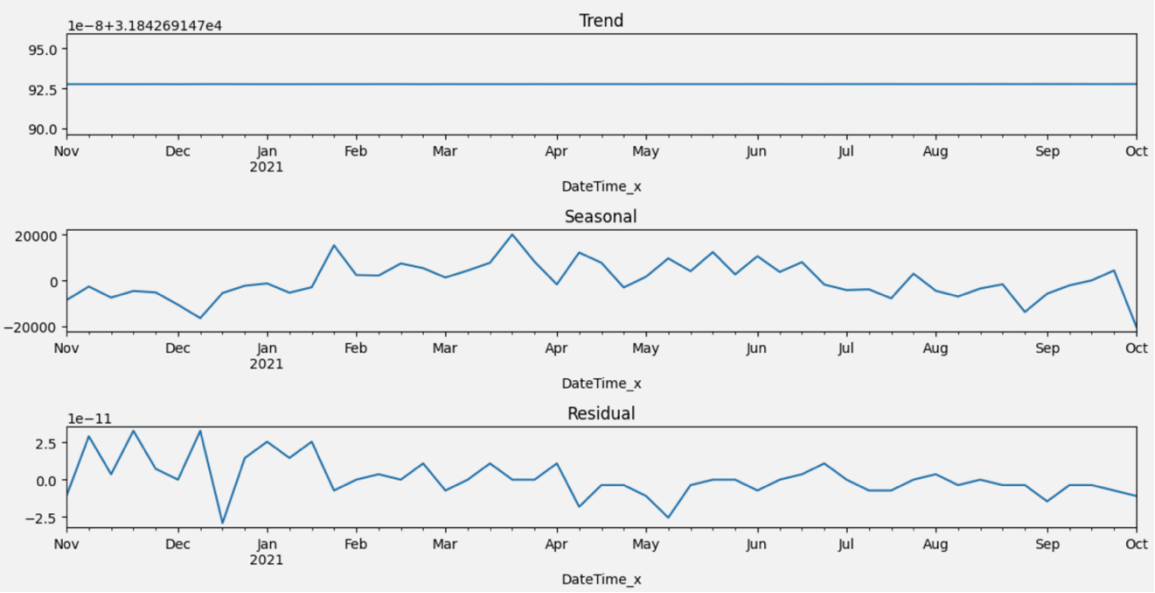



Figure 5. Seasonal and Trend decomposition uses Loess (Locally Weighted Scatterplot Smoothing).

The trend represents the underlying trend in the data. The trend component shows how the data would look if could remove the seasonal fluctuations and random noise. From the plot, the trend seems relatively flat, suggesting that there isn't a strong upward or downward movement in the milk quantity over the period observed.

Seasonal captures the repeating patterns or cycles in the data. The plot shows a consistent repeating pattern, though it's not especially pronounced. This indicates some seasonality in the data, though it's relatively subtle.

Residual is the noise left over after removing the trend and seasonal components from the observed data. The residual plot should ideally look like random noise, which means it shouldn't have any discernible patterns. In the presented plot, the residuals seem to hover around zero with no evident pattern, which is a good sign. This indicates that the decomposition has captured the main structures of the data.

The time series does seem to have some seasonality, though it's not overwhelmingly strong. Based on the obtained results, it is established that machine learning models should be chosen that can be considered the seasonality of the data.

**Data modelling**. The SARIMAX (Seasonal Autoregressive Integrated Moving Average with eXogenous variables) model extends the SARIMA model by including additional external (exogenous) variables. The basic formula of the SARIMAX model is:

$$\phi_p(B)\Phi_P(B^s)(1-B)^d(1-B^s)^D y_t=\theta_q(B)\Theta_Q(B^s)\,\varepsilon_t+\beta X_t \qquad (1)$$

where $\phi$ and $\theta$ are polynomials of the autoregressive and moving average components. $\Phi$ and $\Theta$ are polynomials for seasonal autoregressive and seasonal moving average (AR and MA) parameters, $y_t$ is a predicted variable, and $\varepsilon_t$ is the random error at time t, "X" means the inclusion of exogenous (external) variables in the model; (p, d, q) are the non-seasonal parameters: p is the auto-regressive part, d is the differencing, q is the moving average part.

Parameters (P, D, Q, s) are the seasonal: "P", "D", and "Q" are like "p", "d", and "q" but for the seasonal component; "s" is the number of time steps per season (seasonal order).

The "s" parameter represents a quarterly seasonality in a weekly dataset. A quarter typically has around 13 weeks (since there are 52 weeks in a year and four quarters in a year: 52 ÷ 4 = 13). Therefore, the 's' parameter should be set to 13 to capture the quarterly seasonality. Each calendar year is divided into four quarters, each typically encompassing three full calendar months. Generally, each month has about 4.33 weeks.

We have two types of features: endogenous feature *Daily Milk* and exogenous features: *'Avg_Temp', 'Temp_Spikes', 'Avg_Humidity', 'Humidity_Spikes', 'Avg_Cloud', 'Total_Rain', 'Rainy_Day_Ratio', 'Avg_Age', 'Avg_Lact', 'Avg_DIM', 'Season', 'Rolling_Avg', 'Weekly_Milk_lag_1', 'Weekly_Milk_lag_2', 'Weekly_Milk_lag_3', 'Weekly_Milk_lag_4'*.

## RESULTS

Selecting the best parameters for a SARIMAX model requires understanding both the nature of the dataset and the underlying patterns within it. In this case, the monthly data are for 52 months, with a seasonal pattern given the four seasons.

A SARIMAX model was configured with the parameters (p,d,q) = (4,1,4) for the non-seasonal part and (P,D,Q,s) = (1,1,1,13) for the seasonal component. Using these

parameters, the SARIMAX model was trained and the results with the following error metrics are given in Table 1.

Table 1: Error metrics.

| Model | MAE | RMSE | MAPE |
|---|---|---|---|
| SARIMAX | 342.36 | 467.71 | 2.21 |

The model's predictive accuracy was evaluated using three error metrics:

- MAE (Mean Absolute Error) is the mean absolute error between the predicted and true values. The result of 342.36, indicates the acceptable magnitude of errors in the predictions.
- RMSE (Root Mean Squared Error) is the root mean squared error, which makes large errors weight more heavily [10]. The RMSE of 467.71 is a higher value, suggesting larger errors in the model's predictions. It suggests the possibility of outliers.
- MAPE (Mean Absolute Percentage Error) is the mean absolute percentage error, which indicates that on average the predictions deviate by about 2.21% from the true values. This metric is useful because it is scale-independent and gives an idea of the error relative to the magnitude of the values being predicted. These metrics help evaluate the accuracy of the model. In this case, a MAPE of 2.21% can be considered a relatively low error, meaning that the model generalizes well to its input data. The low MAPE value is encouraging because it suggests that the model is generally accurate to within a few percentage points of the actual values. This is particularly useful in many practical applications where such a degree of accuracy is acceptable.

The SARIMAX model with the given parameters demonstrates a good balance between complexity and predictive power, with some indication of some errors that may need to be investigated further.

## CONCLUSION

In summary, we analysed the problem of forecasting the amount of weekly milk production one period ahead using forecast coefficients. Our analysis has shed light on the key components of time series data and the importance of understanding trends, seasonality, and residual noise. The data we examined revealed a relatively flat trend, suggesting minimal significant fluctuations in milk quantity over the observed period. However, a subtle yet consistent seasonal pattern was evident, highlighting the presence of seasonality in the data.

To address this seasonality, we used to Seasonal Autoregressive Integrated Moving Average with eXogenous variables model, an approach that incorporates external variables. Our SARIMAX model was meticulously configured, with non-seasonal parameters and seasonal parameters, considering the quarterly seasonality within the weekly dataset.

The results of the modeling indicate promising predictive accuracy where the mean absolute error registered at 342.36, signifying a reasonable level of prediction accuracy. The root mean squared error reached 467.71, suggesting the presence of larger errors, potentially stemming from outliers. The mean absolute percentage error was calculated at 2.21% from the true values.

In conclusion, our SARIMAX modeling approach, tailored to the specific characteristics of the dataset, holds the potential to provide valuable insights and predictions in the context of milk quantity forecasting, with room for further refinement to enhance its robustness.

## ACKNOWLEDGEMENTS

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# FORMULATION OF OLEOGELS BASED ON CANDELILLA WAX: PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERIZATION

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**Lecturer PhD Florina Dranca**[1]
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## ABSTRACT

The aim of the study was to characterize the gelling effect of candelilla wax (DW) using different types of cold-pressed vegetable oil (sunflower oil, olive oil, walnut oil, grape seed oil and hemp seed oil). The structuring of the oleogels was achieved by hot incorporation of oil with different percentages of carnauba wax, as follows: 3%, 5% and 11%. Characterization of the oleogels was done by determining the chemical and physical properties of the oleogels, the oil binding capacity and the texture of the oleogels were determined. The color differences were highlighted by the CIE Lab color scale for evaluating the lightness index (L*), components a and b. Candelilla wax showed a strong gelling capacity of over 99.70% for the addition of 11% and capacity minimum of 74.34% for the 5% addition. The value of the peroxide index decreased when the oleogelator concentration increased. The rheological properties were determined by measuring the viscoelastic parameters. The frequency sweep test showed that the storage modulus, loss modulus and viscosity of candelilla wax oleogels were increased with increasing wax concentration. The obtained results demonstrate the fact that candelilla wax can be used in small concentrations to obtain oleogels with a solid texture and easy to incorporate into food products as solid fat substitutes. Harder oleogels, obtained by adding 11% wax, can be effective in delaying oil oxidation during storage. Therefore, these oleogels illustrate that candelilla wax can be of great value in the food field.

**Keywords:** vegetable oils, oleogels; physical-chemical properties; textural properties; rheological properties.

## INTRODUCTION

Oleogelation is a process by which oil in fluid form is transformed into a colloidal or non-fluid polymer network using wax or other oleogelator as an oleogelation agent. As commonly accepted, a gel is essentially a network, the definition of which, as given in any language dictionary, is: "a system consisting of lines, tubes, wires, etc. that cross or are connected to each other'. Oleogels can retain large amounts (up to 99%) of the liquid phase, absorb and release substances, and respond to various physical and chemical

stimuli such as temperature, light, pH, and mechanical deformation. Considering the wide range of fats and vegetable substances with compatible gelling role, the properties and functionalities of organogels are varied. Oleogels have been known and used in the food industry because they retain large amounts of oil, often making them compatible with the properties of animal fats or margarine. Regarding the current state of the food market, structured lipid phases are based almost exclusively on networks of fat crystals. Oil as such does not have an optimal structure to impart specific texture and flavor to foods in a similar way to fats with high levels of trans and saturated fatty acids, making them unsuitable for direct application in many processed foods. Recent studies have shown that organogels can exhibit technological performance very similar to a solid fat, making it possible to use them in some food applications [1-3].

The use of oleogels in food has been imposed with a number of problems currently associated with the use of traditional fats in modern human nutrition, mainly related to the presence of high content of saturated and trans fatty acids [3]. To maintain texture and palatability in the food industry, the amount of saturated fat has increased considerably, which has also been shown to be incompatible with human health. In recent years, studies have shown that diets with high intakes of this type of fat have negative health consequences, contributing to the appearance of several diseases in humans but mainly increasing the risk of developing cardiovascular diseases and diabetes, which significantly reduce life. quality. Due to the strong association of increased risk of cardiovascular disease and other non-communicable diseases with consumption of saturated fatty acids and trans fatty acids be lower over 10% and 1% of total energy consumption, respectively (OMS, 2018 ). Thus, specialists in the field approached ways to develop healthy alternatives to solid fats. In this context, oleogelation has gained increased attention as the most promising oil structuring technique to produce solid-like lipids called oleogels that mimic the properties of traditional solid fats from highly unsaturated oils without trans fatty acids.

Waxes are the most studied oleogelators due to their ability to gel at low concentrations [4-6]. Among the different types of wax, candelilla wax has the property of forming viscoelastic gels at low concentrations [7-8]. From a sensory point of view, the wax used as a gelling agent has the property of being felt when tasting, which is considered a major disadvantage for consumers.This study focused on the development of an oleogel from five varieties of edible oils: grape seed oil, hemp seed oil, olive oil, sunflower oil and walnut oil. Oils that can be found in grocery stores at an affordable price were chosen because the economic criterion is very important when you want to replace margarines in food products. Candelilla wax (DW) is a natural plant wax derived from *Euphorbia antisyphilitica* Zucc., which is grown in the arid regions of northern Mexico. It was reported that candelilla wax is mainly comprised of n-alkanes (26–33 carbons; hentriacontane as the main component) > high molecular weight esters (29–33 carbons) > alcohols and sterols (20–29%) > free acids (7–9%) and > resins (12–14%, mainly triterpenoid esters). According to the Food and Drug Administration (FDA), candelilla wax is classified as a food additive and even if an acceptable daily dose (DZA) has not been established, its use in food has been accepted. Candelilla wax is a promising option for food use and the development of safe and high-quality foods [9].

## MATERIALS AND METHODS

### *1. Materials*

The vegetable oils used for oleogels production were cold pressed ones and were purchased from companies specializing in natural products. The following vegetable oils used in this study were the following: olive oil (OL), grape seed oil (GO), sunflower oil (SO), walnut oil (WO) and hemp seed oil (HO). The gelling agent used was candelilla wax (DW) (Sigma-Aldrich, Hamburg, Germany).

### *2. Methods*

#### *Oleogels production*

Oleogels were obtained using vegetable oils from 5 sources (olive, walnut, sunflower, grape seed and hemp). Candelilla wax were used as oleo-gelation agents in percentages of 3%, 5% and 11%. The oleogels were obtained according to the method described by Ropciuc et al., 2023. The oils and wax were heated to a temperature of 90ºC using a hotplate with an orbital stirrer until the wax completely dissolved. The oleogel samples were quantitatively placed in glass tubes and left at a temperature of 22-23ºC for cooling. Afterwards, the oleogel samples were kept in the refrigerator for stabilization. In order to evaluate the various parameters of the oleogel, it is necessary to bring back the samples to room temperature at least 1 h before starting the tests.

#### *Physicochemical analysis*

*The colour characteristics*

The color characteristics namely the darkness/brightness (L*), shade of blue/yellow (b*), shade of red/green (a*) were determined using the CIELab system (Konica-Minolta 200, Tokyo, Japan). The method used for analysis was previously described by Ropciuc et al. [10].

*Peroxide index evaluation*

The determination of the peroxide index was determined using the international standard method ISO 3960:2007 after the method previously described by Ropciuc et al. [10].

*The oil binding capacity*

The oil binding capacity was determined according to the method described by Ropciuc et al. [10]. For this purpose, 1.0 g of oleogel was centrifuged using a microcentrifuge (Hermle Z206A) for 15 minutes at 4000 rpm at 24℃.

#### *Rheological behavior of oleogels*

The rheological measurements were performed using a Haake Mars 40 dynamic rheometer (Karlsruhe, Germany) equipped with Peltier system and circulating water bath (Huber, Germany) for temperature control. A glossy plate rotor with a diameter of 40 mm was used, the distance between the plates being 1 mm. The linear viscoelastic region (LVR) of the oleogels was determined by logarithmic growth the oscillation stress from 0.01 to 1000 Pa at a frequency of 1.0 Hz. The frequency sweep range was set from 0.1 to 100 Hz [.

#### *Statistical Analysis*

All data were expressed as mean ± standard deviation. The statistical significance of differences between samples was tested by using one way analysis of variance (ANOVA) with Tukey's test, to determine the significant differences at a 5% level. Results reported in this study are mean values, and significant differences between

samples were analyzed by one-way analysis of variance (ANOVA) by Tukey's test ($p \leq 0.05$) using XLSTAT software, ver. 2023 (Addinsoft, New York, NY, USA).

## REZULTS AND DISCUSSION

Physical-chemical composition of different types of oleogels based on carnauba wax is shown in Table 1. DW is capable of producing a strong oleogel when used at very low concentrations (1.5%)[11-12]. The low polarity, long chain length and high ability to bind the oil in the structure provide excellent crystallization properties of the liquid oil even at low concentrations (3%) makes this type of wax successfully used for structuring the oil in a structure stable. This property results from the fact that the wax has as its main component hentriacontane, as well as other n-alkanes in smaller quantities, such as nonacosane and tritriacontane, which have the capacity to self-assemble in various non-polar solvents, resulting in the development of microcrystalline structures that form a type of plate in all cases. These microcrystalline units are the primary structures that form the three-dimensional crystalline network of n-alkanes in oleogels [9]. The low polarity, long chain length, and high melting point of the components found in waxes provide excellent crystallization properties to the liquid oil, and therefore even at low concentrations, they can crystallize into a well-formed network with strong oil-binding properties [11] .

The color parameters for the oleogel samples obtained from different oils differ depending on the color of the oil sample. The value of the brightness parameter (L) decreases in the samples obtained with hemp oil (HO) and olive oil. They have a darker color, a fact that is also noticeable by the low value of this parameter. Oleogels with grape seed oil (GO) show the highest values of the L parameter because it has the lightest color. When the percentage of added wax increased, the product was slightly brighter and would have a matte appearance, similar to margarine due to the intense crystallization of the oil in a highly stable network. Parameters a and b vary with the percentage of added wax and the color of the oil. When the percentage of wax increases, the oleogels become lighter in color. The high value of b* indicates yellow variations.

Table 1. Physical-chemical composition of different types of oleogels based on candelilla wax

| SAMPLE | L* | a* | b* | IP, meqO2/kg | OBC, % |
|---|---|---|---|---|---|
| **GO_3DW** | 54.464 $^{b}$ | -6.654 $^{cde}$ | 17.104 $^{h}$ | 0.445 $^{a}$ | 95.584 $^{ab}$ |
| **GO_5 DW** | 63.825 $^{ab}$ | -7.876 $^{de}$ | 22.139 $^{de}$ | 0.552 $^{a}$ | 97.778 $^{ab}$ |
| **GO_11 DW** | 68.216 $^{a}$ | -8.211 $^{e}$ | 25.534 $^{cd}$ | 0.274 $^{a}$ | 99.692 $^{a}$ |
| **HO_3 DW** | 47.929 $^{cd}$ | -5.856 $^{bcd}$ | 25.383 $^{cd}$ | 0.944 $^{a}$ | 70.606 $^{d}$ |
| **HO_5 DW** | 48.188 $^{d}$ | -5.187 $^{ab}$ | 20.819 $^{fg}$ | 0.914 $^{a}$ | 77.786 $^{cd}$ |
| **HO_11 DW** | 50.189 $^{c}$ | -7.503 $^{bcd}$ | 24.082 $^{de}$ | 0.982 $^{a}$ | 89.789 $^{a}$ |
| **OL_3DW** | 49.512 $^{d}$ | -6.680 $^{cde}$ | 30.739 $^{b}$ | 1.292 $^{a}$ | 54.995 $^{e}$ |
| **OL_5 DW** | 48.174 $^{cd}$ | -7.259 $^{cde}$ | 28.117 $^{bc}$ | 0.865 $^{a}$ | 57.581 $^{e}$ |
| **OL_11DW** | 48.934 $^{cd}$ | -7.606 $^{de}$ | 34.124 $^{a}$ | 0.488 $^{a}$ | 87.592 $^{c}$ |
| **SO_3 DW** | 51.008 $^{bc}$ | -5.560 $^{cde}$ | 17.689 $^{gh}$ | 0.859 $^{a}$ | 90.045 $^{ab}$ |
| **SO_5 DW** | 57.158 $^{b}$ | -6.589 $^{bcd}$ | 24.072 $^{de}$ | 0.603 $^{a}$ | 95.855 $^{ab}$ |
| **SO_11 DW** | 57.389 $^{bc}$ | -6.345 $^{bcd}$ | 27.127 $^{cd}$ | 0.292 $^{a}$ | 99.009 $^{a}$ |

| | | | | | |
|---|---|---|---|---|---|
| **WO_3 DW** | 52.742 $^{bc}$ | -3.050 a | 25.920 $^{cd}$ | 0.860 $^{a}$ | 85.087$^{c}$ |
| **WO_5 DW** | 50.467 $^{c}$ | -3.629 $^{ab}$ | 24.204 $^{de}$ | 0.789 $^{a}$ | 87.722 $^{ab}$ |
| **WO_11DW** | 50.848 $^{c}$ | -4.672 $^{abc}$ | 19.829 $^{fg}$ | 0.424 $^{a}$ | 89.838 $^{ab}$ |

OL – oleogel with olive oil, GO – oleogel with grape seed oil, WO – oleogel with walnut oil, HO – oleogel with hemp seed oil, SO – oleogel with sunflower oil, L* – lightness (L* = 0 is black and L* = 100 is white), a* (-a = green and +a = red), b* (-b = blue and +b = yellow), IP-peroxide index, OBC-oil binding capacity; p<0.0001, a-g different letters in the same column indicate significant differences among samples, according to the LSM test with α=0.05.

These shades of yellow are noticeable in walnut oils (WO) and sunflower oil (SO) where carotenoid compounds are also present. The color of oleogels is an important sensory indicator for use in food and attracting consumers. The peroxide index is an indicator of the oxidation of oleogels. This value is within the limits of 0.292 mg/mL (SO_1DW) and 1.292 mg/mL (OL_3DW). The addition of candelilla wax decreases the value of the oxidation index, this aspect can be considered as a method of improving the oxidative stability for these categories of oils.

The OBC study evaluated the structuring capabilities of the oil in the oleogel network, as well as the gel stability of each formulation. As shown in Table 1, the concentration of beeswax as oleogelator significantly influenced the OBC percentage of the oleogel. As the percentage of candelilla wax increased, so did the stability of the oil in the structure. The samples with DW and sunflower oil (SO), grape seed oil (GO) and walnut oil (WO) in percentages of 5-11% produced an oleogel that presented a structure with higher hardness compared to other evaluated oils, which may lead to delayed oil oxidation during storage [12]. Olive oil (OL) oleogel showed higher oil loss (54.995%) than GO grape seed oil oleogels (99.778%). This is probably due to the composition of grape seed oil in fatty acids capable of forming an extremely stable structure. The same results were reported in other researches [12-13].

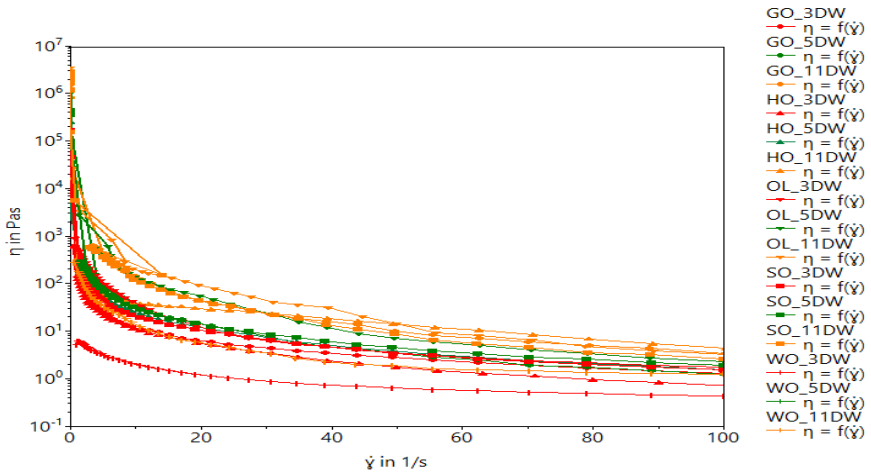



Figure 2. Behavior of the oleogel viscosity with respect to the shear rate

All oleogels showed shear-thinning behavior (Figure. 2). This behavior is in agreement with that reported for oleogels based on candelilla wax (Jang et al., 2015). The viscosity in the shear rate range of 0.0–100 s -1 is well described by the power law $\eta=\gamma^{n}$, Where γ is the shear rate and is the flow behavior index. Oleogels made from 3% DW show remarkable stability without oil separation [14]. The viscosity reduction effect is noticeable in the first stages of shearing. This aspect can be associated with the specific

crystallization structure of candelilla wax. As research describes, candelilla wax produces granular crystallization, large crusts that break under shear.
Figure 2. A, B, and C a show the behavior of the storage and loss moduli with respect to strain frequency. Graph A- represents the vascoelastic moduli for 3% wax oleogels and are expressed as predominantly linear values. The predominantly elastic behavior of the oleogel samples is observed.G′ predominated over G″ over the entire frequency range. Graphs B and C represent the vascoelastic properties for the 5 and 11% oleogels [15].

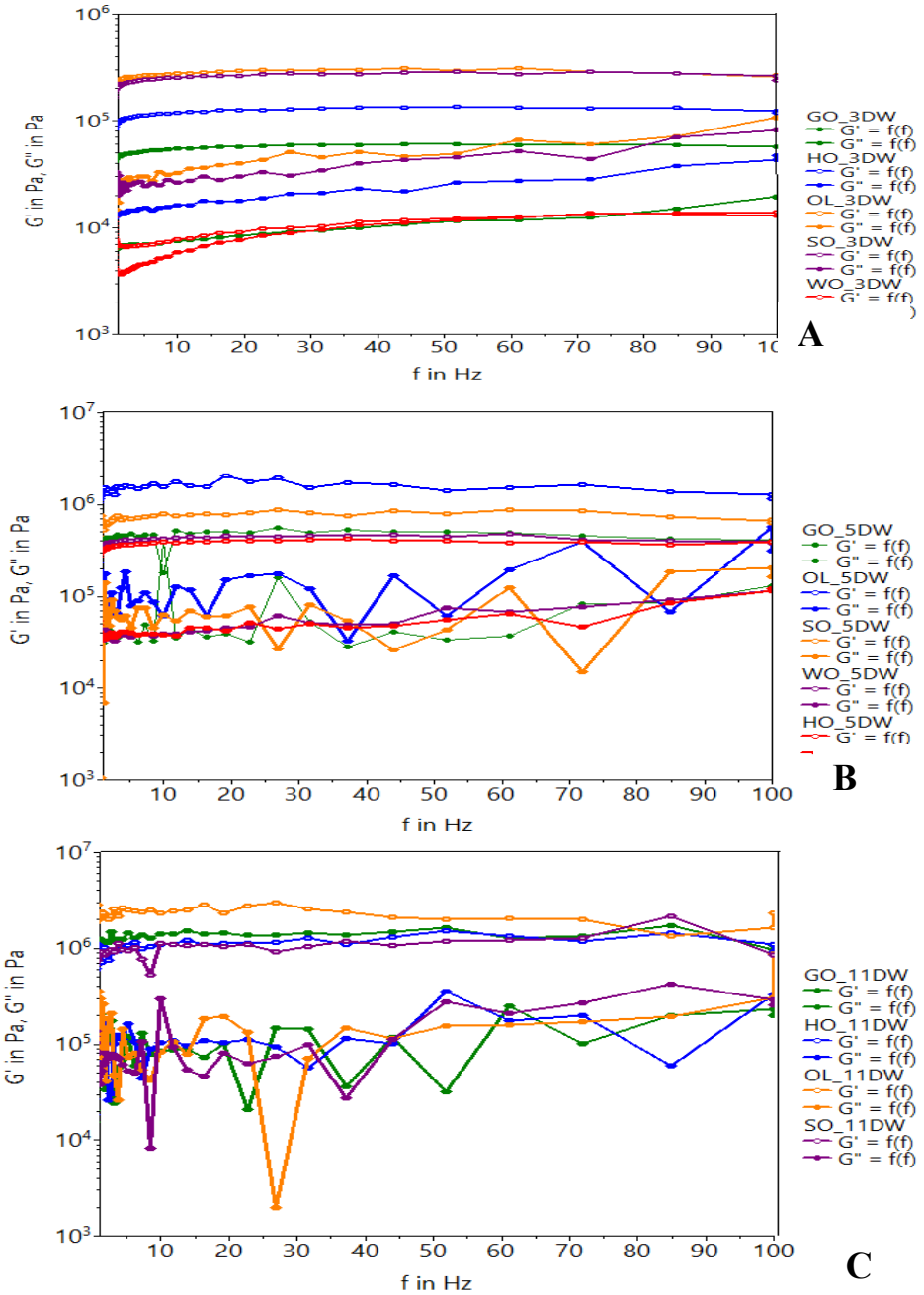



Figure 3. Graphic representation of storage modulus (), loss modulus (G") depending on frequency. A- G' G" modules for oleogel with 3% candelilla wax; B- G' G" modules for oleogel with 5% candelilla; C- modules G' G" for oleogel with 11% candelilla wax

The G″ modulus underwent a significant change in the additions used reflecting the perturbation of the oleogel structure induced by large shear deformations. The G″ modulus underwent a significant change in the additions used reflecting the perturbation of the oleogel structure induced by large shear deformations. Both the storage modulus (G′) and the loss modulus (G″) underwent a perturbation of the linear structure due to the gelling mode of the candelilla wax. Research suggests that candelilla wax gels as platelets and not as crystals as other waxes [14,15].

## CONCLUSION

This work investigated the effect of candelilla wax as a gelling agent for five different vegetable oils (grapeseed, hempseed, olive, sunflower and walnut). The basic idea was that vegetable oil is better for human health in terms of chemical composition than margarine, because it contains a high amount of unsaturated fatty acids. The results of this study showed that the oleogels obtained have different physicochemical properties in terms of colour, oil retention capacity in the structure and acidity index. Colour is an important property because oleogels with variations of green or intense yellow introduced in different food products can negatively influence the consumer's decision. The oil binding capacity in the structure is a property that describes the stability and structure characteristics of the oleogel obtained. According to the results obtained, the percentage of 3% candelilla wax is considered sufficient for stable gelling and ideal rheological properties. According to the results obtained, the percentage of 3% candelilla wax is considered sufficient for stable gelation and ideal rheological properties. The addition of wax in percentages of 5 and 11% leads to obtaining strong oleogels that are difficult to incorporate into food products at temperatures of 20 ºC. The rheological properties demonstrate the fact that oleogels have more elastic than viscous properties. The addition of wax in a percentage of 3% leads to obtaining oleogels with stable and specific rheological properties. The chemical composition of the oils and the type of oleogelator dictate the properties of the oleogel and its applicability in food systems in place of unwanted solid fats.

## ACKNOWLEDGMENT

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# IMPACT OF TEMPERATURE AND SOWING DEPTH ON GERMINATION, GROWTH AND DEVELOPMENT OF THE INVASIVE SPECIES *ASCLEPIAS SYRIACA*

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## ABSTRACT

*Asclepias syriaca* is a perennial species, native to North America. In Romania, being introduced as an ornamental species, in present being found in different habitats (agroecosystems) and on ever wider areas causing damage. Although the basic biological characteristics of *Asclepias syriaca* are known, its control is a significant problem. As a result, we hypothesized that temperature and sowing depth can be used to reduce the population of *Asclepias syriaca*. To test this, the seeds were put at 5ºC, 27ºC, room temperature, after which they were sown at a depth of 0.5 cm and 1 cm respectively, the responses regarding the germination and growth of *Asclepias syriaca* plants were studied in the laboratory. Following protocol was determined: the length of the roots, the height of the plants and the fresh weight. *Asclepias syriaca* seeds sown at 1cm depth showed a higher germination percentage compared to those sown at 0.5 cm. The seeds exposed at 27ºC were the first to germinate exerting a higher height. Those subjected to the clipping method did not show a stimulation of the germination percentage and the roots and height showed the lowest values. At 5ºC the germination percentage is reduced.

**Keywords:** *Asclepias syriaca*, germination, temperature, depth

## INTRODUCTION

*Asclepias syriaca* is a perennial dicotyledonous plant belonging to *Apocynaceae* family. The species is native to North America [1]. In Europe, *Asclepias syriaca* was introduced as an ornamental plant in the 17th century [2, 3] as well as for beekeeping, flowers being an important source of nectar for bees. The first report of the species *Asclepias syriaca* in Romania dates back to 1836 (as a species cultivated for ornamental purposes), and since 1939 it appears as a sub spontaneous species [4]. From the point of view of distribution, in the historical regions of Romania, *Asclepias syriaca* is present

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in: Transylvania, Banat, Crisana, Maramureș, Oltenia, Muntenia, Moldova, Danube Delta [4]. Currently (in Romania) this plant is present in natural, semi-natural and artificial habitats (sunflower, corn, alfalfa, soy, rape, wheat, barley) [5, 6, 7].

Research on the reproduction and morphological characteristics of the species *Asclepias syriaca* was carried out by: Bhowmik [8], Sárkány [9], Mojzes [10], Dvirna [11], Follak [3]. The main characteristics of the plant: milky sap (latex), seeds with pappus, propagation by rhizomes [8, 11, 3] and allelopathic substances (asclepiadin, sitoserol, quercetin and nicotine) [3], were decisive in the approach to studies related to this species, so in the specialized literature we will find studies regarding the positive and negative economic impact (on biodiversity and ecosystem functionality, on health, on agriculture).

This weed propagates by seeds and rhizomes with adventitious buds [8]. Infestation in neighboring areas comes from sprouting root buds, while seeds become a source of infestation in more distant areas.

The aim of this study was to obtain information on (a) seed germination in relation to temperature and sowing depth (b) plant growth and development seedling emergence as a function of sowing depth and temperature.

## MATERIALS AND METHODS

### Site Description

The seeds of *Asclepias syriaca* plants were collected from the Pișcolt site (Figure 1), Satu Mare county (România, Nord-Vest, N 47°34'50,19636, E 221733,38232, altitude 128 m) in 24th October 2021.




Figure 1 – The area from which *Asclepias syriaca* fruits were harvested



Figure 2 Harvesting the fruit and papus removal
Photo Kincel K. and Ștef R.

### Germination Study

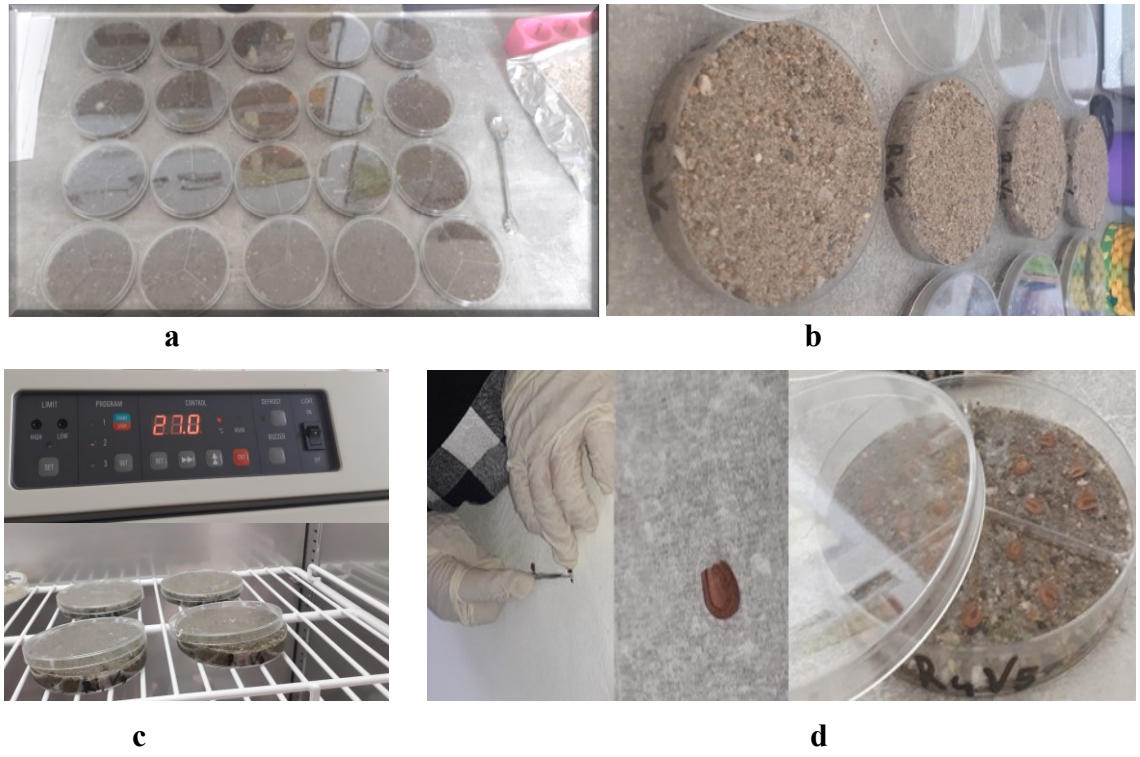
The seeds were transported to the laboratory, where the papus was removed (Figure 2) and stored at room temperature (20 and 22°C) until June 2022. Germination testing was performed in the laboratory. For this we used: sterile Petri dishes with 3 compartments, *Asclepias syriaca* seeds and sterile sand. Before placing the seeds in Petri dishes, I sterilized the sand in an oven at a temperature of 121°C for 60 minutes. 15 seeds of *Asclepias syriaca* were placed in each Petri dish, at 0.5 cm (one variant – V3) and 1 cm (4 variants – V1, V2, V4, V5) depth. Before placing the seeds in Petri dishes, they were exposed to room temperature (Variant 1, Variant 3, Vaianta 4, Vaianta

5) and those in variant 2 were exposed to a temperature of 5°C (for 14 days). In variant 5, the seeds were kept at room temperature, and before being placed in the Petri dishes, they were subjected to the clipping method (figure 3d), which involves removing (cutting) a part of the seed (the narrower part of the protective layer) with a scalpel in order to interrupt the state of vegetative rest, without destroying the embryo.

The seeds from variant 4, after being placed in Petri dishes, were placed in the incubator at 27°C (figure 3c) for the entire duration of the experiment, the other 4 variants were placed in the laboratory at room temperature (21°C) (figure 3a-b).

After placing the experiment and watering them, the pots were covered to maintain humidity, until the first germinated seeds were spoted.

The experimental variants were watered with 10 ml of distilled water, once every two days until the seeds germinated. After the germination of the first seeds, the lids of the Petri dishes were removed and they were watered daily with 15 ml of distilled water (figures 4).



**a** **b**

**c** **d**

Figure 3 – Trial set up in order to establish the germination of *Asclepias syriaca* seeds (a-b - placement of the seeds in Petri dishes; c-variant placed in the incubator; d - the variant in which the clipping method was used) (Photo Kincel K.)

Germination assessments were performed 7-10-12-19 days after setting up the experiment. Vegetative growth and plant weight were determined on the last day of the experiment (day 19). The weight of the seedlings was determined with the analytical balance (figure 5), and the root length and the height of the seedlings were measured with the graduated ruler.

The seed germination percentage was determined according to the formula:
**FPG** = (germinated seed /seeds total) x 100

**Germination index** (GI) calculated as: (GI) = (20xN1)+ (19xN2)+… + (1xN20); where N1, N2…N20 is the number of germinated seeds on the first, second and subsequent days until 20th day and the multipliers (e.g. 20, 19 etc.) represent weights given to the days of the germination [12]



Figure 4. Watering the experimental variants (Photo Kincel K.)




Figure 5. Performing biometric measurements (Photo Kincel K.)

**Mean germination time (MGT)**: MGT = ∑(n × d) / N, where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment [13]
**Speed of germination** - Speed of germination= n1/d1+n2/d2+n3/d3+--- Where, n = number of germinated seeds, d= number of days [12]

## RESULTS

Observations on the germination of *Asclepias syriaca* seeds began 24 hours after placing the experiment, but the first germinated seeds were recorded after 7 days (Figure 6) in variant 4 (the seeds were kept at room temperature and placed at a depth of 1 .0 cm - during the experiment were placed in the incubator at 27°C). Late emergence of *A.syriaca* seeds was also reported by Bhowmik [8] who stated that the first seeds started to germinate 6 days after sowing at 27°C and 21°C, the longer period of time (18-25 days ) was recorded in the variants where the seeds were exposed to 10°C and 15°C. At 10 days the number of germinated seeds/total variants was 46 distributed as follows: V1 – 7 seeds; V2 – 1 seed; V3 -21 seeds; V4 – 15 seeds; V5 – 2 seeds. From figure 6 it can be seen that in all the variants *Asclepias syriaca* seeds germinated, the most germinated seeds were registered in variant 3, it is also noteworthy the number of germinated seeds (15) in variant 4 (the Petri dishes were inserted in incubator 27°C). Seeds placed at the soil surface (0.5 cm) showed the lowest germination (1 seed).

On the eleventh day the number of germinating seeds was identical to that recorded on the tenth. The number of germinated seeds increased to 109 on the twelfth day. Figure 6 shows that the clipping method did not stimulate the germination of *Asclepias syriaca* seeds (10 seeds). At the last evaluation (19 days) it was determined that 155 seeds/total experience had germinated out of the 300 used in the experience.

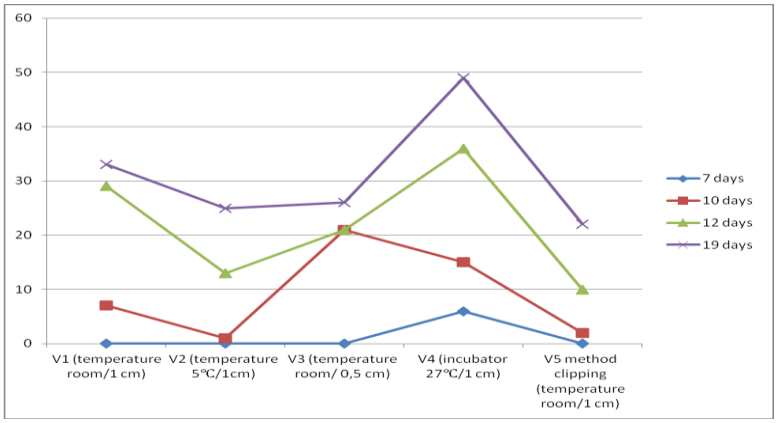



Figure 6 – Sum of germinated seeds /4 repetitions

Germination percentages (FPG) recorded at the end of the experiment were between 36.67 - 81.67% (Table 1). The germination index of *Asclepias syriaca* seeds lays between 118 – 372, the highest value was recorded in the variants placed in the incubator at 27°C. The seeds subjected to the clipping method presented a GI of 118. The mean germination time (MGT) of *Asclepias syriaca* seeds was 11.73 days in the variant sown at 0.5 cm (V3). The seeds exposed to the temperature of 5°C for 14 days needed more days to germinate (MGT 15.28 days). The clipping method did not stimulate germination, MGT being the highest in this variant (15.64 days). The germination rate, recorded in the experience, was 2.19-5.16 (Table1).

Table 1

Seed germination of *Asclepias syriaca* and related germination parameters

| Variant | No. total of germinated seeds/variants | FPG (%) | IG | MGT (days) | SG |
|---|---|---|---|---|---|
| V1 (temperature room/1 cm) | 33 | 55 | 283 | 12,42 | 4,85 |
| V2 (temperature 5°C/1cm) | 25 | 41,67 | 133 | 15,28 | 2,5 |
| V3 (temperature room/ 0,5 cm) | 26 | 43,33 | 157 | 11,73 | 3,47 |
| V4 (incubator 27°C/1 cm) | 49 | 81,67 | 372 | 12,88 | 5,16 |
| V5 method clipping (temperature room/1 cm) | 22 | 36,67 | 118 | 15,64 | 2,19 |

The highest vegetative growth (3.10 cm) was recorded in the treatment placed in the incubator at 27°C, the differences being significantly positive compared to the control (V1 control). The biometric measurements placed the plants, in terms of height, between 1.56 cm and 3.10 cm. The seeds subjected to the clipping method determined the lowest vegetative growth (1.53 cm) (Figure 8).

The seeds subjected to the clipping method (0.31 cm) and those placed in the incubator (0.56 cm) failed to develop their root system, the results obtained showing very significantly negative differences compared to the control (V1 - 2.87 cm).

The maximum root length was recorded in the variant heat-treated at 5 °C (2.91 cm), followed by the control variant (2.87 cm) and the variant where the seeds were placed at germination at a depth of 0.5 cm (2.77 cm ) (Figure 9).

In order to establish the green mass, the plants from each variant were weighed, 19 days after the placement of the experiment, they presented values of 0.122 g - 0.550 g/average of the repetitions (figures 10).

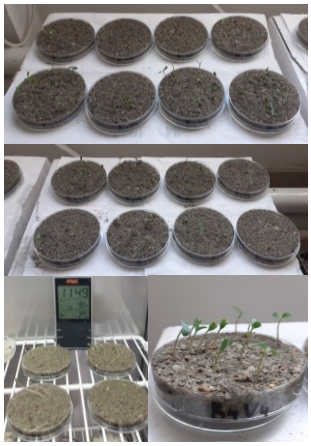
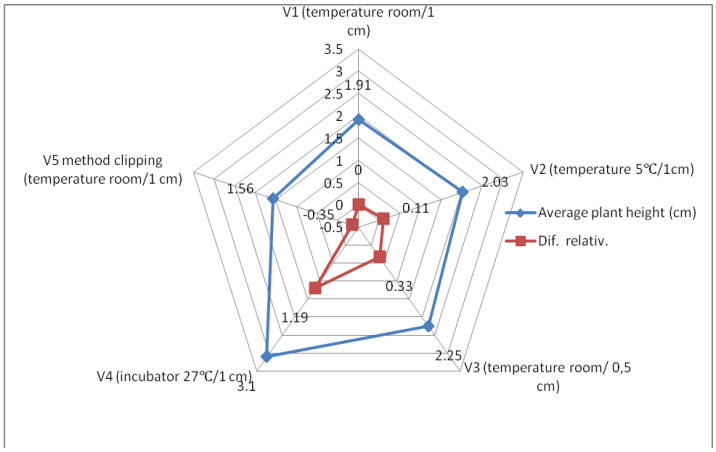
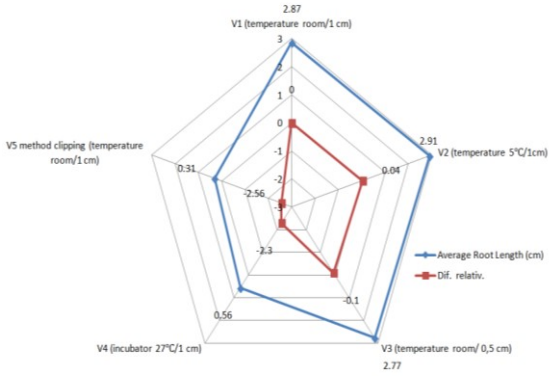


Figure 7. Aspects from the experimental variants



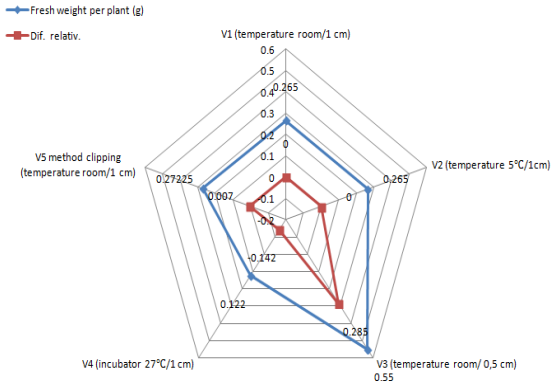

DL 5% = 1.04; DL 1% = 1.46; DL 0.1 = 2.07

Figure 8. Average height of *Asclepias syriaca* plants 19 days after placing the experiment




DL 5% = 0.99; DL 1% = 1.39; DL 0.1 = 1.97

Figure 9. Influence of temperature and sowing depth on root length of *Asclepias syriaca*




DL 5% = 0.251; DL 1% = 0.366; DL 0.1 = 0.549

Figure 10. Influence of temperature and sowing depth on plant weight of *Asclepias syriaca*

The highest value, regarding the green mass of *Asclepias syriaca* plants, was recorded in the variants where seeds were placed at a depth of 0.5 cm, the differences being significantly positive. The exposure of the seeds to 27°C in the incubator determined the etiolation of the plants (manifested trough the elongation of the stems, the shrinking of the leaves), so that in these variants the lowest vegetative mass was recorded. In variants 1, 2 and 5, the weight of green mass presented close values, which led to the lack of statistical significance.

## DISCUSSION

Temperature is an abiotic factor that plays an important role in plant growth and development, and its effect on germination is complex [14]. The minimum germination temperature varies according to the species [14], tropical plants show optimal germination between 15°C and 30°C, temperate plants between 8°C and 25°C, and alpine plants between 5°C and 30°C .

While *A. syriaca* seeds germinate in a wide range of temperatures, the minimum temperature for germination varies between 15 - 20°C and the maximum 35-40°C [15]. In the present study, the highest germination percentage (81.67%) was recorded in the variants placed in the incubator at 27°C. The result being consistent with the study carried out by Radivojevic [16] who tested the germination of *A. syriaca* seeds at temperatures of 20°C, 25°C, 30°C and 35°C, finding that most of the seeds germinate at temperatures of 21°C - 26°C. And the study carried out by Bhowmik [8] claims that at 27°C the highest percentage of germination was recorded. Seeds germinated for 14 days at 5°C germinated 41.67% and required 15.28 days for emergence. These results are not consistent with information published by Evetts and Burnside [15] supporting the stimulation of germination by exposure to 5°C. The seeds exposed to room temperature determined the emergence of 55% of the seeds sown at 1 cm, respectively 43.33% for those sown at 0.5 cm.

The seeds exposed to the temperature of 27°C, although they stimulated the emergence and growth of Asclepias syriaca plants, did not result in vigorous plants (root system and plant weight showing negative meanings).

## CONCLUSION

Exposing the seeds to 27°C, there was a stimulation of germination (FPG, IG and SG parameters showed the highest values) and the height of *A. syriaca plants*. The decrease in temperature conducted to a lower emregence.

Placing the seeds at a depth of 0.5 cm led to a decrease in the average germination time (11.73) stimulating vegetative growth (plant weight being maximum in this variant).

Stratification of the seeds at 5°C for 14 days determined a stimulation of the root system, *A. syriaca plants*, from this variant, presented the highest values (2.91 cm).

The seeds that were subjected to the clipping method showed minimal values regarding germination and vegetative growth of *Asclepias syriaca* plants.

Such information may be useful in guiding future research on the control of this weed.

## ACKNOWLEDGEMENTS

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# IMPROVING THE QUALITY OF UNHEATED TOMATO JUICE BY USING PLANTS RICH IN BIOACTIVE COMPONENTS

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## ABSTRACT

Tomatoes (Solanum Lycopersicum) are fruits rich in bioactive substances, especially compounds with antioxidant characteristics. Tomato fruits are a source of vitamin C, having beneficial properties for health. Current indications lead us to the conclusion that tomato fruits are potential candidates for the study of bioactivity, as well as for functionally developed fruits.

The activity of these bioactive compounds is to combat the activity of free radicals. Tomatoes are the most popular vegetable crop in the world. These vegetables are important due to their high contribution to human health and nutrition. The attractive colour and flavour of tomato have made it a dietary staple in many parts of the world One of the most popular options for consuming tomatoes is tomato juice. Raw tomato juice is known as a good energy drink, tasty, and filled with health benefits. The motivation, in this case, was to obtain food products with superior taste and nutritional properties that fall within the scope of food engineering. The main objective of our study was to obtain and reveal some physicochemical and nutritional properties of some varieties of raw tomato juice with the addition of green chillies and horseradish. These additions increased the antioxidant content of the juice and also improved the sensory characteristics of the two types of juice.

**Keywords:** tomato juice, bioactive components, green chillies, horseradish

## INTRODUCTION

Tomato is the second most important vegetable around the world, after potatoes, economically and planted in nearly all countries [1]. This fruit is very common in the Romanian diet and is one of the most consumed vegetables as fresh as the processing industry. Tomato is an excellent source of important health compounds due to its balanced content of minerals and antioxidants (e.g.: vitamins C and E, carotenoids, lycopene, lutein, phenolic compounds, and phenolic acid, and flavonoids) [2]. The nutraceutical value of tomato fruit is offered by the content of compounds organic acids,

polyphenols, carotenoids, soluble sugars, minerals and vitamins (especially vitamin C and E). and also volatile compounds [1,2].

Lycopene, a pigment, is the most important chemical compound in tomatoes, capable of neutralizing free radicals in the human body with its antioxidant characteristics and preventing human beings affected by cancer, accelerated ageing, cardiovascular diseases, osteoporosis, diabetes, and lots of other diseases [3]. The color of tomatoes is ensured by the existence of pigments

Thermal processing of food products is one of the basic operations in the food industry, applied for improvement of their qualitative properties and extending their storage time.

During thermal processing, structural changes occur and, therefore, physical, chemical and biochemical changes occur in food matrixes. That's why we wanted to make tomato juice without heat treatment by using as an addition some plants (hot peppers or horseradish root) rich in bioactive compounds [3,4,5].

Hot pepper (*Capsicum annuum var. annuum L.*) is a popular crop worldwide because of its typical flavour, aroma, pungency and nutritional contents. The crop is widely grown in tropical and subtropical conditions for home consumption, domestic and export purposes. Hot pepper has been grown in Romania for centuries. Peppers are consumed in fresh or powder form. Dry fruit is consumed in the country, as a spice, especially in powder form. Nowadays, there is a widespread scarcity of dry fruit demands [5].

Pepper has a high nutritive value and is an excellent source of ascorbic acid and powerful antioxidants – carotenoids, poly-phenols, and other phytochemicals [5].

Horseradish (Armoracia rusticana), belonging to the Brassicaceae family, is a perennial plant that has been used in Egyptian cuisine as far back as 1500 BC. In Europe, it has been used in cooking since the Middle Ages [6]. Both its leaves and roots of horseradish are edible and have been consumed as food and spice due to their spicy flavour, along with their use as a popular medicinal plant [7]. The dried and ground horseradish root is used in spices.

Horseradish is recognized for its health-promoting phytochemicals and beneficial effects as anticancer agents due to glucosinolates and their isothiocyanate derivatives and vitamins [6]. It also contains coumarins, phenolic acids and vitamin C [5,6].

Horseradish plantlet leaves were reported to have aliphatic glucosinolate sinigrin as the dominant glucosinolate being as much as more than 80% of total glucosinolates [7].

The plant belongs in the category of GRAS (generally recognized as safe) for human consumption to be used in kinds of ketchup and mustards or as a natural seasoning and flavouring.

The motivation, in this case, was to obtain food products with superior taste and nutritional properties. Our objective was to obtain and characterize a functional food, with increased nutritional value, raw tomato juice with the addition of green chillies and horseradish, which have antibacterial and antifungal properties. These additions increased the antioxidant content of the juice and also improved the sensory characteristics of the two types of juice.

## MATERIALS AND METHODS

***Chemicals and equipment.*** All reagents used in this research were of analytical grade – from SigmaAldrich and Merck (Germany).

In order to conduct the experimental research, tomato juice was prepared without heat treatment.

***Tomato Juice preparation***

The unheated tomato juice was prepared according to a traditional recipe from the Oltenia area of the Dunarii Meadow (Poiana Mare) – area recognized for high tomato production.

Mature, fresh and ripe tomatoes ("ox heart") were obtained from the Oltenia area – the farms of the Danube meadow (Poiana Mare).

The "ox heart" tomatoes were rinsed, and sorted and seeds and skin removed. The fruit pulps were then blended using a control blender, added salt (in a ratio of 1: 150) and sieved through a cotton cloth to have relatively fine juice. After filtration, a percentage of 60% juice was obtained. The obtained tomato juice was divided into 3 parts. The first part (TJ1) was packed in bottles.

Finely chopped hot pepper was added to the second part of the juice, and finely chopped fresh horseradish root was added to the third - TJ3. After being mixed with tomato juice, the obtained juices were packed in glass jars. Both hot pepper and horseradish were fresh and washed and cleaned before use. The proportion of hot peppers and horseradish added to the tomato juice was 3:20.

In order to carry out the experimental part, we analyzed from a physico-chemical point of view samples of vegetables used as raw material to obtain unheated tomato juice: tomatoes, hot peppers and horseradish, as well as tomato juice.

**Table 1**. Some morphologic characteristics and descriptions of tomato used in prepared tomato juice

| Predominant fruit shape | Fruit size | Fruit firmness | Seeds number | The exterior color of mature fruit | Fruit weight (average), g |
|---|---|---|---|---|---|
| Heart-shaped | Intermediate (4–10 cm) | Firm | Small | Red -pink | 310 |

We obtained tomato juice not thermally treated simply and with the addition of hot peppers and horseradish. This combination is made from the desire to obtain a functional food, with increased nutritional value. Green chillies and horseradish have antibacterial and antifungal properties. These additions increased the antioxidant content of the juice and also improved the sensory characteristics of these types of juice.

***Physical and chemical analyses***

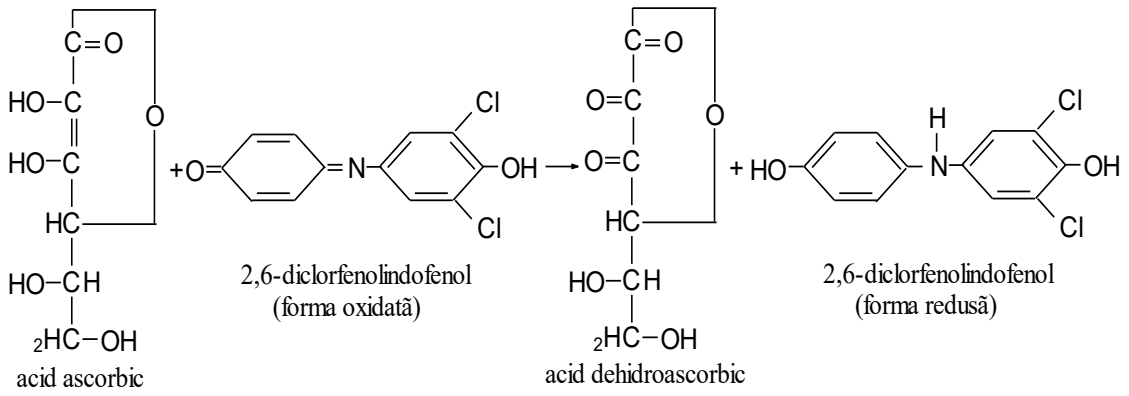
To determine *the weight of the fruit*, we used a digital scale with a sensitivity of 0.01 g. Each determination was performed three times (in triplicate). The weight of the fruit was measured in triplicate.

*The determination of the dry matter and water content* was made by using a Memmert oven (tomato, chilli pepper, horseradish, tomato juice). The water content actually represents the weight loss achieved by heating at the temperature indicated by the analysis method until reaching the constant mass. [1, 9].

*The ash content* (total mineral content) was evaluated by incineration at 500±15$^{o}$C.

*The determination of the total soluble solids* TSS ($^0$Brix**)** in the these samples has been achieved by employing a handheld refractometer previously adjusted to zero with distilled water (the KRÜSS Instruments, Germany). The prism of the refractometer was cleaned with distilled water after each analysis. *Titratable acidity* of the samples was determined according to the method of AOAC (2005) [9].

*Ascorbic acid determination.* Determination of ascorbic acid content in sample (tomato, hot pepper, horseradish root, juice) was accomplished by titration with 2.6-diclorophenolindophenol (2,6-DFI). The method is based on the color change of the reagent, following the redox reaction.




Thus, 2,6-diclorophenolindophenol in the ionized form is red in acid and blue in the basic medium. Ascorbic acid was extracted using a weak acid (solution of oxalic acid in the presence of hydrochloric acid) and titrated against solution using 2,6-DFI solution until we observed a light pink-purple color [1,3,9].

***Sensory evaluation***

For sensory evaluation we have selected twenty panellists to evaluate aroma, colour, taste, smell, consistence and appearance. This group consisted of 10 men and 10 women who were undergraduate and graduate students. The samples (30 mL) were served cold (10 ± 2 °C) in 50 mL plastic cups codified with three-digit random numbers (each sample being three times given for tasting, without the panelists' awareness regarding this aspect). Mineral water at room temperature and saltwater biscuits were served to allow the assessors to cleanse their palates between samples. Each consumer evaluated the three samples (TJ1, TJ2, TJ3) in a single session. The samples TJ1 is toamto juice simple samples obtained and TJ2 and TJ3 are toamto juice samples obtained by adding hot pepper (TJ2) and respectively horseradish.

***Statistical analysis***

All determinations were performed in triplicate, calculating their arithmetic mean of three separate determinations. The data were statistically analyzed using the program Microsoft Excel.

**RESULTS**

The results of our study are presented in the following figures:

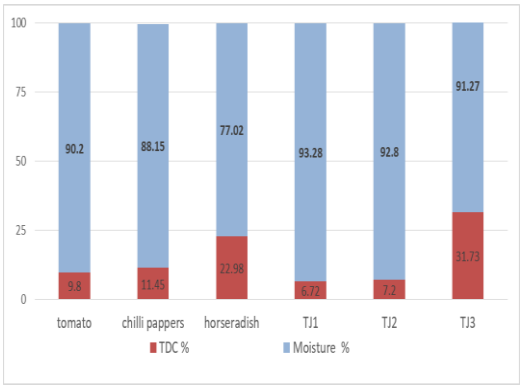



**Figure 1.** Moisture and total dry weight contents of sample (TDC)

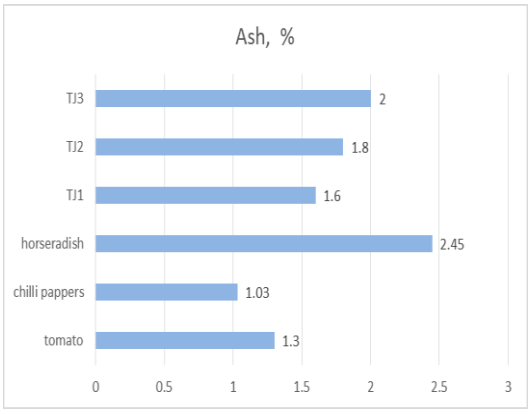



**Figure 2.** Total mineral content (ash) of sample

Note: The samples **TJ1** is tomato juice,
The samples **TJ2** is tomato juice with hot pepper added,
The samples **TJ3** is tomato juice with horseradish added.

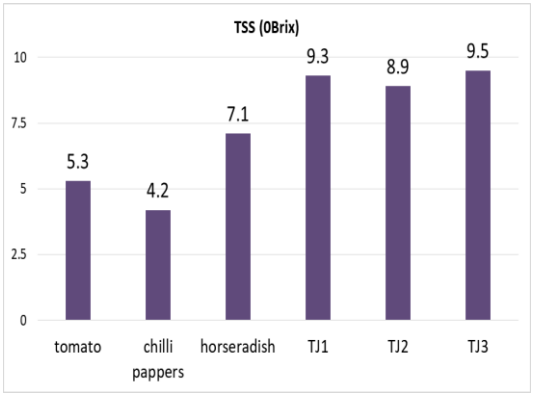



**Figure 3.** Total soluble solids **(TSS)** (Brix°) in samples

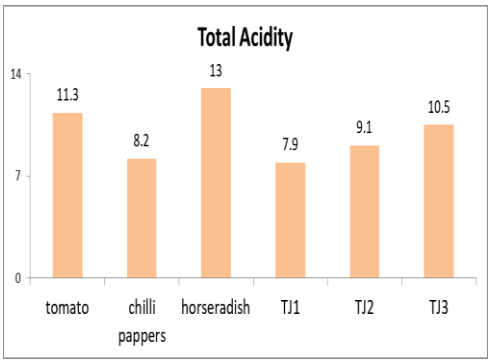



**Figure 4.** Acidity of samples

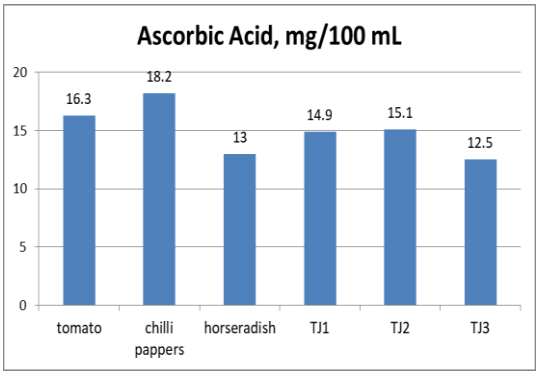



**Figure 5.** Ascorbic acid content in samples

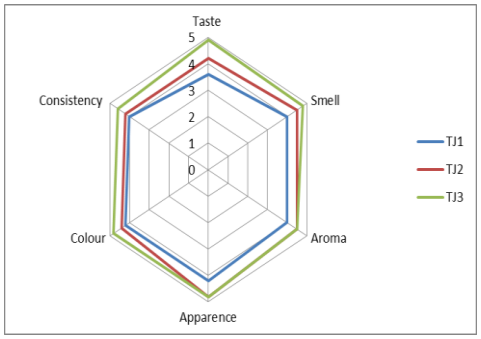



**Figure 6.** Sensorial aspects of tomato juice

## DISCUSSION

The total dry weight content and moisture of the tomato fruits and juice taken in the study are given in Figure 1. The moisture content of samples varied between 77.02 and 93,5g/100g FW (fresh weight- FW). The highest moisture content (93.2 g/100g FW) was identified for simple tomato juice. The lower moisture content (77.02 g/100g FW) was identified in the horseradish sample.

The ash content (g/100g FW) is the indicator of the total mineral content as a criterion of quality that can offer information about the analysed fruits' origin, in the case of tomato fruits. The differences in the ash content of fruit could be due to the way of harvesting and storage conditions [2]. As can be seen from Figure 2, where is presented our data, the higher ash content (total mineral content) for the analysed samples was found in the horseradish (2.45 %, g/100g FW ) and the lowest in the hot pepper (1.03 %, 2.45 g/100g FW). It had to be like that, especially since horseradish has the lowest moisture content. From the data displayed in Figure 3 we can note that the value of total soluble solids content (TSS content – brix degree) was prominent in TJ3 sample and the lower value measured for pepper sample. From the analysis of the experimental data, Figure 4, high acidity is observed in horseradish (13°), and the lowest acidity occurs in tomato juice TJ1 without the addition (7.9°).

The ascorbic acid content in the fruit of the tomato, hot pepper and horseradish root was determined, and data obtained are shown in Figure 5. To determine the ascorbic acid content, the titrimetric (with 2,6-DFI ) method of sample extracts was applied.

As can be seen from figure 4, the ascorbic acid content of analysed tomato fruits was found to range between 12.00 mg /100g FW (TJ3, juice) and 18.5 mg /100g FW (chilli pepper) – figure 5. Ascorbic acid was in all the samples, and chilli pepper had the highest concentration (18.05 mg/100 g FW).

The values found in our study for tomato fruits (16.3 mg/100 g FW) were in concordance with the study of George et al. [10] vitamin C content varied from, 8 to 32 mg/100 g in the pulp and 8 to 56 mg/100 g in the skin of tomatoes grown in India. The content of ascorbic acid found by the studies of Toor and Savage [11] is raged from 6 to 18 mg/100 g FW) and Pinela et al. [12] found the ascorbic acid content between 10.85 and 18.56 mg/100 g FW in portuguese tomato varieties. The ascorbic acid role in the prevention of diseases regarding oxidative damage occurs due to its capacity to neutralize the action of free radicals in biological systems [1,8,12]. Ascorbic acid (vitamin C) is found in abundance in many fruits and is a hydrophilic antioxidant.

The results of the sensorial assay are presented in Figure 6. One of the objectives of our research was to relate the physical and chemical characteristics of tomato juice to its sensory acceptance.

The sensorial properties were evaluated by a scoring scale (1 – 5) (1-low, 5-excellent). From a sensorial point of view, the tomato juice was well appreciated.

As expected, the least appreciated was the tomato juice prepared by us without any plant addition (TJ1), since it does not contain any addition meant to improve its sensorial qualities. The lowest score was registered on the taste level (3.5) and the highest score was recorded by the TJ3 sample, for the taste, (4.9). On the aroma, the lowest level score was 4 for TJ1 and the highest score was 4.8 recorded by the TJ3 sample The tomato juice prepared by us can improve its sensorial qualities by adding some components (plant adding). The pepper and horseradish managed to change the

sensory characteristics of the juice through its aroma. The tomato juice with the addition of horseradish was very well appreciated.

## CONCLUSION

In our work, investigations of physicochemical characteristics were carried out to reveal the nutritional values, chemical composition, and antioxidant capacity (ascorbic acid content) of tomato, tomato juice, tomato juice with the addition of plants and the plants used as addition (hot pepper, horseradish) used to enrich them and increase the quality of raw prepared tomato juice.

From these results, it can be noted that tomato fruits represent valuable products, due to their rich and beneficial nutritional composition and can be useful in a balanced diet. Furthermore, this work provides many arguments for the use of tomato fruits as healthy foods and their use not only in the food industry but also in the pharmaceutical industry.

It is difficult to create a functional food intended for all categories of consumers, because a balanced combination, on a scientific basis, of the basic ingredients and biologically-active substances must be achieved.

The vegetables added to the unheated tomato juice, due to their multiple qualities but especially because they have a high content of antioxidants, contributed to increasing its nutritional value, their consumption having a beneficial effect on consumers.

## ACKNOWLEDGEMENTS

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# JAPANESE HONEYSUCKLE (*LONICERA JAPONICA L.*) – A VALUABLE SOURCE OF ANTIOXIDANT AND ANTIBACTERIAL METABOLITES

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## ABSTRACT

The use of natural products for healing purposes has gained great attention worldwide. Increased interest in certain plant products has been achieved mainly due to their ability to limit antibiotic resistance. Also, as the hypothesis that all biological processes at the cellular level are severely affected by the formation of free radicals has been confirmed, it is attempted to highlight some active principles in plants and their use as agents to counteract the oxidative process [1, 2]. This paper aims to demonstrate the antioxidant and antibacterial capacity of the alcoholic extracts from the flowers and leaves of *Lonicera japonica*, a widespread ornamental plant, as well as to carry out volatile compounds profiling from the essential oil of *L. japonica*. For the quantification of the antioxidant properties, the following assays were carried out: total antioxidant capacity (TAC), superoxide dismutase activity (SOD), total polyphenolic content and total flavonoid content. The antibacterial and antibiofilm activity of the extracts and essential oil were evaluated on multidrug-resistant *Staphylococcus aureus* and *Escherichia coli* strains. For the identification of the biologically active volatile compounds, GC-MS chromatographic analysis was carried out, with the NIST database as the identification source.

The obtained results support the antioxidant, antibacterial, and antibiofilm potential of *Lonicera japonica* [3]. Regarding the flowering stage of the plant, the silver flower extract demonstrated a significant antimicrobial effect, mainly against *E. coli.* Thus, during the evaluation of the antioxidant potential of the extracts, considerable values were obtained for all tested parameters, supporting the antioxidant potential of this plant.

**Keywords:** Lonicera japonica, antioxidant potential, antibacterial capacity

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## INTRODUCTION

The genus *Lonicera* (belonging to the *Caprifoliaceae* family) includes over 150 species of cosmopolitan plants. *Lonicera japonica* Thunb., a medicinal and ornamental plant native to Asia, is an important source of remarkable bioactive compunds and is widely used in herbal medicine for its antioxidant, antimicrobial, and anti-inflammatory properties [4]. *L. japonica* flowers benefit from remarkable bioactivity and many important functions such as antibacterial, anti-inflammatory [5], antioxidant and hepatoprotective [6]. Research in recent years has also confirmed the antitumor properties of the plant. In addition, probably the most important is the antioxidant function of *L. japonica* [6]. In 1985, Helmut Sies defined the concept of oxidative stress as the result of a disturbance of the balance between pro-oxidant and antioxidant species, in favor of the former, thus causing possible damage [2]. At the opposite pole are antioxidants, which are molecules that have the ability to inhibit the oxidation of other molecules by preventing or reducing the formation of reactive species. Studies have shown the isolation of about 320 such compounds from the buds and flowers of the plant [4]. Predominantly, those are represented by phenolic structures, flavonoids, as well as numerous volatile compounds [7]. Also, *L. japonica* has in its composition iridoids, glycosides [8], saponins and cerebrosides [9]. Thus, this paper aims to demonstrate the antioxidant and antimicrobial activity of the alcoholic extracts from the leaves and flowers of *Lonicera japonica*.

## MATERIALS AND METHODS

### *Sample collection location*

The location selected for the collection of the samples was in the range of the Banat region, Romania, having the following coordinates: 45°21'24"N, 21°43'35"E.
The plant material was collected during the period when *L. japonica* finds itself in the second flowering interval (June-September). The samples consisted of flowers in different flowering stages, being represented by buds (juvenile, green and white buds), silver flowers (in the intermediate stage of flowering), golden flowers (in the advanced stage of flowering) and leaves.

### *Extract preparation*

The plant material, namely the buds, flowers and leaves of *L. japonica* were freeze dried with the use of a Kryptonstraat 11 (ilShin Europe) freeze-dryer. The extracts were prepared in 70% methanol in a ratio (plant:methanol) of 1:20. The next step was the homogenization of the plant-methanol mixture with the use of an orbital stirrer (30', 200 rpm) and the filtration of the extract through a 90 mm filter paper.

### *Determination of the total polyphenolic content*

In order to determine the total polyphenolic content of *Lonicera japonica* extracts, the method described by Tamas-Krumpe O.M. et al. [10], with modifications [11] was used. This method consisted of the use of the 10% Folin-Ciocalteu reagent and sodium carbonate, with gallic acid in different concentrations (0; 3,9; 7,8; 15,62; 31,25; 62,50; 125 and 250 μg/mL) as a standard. 25 μL of each alcoholic extract were pipetted into a 96 wells microplate followed by the addition of 125 μL of the Folin-Ciocalteu reagent and 100 μL $Na_2CO_3$. The resulting complex was kept at room temperature in a place away

from light for 30 minutes. Following the calibration curve described by the equation $y= mx+b$, a correlation coefficient ($r^2$) equal to 0,9988 was obtained.

### *Determination of total flavonoids*

The determination of the total flavonoid content of the alcoholic extract of *L. japonica* was carried out after the method described by Al-Matani S.K. et al. [7] with modifications: 500 µL of each sample was pipetted into 5 ml volumetric flasks, 150 µL of freshly prepared $NaNO_2$ 5% were added, and the resulting mixture was stored for 5 min at room temperature; after 5 min, 250 µL of $AlCl_3$ 2% were pipetted, the reaction time being 6 min, at room temperature. 250 µL NaOH were added to the newly formed complex, the reaction time being 10 min; the samples were brought to a final volume of 5 mL with distilled water, and the absorbance of the four extracts (leaves, buds, silver flowers and golden flowers) was read at 510 nm. Regarding the reference standard, rutin (1 mg/mL) was used in the following concentrations: 0; 62.5; 125; 250; 500 and 1000 µg/mL. On the standard curve, a correlation coefficient ($r^2$) equal to 0.9980 was obtained.

### *Determination of the total antioxidant capacity*

The total antioxidant capacity for the alcoholic extracts was determined with the use of a biochemical analysis kit (*Elabscience E-BC-K136-M*), the optical density of the samples being at 520 nm wavelength. The results were calculated with the following formula:

**T-AOC (U/mL)=** $\frac{\Delta A}{0{,}01} \div 30^* \times \frac{V1}{V2} \times f$,

*where* $\Delta A = OD_{Sample} - OD_{Control}$

** = the reaction time (30'),*

$V_1$ *= the total volume of reaction (mL),* $V_2$ *= the volume of the sample added to the reaction (mL),*

*f = dilution factor of sample before tested.*

### *Determination of the superoxide dismutase activity*

Superoxide dismutase activity was determined with the use of a biochemical analysis kit (*Elabscience E-BC-K019-M*), based on the hydroxylamine oxidation method. This reaction results in the formation of a purple complex whose absorbance was quantified at 550 nm wavelength. The results were calculated with the following formula:

**T-SOD activity (U/mL)= i ÷ 50% ×** $\frac{V1}{V2} \times f$,

where i= $\frac{(A1-A3)-(A2-A3)}{(A1-A3)} \times 100\% = \frac{(A1-A2)}{(A1-A3)} \times 100\%$.

$A_1$ = the OD value of control well at 550 nm;

$A_2$ = the OD value of sample well at 550 nm;

$A_3$ = the OD value of $Control_{blank}$ well at 550 nm;

$V_1$ *= the total volume of the reaction solution (mL);*

$V_2$ *= the volume of the sample added into the reaction system (mL);*

*f = dilution factor of sample before tested*

### *Identification of the volatile compounds via GC-MS*

The essential oil (EO) of *Lonicera japonica* was obtained from a certified international bio-producer and the volatile compounds were identified via GC-MS analysis. The sample was diluted with hexane in a ration of 1:20. The device used for the analysis was a 7890A Gas Chromatograph (*Agilent Technologies USA*) coupled to a 5975C Triple-Axis MS Detector (*Agilent Technologies USA*) and equipped with a *HP-5MS* capillary column (30 m x 0.25 mm i.d x 0.25 µm). The sample was introduced into the injection port with a 5 µL syringe, in the split mode (the split ratio was 50:1) and a volume equal to 2 µL. Helium was the carrier gas with a flow rate of 1 mL/min. The temperature was initially programmed at 50°C for 3 min, raised to 120°C with 4°C/min and held there for 30 min, raised one more time to 180°C with 8°C/min and held there for 10 min.

***Determination of the antibacterial and antibiofilm activity***

Antimicrobial activity of the alcoholic extracts (flower buds, silver flowers, golden flowers and leaves) and essential oil of *L. japonica* was tested on two multidrug-resistant bacterial strains: *Staphylococcus aureus* CP8150 (methicillin resistance phenotype), respectively *Escherichia coli* CP7921 (fluoroquinolone and sulfonamide resistance phenotypes, penicillinase production), using the broth microdilution method in 96-wells microplates. The bacterial isolates were grown overnight on Columbia agar at 37°C. Colonies from overnight cultures were suspended into physiological saline and vortexed to obtain a bacterial suspension of 0.5 McFarland turbidity (1.5-2 x $10^8$ CFU/mL). The extracts were brought to an equal final concentration of 1%. Each testing well was filled with 90 µL of sterile BHI broth, 10 µL of bacterial suspension and 50 µL of 1% plant extract, to obtain a final working volume of 150 µL. In the wells corresponding to the positive controls, instead of 50 µL plant extract, 50 µL of methanol 1% were added. The blanks consisted of 90 µL of sterile BHI broth, 10 µL 0.9% NaCl and 50 µL of 1% plant extract. For the antibacterial activity of the EO, the wells were filled with 10 µL of bacterial suspension, sterile BHI broth and EO, in order to obtain final concentrations of 2%, 1.3%, 0.66% and 0.33% EO to 150 µL working volume. The positive controls consisted of 140 µL of sterile BHI broth and 10 µL of bacterial suspension. In the wells corresponding to the blanks the 10 µL of bacterial suspension were replaced by the same volume of 0.9% NaCl. The plates were incubated at 37°C for 20 hours in normal atmosphere. The optical density (OD) was measured at 590 nm. After the determination of the antimicrobial activity, the microplates were reincubated for another 20h and crystal violet (CV) assay was performed to assess the antibiofilm activity of the EO. The wells were washed twice with 200 µL of distilled water to remove planktonic cells. Plates were then incubated for heat fixation at 60°C for 20 min. Staining was made with 155 µL of 0.1% crystal violet for 20 min. The CV bound to the adhered biofilm was solubilized with 160 µL of 96% ethanol and incubated for 30 min at room temperature with gentle shaking. The absorbance was recorded at 540 nm.

## RESULTS AND DISCUSSIONS

The first results regarding the antioxidant properties of the methanolic extracts of *L. japonica* concern the total polyphenolic content. The highest concentration of total polyphenols (11.27 ± 0.01 mg/g) was expressed in the case of the silver flower (SF) extract, decreasing to 9.07 ± 0.13 mg/g with the transition to the next stage of flowering, namely that of golden flowers (GF). This variation is also mentioned by Chen Bo-Ching et al. [4]. Regarding the polyphenolic content of the extracts from the flower buds (FB), the obtained value (10.43 ± 0.05 mg/g) was lower than in the case of silver flowers but higher than the flowers' in the last development stage.

In the case of total flavonoids, the highest level was found in the case of the alcoholic extract from the flower buds (FB) (45.19 ± 0.04 mg/g). With a decreasing tint, this value was followed by that of the extract from the silver flowers (SF) (41.90 ± 0.01) and the golden flowers (GF) with the lowest value (34.38 ± 0.01 mg/g). For the analyzed extracts, the values of the total antioxidant capacity were relatively close to each other. However, in the case of silver flowers (SF) the indicated value was the highest, reaching 1219.69 ± 0.09 U/mL. It was followed by the extract from the flower buds (FB), with a value of 1159.28 ± 0.76 U/mL, respectively from the golden flowers (GF) with 813.45 ± 0.51 U/mL.

For the superoxide dismutase activity, the highest value was obtained for the silver flower (SF) extract, 204.59 ± 0.87 U/mL, which is considered much higher than the values of the other samples which indicated 63.86 ± 0.50 U/mL for the flower buds (FB), respectively 50.13 ± 1.70 U/mL for the golden flowers (GF).

The total polyphenols, as well as the activity of superoxide dismutase and the total antioxidant capacity, indicated an ascending plateau throughout the whole period of flower buds development and their transformation into silver flowers; with the transition to the golden flower stage, the values indicated a downward trend. Another aspect concerns the amount of total flavonoids which indicated a higher value in the case of the flower buds extract and not in the silver flower extract as expected (Figure 1).

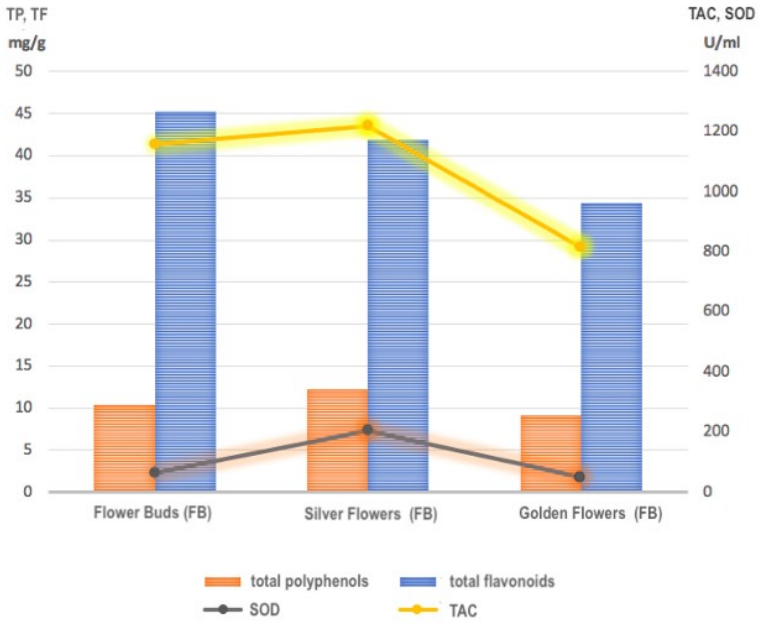



**Figure 1**. The variation of the results for the studied parameters during the flowering stages

The main volatile compounds identified by GC-MS are presented in Figure 2. The obtained chromatogram shows the presence of thirty-nine compounds, representing 99,31% of the total volatile content.

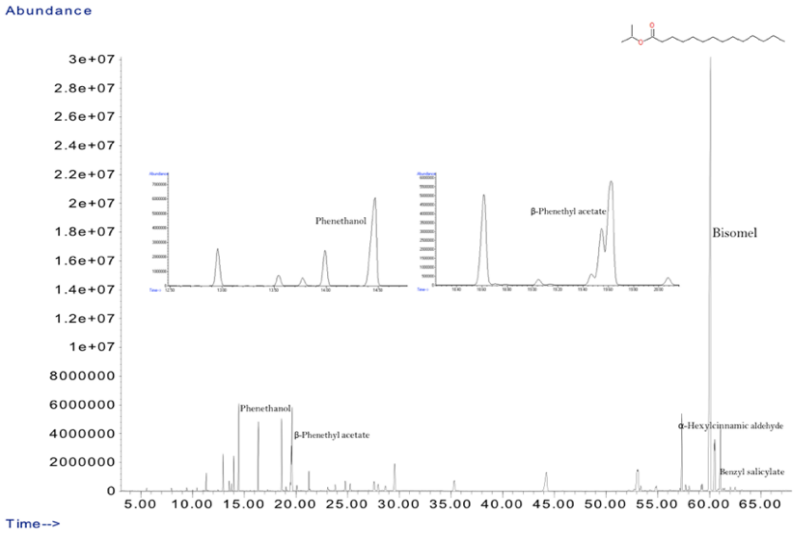



**Figure 2.** GC-MS analysis results of the *Lonicera japonica* essential oil

The main compound, at a retention time of 60.118 min was bisomel, with a percentage of 49.56%. It was followed by phenethanol (4.36%) and β-Phenethyl acetate (3.92%).

Regarding the antibacterial capacity of the extracts, a significant inhibition rate on *Escherichia coli* was recorded. The highest value, 57.44%, was obtained in the case of extract II, representing the silver flowers. This was followed by extract III, from the golden flowers, with a percentage of 56.87% (without a significant difference between the two, $p>0.05$), then extract IV, from the leaves, which indicated an inhibition rate of 25.34% and extract I, from the flower buds, with a value of 22.22% (Figure 3). Considering the fact that methanol has potentiated the growth of the *E. coli* strain, it is possible to emphasize the even greater inhibitory capacity of the extracts on bacterial growth.

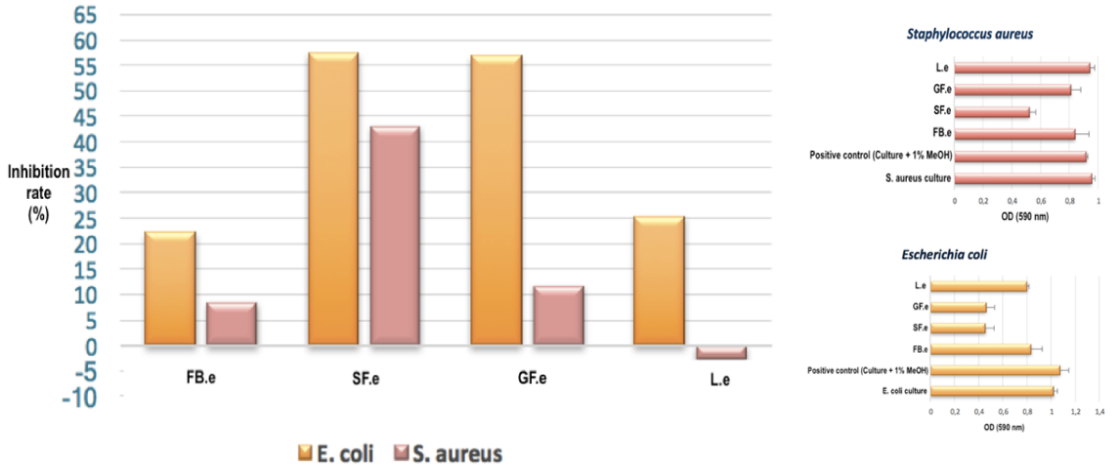



**Figure 3.** Graphical representation of the inhibition rate (%) of the extracts on the two bacterial strains, respectively *Escherichia coli* and *Staphylococcus aureus* (L.e = leaves extract, GF.e = golden flowers extract, SF.e = silver flowers extract, FB.e = flower buds extract)

In the case of the *Staphylococcus aureus* strain, compared to controls, the highest inhibition rate recorded was for extract II (silver flowers), 43.12%, followed by extract III (golden flowers), with 11.74% and extract I (flower buds), with a percentage of 8.62%. Regarding the extract from the leaves (IV), a significant potentiation of the bacterial growth was registered ($p<0.05$). The *L. japonica* essential oil (EO) exhibited a lower antibacterial activity compared to the silver flowers alcoholic extract, with inhibition rates between 13.87% and 38.37% in the case of *S. aureus* and between 22.82% and 40.67% for *E. coli*. At lower concentrations of EO a potentiation of biofilm formation was registered (Figure 4).

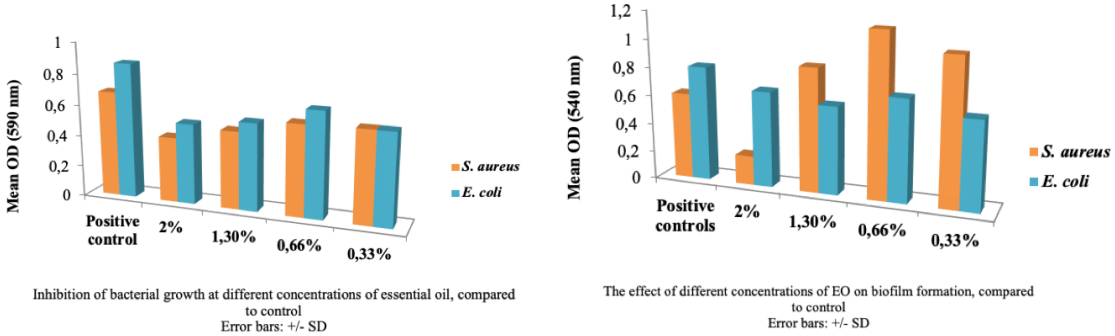



**Figure 4.** Antibacterial and antibiofilm formation capacity (inhibition rates %) of *L. japonica* essential oil

## DISCUSSION

The antioxidant capacity of the extracts is primarily due to the presence in the structure of the plant's organs of phenolic compounds such as polyphenols, phenolic and terpenic acids. Flavonoids support the antioxidant process, but their involvement is not an essential factor, a fact indicated by the results obtained. Although the total flavonoid content of silver flower extract did not show the most significant value, both total antioxidant capacity and superoxide dismutase activity reached the highest thresholds. Regarding the antimicrobial activity of the extracts, until now the compounds responsible for the inhibition of the bacterial status are not known with certainty [12, 13], but our results indicated remarkable percentages of inhibition of bacterial development, especially on Gram-negative bacteria. The results support the data from the current literature according to which *L. japonica* has a remarkable antimicrobial activity, a more significant antibacterial effect being recorded on Gram-negative bacteria [3]. In the evaluation of extracts from flowers in different stages of development, the highest value of bacterial growth inhibition was obtained for the alcoholic extract from silver flowers (43.12% on *Staphylococcus aureus*, respectively 57.44% on *Escherichia coli*). It should be noted that the differences between the inhibition percentages demonstrated by this extract and that of the golden flowers were not significant, the p value being >0.05. Regarding the evaluation of the leaf extract for antimicrobial purposes, its use is not recommended because, in the determinations carried out, it was found to potentiate bacterial growth.
In terms of the essential oil, it has been suggested that the antibacterial activity is closely associated with high concentrations of linalool and α-terpineol [14]. Tao et al. [15] suggest that the essential oils of *Flos Lonicerae* have a bacteriostatic effect against Gram-negative bacteria, with a MIC of 20.0 µL/mL against *S. aureus* and of 37.0 µL/mL against *E. coli*. The major compounds of *Flos Lonicerae* essential oils were linalool (10.4%), palmitic acid (8.0%), geraniol (6.9%), hexanal (2.5%), and α-terpineol (2.2%). In the commercial product analyzed in the present study, α-terpineol (0.15%) and linalool were found in much lower concentrations, the last mainly in the form of linalool oxide (1.30%) and β-linalool (1.27%) which may explain its poor antimicrobial and antibiofilm activity.

## CONCLUSION

The results obtained after testing the antioxidant and antibacterial activity of the alcoholic extract of *L. japonica* demonstrates the possibility of its successful use for both purposes: as an antioxidant and antibacterial agent. All the determinations aimed to evaluate the possibility of using the plant in the process of preventing or reducing the formation of free radicals in the body, and the results obtained, as well as the data from the current specialized literature, confirm this hypothesis. Regarding the antimicrobial activity of the extract, until now the compounds responsible for the inhibition of the bacterial status are not known with certainty, but the results indicated remarkable percentages of inhibition of bacterial development, especially on Gram-negative bacteria. With a view to channeling some future research directions, there are several aspects worth pursuing regarding the evaluation of the functions of this plant. A first step would be to perform a more advanced analysis of the therapeutic principles that *L. japonica* possesses and to identify some methods of conditioning and administering them. Regarding the antimicrobial activity, it is necessary to test the extracts on other bacterial strains and also to evaluate the antiviral capacity of *L. japonica*.

## ACKNOWLEDGEMENT

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# MODELING OF THE BOILING PROCESS IN CORRUGATED PIPES BY THE FINITE ELEMENT METHOD

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## ABSTRACT

The article examines the possibility of using corrugated pipes in the evaporator to intensify the heat exchange process. A computer model was constructed using the finite element method in Fluent ANSYS. Performed computer simulation of the boiling process of liquid in a smooth and corrugated pipe and was check it for adequacy. According to the modeling results, was carry out a comparative analysis of quantitative and qualitative indicators for a smooth and corrugated pipe. The temperature value in smooth and corrugated pipes was set. The volume of the vapor phase was determined and the heat transfer coefficients for both pipe types were calculated. The value of the heat transfer coefficient for the corrugated pipe is 3296 W/($m^2$· K), which is almost 52% more than the value for smooth one. The adequacy of the obtained values was checked by comparing the results with the calculated theoretically. Provided recommendations on the feasibility of using corrugated pipes and the possibility of optimizing their geometric configuration in the future. This study can be used by engineers at the stages of designing heat exchange equipment. The further stage of this study will be the analysis of the effect of corrugation geometry on the efficiency of heat transfer.

**Keywords:** evaporator, heat transfer coefficient, Fluent, ANSYS.

## INTRODUCTION

The classic heat exchanging elements of industrial evaporators are heat exchange pipes. The efficiency of such surfaces is considerable because in the heating chamber of a small volume, a large specific surface of heat exchange could be developed. However, designs of this type have some disadvantages that are associated with the formation of condensate film during condensation of water vapor on the pipe surfaces, which in turn is a thermal resistance for heat exchange and may reduce the intensity of the phase transition of the vapor-liquid. From the other side, in the middle of the pipes, there is a boiling of the

concentrated solution, over time the value of the heat transfer coefficients is directed to the decline, which also has a negative effect on heat exchange.

The reduction of the heat transfer coefficient from the side of the solution, which boils mainly caused by the change in thermophysical parameters caused by its evaporation and increased concentration. To improve the heat and power features of evaporators the heat exchanging pipes of modified geometry could be used. Corrugated pipes of various modifications can be an effective design way to improve the thermal and hydraulic characteristics of the equipment. They are able to break the flow of condensate film on the outer surfaces of heat exchanging pipes, which can reduce the thermal resistance of the condensate layer. From the inner surface of the pipes, serve as additional boiling centers and vaporization, which will positively affect the parameters of heat transfer [1-2].

The state of the problem. The study made by Santa R. and co-authors presents the results of the verification of the adequacy of the proposed mathematical model of the physical process in the tubular evaporator of the heat pump [1]. The adequacy of the proposed mathematical model in the design of the heat pump was evaluated by comparing the results of experimental testing of the tubular evaporator of the heat pump with the numerical results obtained as a result of the use of the proposed mathematical model. Theoretical results were obtained by dividing the evaporator into control volumes and solving the appropriate system of equations of the proposed mathematical model using the Runge-Cutta and Adams Moulton method. As a result, the greatest deviation of the thermal power of the evaporator according to the calculations and experimental studies was from +9.39% to −6.31%. This confirms the efficiency of numerical modeling for heat exchange elements optimization.

Yuan H. and co-authors has developed a new thin film evaporator, which is combined with the compression refrigeration system, as well as its heat transfer [2]. The results of the work show that the cooling capacity of both types of evaporators increases linearly with increasing evaporation temperature. And under the same conditions, the cooling power of the thin film evaporator is twice as high as the standard evaporator with a smooth tube. Experimental data show that the productivity of heat transfer of a thin film evaporator is much better than a smooth tubular evaporator. This study confirms the possibility of increasing the energy efficiency of heat exchange elements by changing their configuration.

The numerical study [3] analyzes the behavior of the flow, the pressure drop and incensement the heat transfer of fluid in the corrugated pipe with different geometric configurations by using computer simulation technique. Numerical modeling is used the computational fluid dynamics (CFD), which provides a current model with different structural parameters. This study was conducted for a wide range of Reynolds numbers and for different sizes of heat exchange corrugated pipes. On the basis of the data obtained, correlation expressions were developed for the friction coefficient and Nusselt number with different geometric parameters of the corrugated pipe. However, in this study there are no data for heat transfer in the event of a change in the aggregate state, that is, the calculation range was concerned with temperature values and pressure to the phase transition.

For the studying of heat exchange in the pipes of new design it is necessary to go from the development of technical documentation to conducting physical experiments and

confirming the correctness of the assumptions. Reducing the costs related to full-time experiments and the time spent on the preparation of stands and the production of experimental samples can be realized by using computer modeling [4-5].

The most progressive method of computer modeling of hydrodynamic, heat and mass exchange processes is the finite element method. This method is implemented in the following programs ANSYS, COMSOL, SolidWorks, SimScale [6-8].

The purpose of this study is to analyze the possibility of using a corrugated pipe as an element of improving the efficiency of the evaporators.

The relevance of the study is due to the use of evaporators in most lines of biotechnological industries, the implementation of energy savings during technological operations and rigid control of process parameters for obtaining products of appropriate quality.

The purpose of the article is to use computer modeling methods to evaluate the possibility of using corrugated pipes of the proposed geometry to intensify heat exchange during the boiling of the liquid in the heat exchange pipes of the evaporation apparatus.

## MATERIALS AND METHODS

Fluent ANSYS was selected for modeling. The modeling was carried out for 2D models, taking into account the symmetry of the pipes and the need to reduce the load at the computer workstation (Fig. 1).

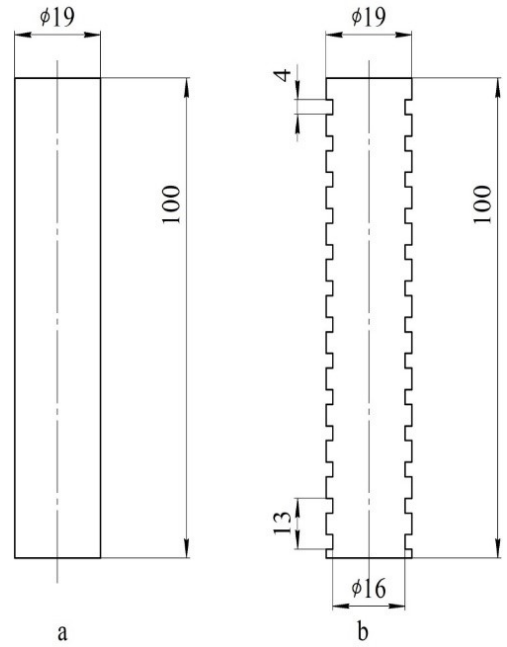
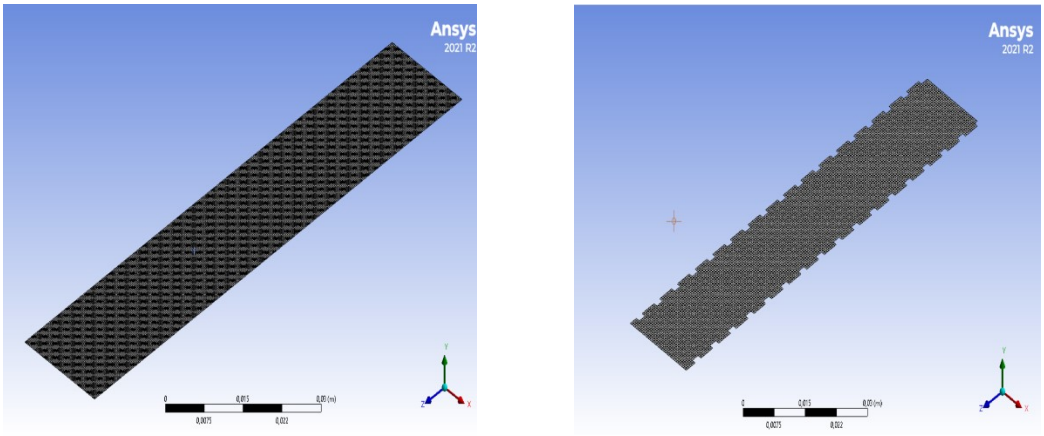



Fig. 1. Geometric dimensions of the modeling zone a) smooth pipe; b) corrugated pipe

To implement the finite element method geometric models were divided into the final set of elements in the ANSYS "Mesh". The “Use Adaptive Sizing” feature with the value of

Resolution = 7 was used during meshing. To improve the mesh “Refinement” was activated, with the value Refinement = 3 (Fig. 2).




a b

Fig. 2. The mesh of the calculated area: a) smooth pipe; b) corrugated pipe

The type of modeling was "Transient", Timestep set to 0.0001 second, which provides the conditions of convergence and value of Courant Number less than 10.

In general settings next features were activated [9-10]:

• In the “Multiphase” model – “Volume of Fluid”; the main and secondary phases were determined; the interaction between the phases was given;

• In the “Energy” model – “Energy Equation”.

For modeling the boiling process, the material "Water" was selected as a liquid, for which the physicochemical parameters are automatically determined, depending on the temperature and the critical points [11-12].

The boundary and initial conditions of the process:

• At the side ends of the model a boundary condition "Wall" was set with the temperature of 150˚C;

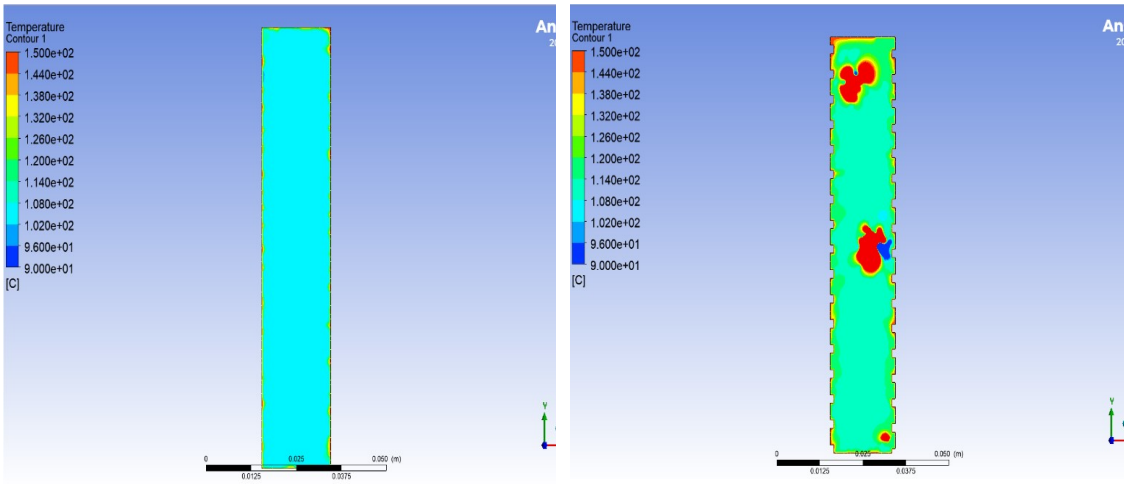
• At upper and lower ends a boundary condition "Wall" was set without heat exchange;

• Initial fluid temperature 98˚C.

## RESULTS

As a result of computer simulation, the temperature, the volume of vapor and water, and the value of heat flow from the wall to boiling liquid were obtained.

The outline of temperature distribution in pipes of different configuration is shown in Fig. 3.

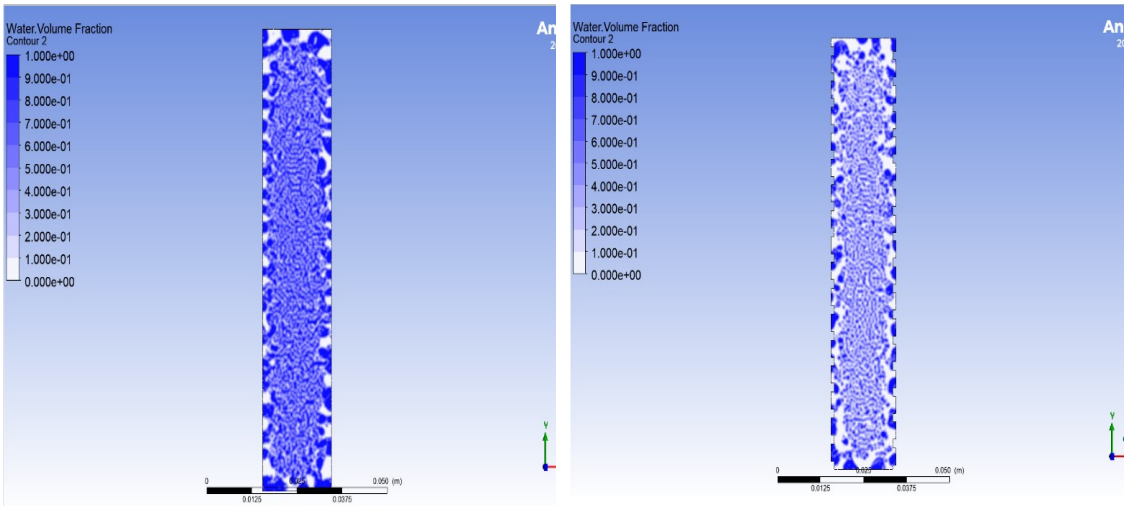



a b

Fig. 3. Contour of temperature distribution a) smooth pipe; b) corrugated pipe

During the simulation in the smooth tube, there is a uniform heating of the liquid to an average temperature of 110 ˚C, and the temperature of the temperature is higher (Fig. 3, a). When using a corrugated pipe, the temperature in the pipe is significantly increased. Local zones with overheating up to 150 ˚C are observed in the corrugated pipe, with an average temperature of 123 ˚C (Fig. 3, b).

The volume of the liquid phase in the volume of the pipe is shown in Fig. 4.




a b

Fig. 4. Volume fraction of liquid: a) smooth pipe; b) corrugated pipe

There is a uniform heating of the liquid in the smooth tube, an average value of temperature is 110 ˚C, the temperature near the wall is higher (Fig. 3, a). When using a corrugated pipe, the temperature in the pipe is significantly increased. Local zones with

overheating up to 150 ˚C are observed in the corrugated pipe, with an average temperature value 123 ˚C (Fig. 3, b).

The volume of the liquid phase in the volume of the pipe is shown in Fig. 4. For a smooth pipe, taking into account the uniform distribution of temperatures, the phase transition occurs evenly throughout the volume, the most intensively bubbles are formed near the walls of the pipe (Fig. 4, a). When modeling the boiling process in the corrugated pipe, there is higher volume fraction of the vapor phase in pipe due to the higher average temperature and more vapor centers on corrugations.

In order to compare the efficiency of the use of corrugated pipes, the coefficient of heat transfer from the wall of the boiling pipe was calculated. The calculation of the heat transfer coefficient is based on the use of the formula of the specific heat flux:

$$q = \alpha \cdot \Delta t$$

whence:

$$\alpha = \frac{q}{\Delta t}$$

By using "Expression" the average value of the specific heat flux on the side walls of the pipes and the temperature near the fluid wall were defined, the results of the calculation are given in Table 1.

## DISCUSSION

To confirm the adequacy of the computer model, the heat transfer coefficient from the wall to a liquid, which boils was calculated by the empirical formula [1]:

$$\alpha_2 = 780 \cdot (\alpha_1 \cdot \Delta t_1)^{0,6} \frac{\lambda^{1,3} \rho_l^{0,5} \rho_v^{0,06}}{\sigma^{0,5} r^{0,6} \rho_0^{0,66} c^{0,3} \mu^{0,3}},$$

where $\alpha_1$ is the heat transfer coefficient from the heating steam to the pipe wall; $\Delta t_1$ is the difference between the wall temperature and condensation temperature; $\lambda$ is fluid thermal conductivity coefficient; $\rho_l$ is liquid density; $\rho_v$ is water vapor density; σ is surface fluid coefficient; $r$ is specific heat of vaporization; $\rho_0$ is water vapor density at 100˚C; $c_2$ is specific heat of fluid; $\mu$ is the coefficient of dynamic viscosity of the liquid.

The determining temperature is the boiling point of the liquid at a given pressure.

Substituting the process parameters and the values of the properties of the liquid corresponding to the computer modeling we obtain the calculated value of the heat transfer coefficient from the wall to the boiling liquid $\alpha_2$=2412 W/(m$^2$·K).

The value of the heat transfer coefficient received in computer modeling is 2162 W/(m$^2$·K), that only 11% differs from theoretical value. Given the permissible error of 30 % for the formulas obtained empirically, it can be argued that the computer model adequately describes the process of heat transfer from a wall to boiling water.

Table 1. Results of calculation

| Type | specific heat flux, W/m$^2$ | Temperatures Difference, ˚C | Heat transfer coefficient, W/(m$^2$·K) |
|---|---|---|---|
| smooth | 86 504 | 40 | 2162 |
| corrugated | 89 006 | 27 | 3296 |

## CONCLUSION

The computer model adequately describes the boiling process of fluid in the pipe, so it can serve to analyze the feasibility of using a corrugated pipe in the evaporator and to further optimize its geometry.

Simulation results demonstrate the feasibility of using corrugated pipes. Comparing the presented models, including the indicators of the heat transfer coefficient, it should be noted that the corrugated surface pipe has better indicators than a smooth surface.

The value of the heat transfer coefficient for the corrugated pipe is 3296 W/(m2·K), which is almost 52 % higher than the smooth pipe. The use of these pipes in evaporators will reduce the metal intensity of the structure and increase its technical and economic indicators.

The further development of this study will be the analysis of the effect of corrugation geometry on the efficiency of heat transfer.

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# NUTRITIONAL AND PHYTOCHEMICAL ENRICHED MUFFINS BASED ON WHEAT FLOUR AND BAOBAB PULP FLOUR (*ADANSONIA DIGITATA* L.)

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## ABSTRACT

This study was examined to assess the beneficial effects of baobab flour (BF) on wheat flour (WF) muffins. Four (4) types of muffins, including control and 3 with 10, 20, and 30% BF, were produced and analyzed from a nutritional, phytochemical, and organoleptic point of view. The results of the nutritional analyses revealed that partial substitution of WF by BF significantly improved the nutritional characteristics of the muffins. There was a significant increase in mineral and lipid content in the muffins as BF became more abundant. The muffin with 30% BF (BWM3) was the richest in macro and microelements apart from Mn (Zn: 11.12±0.12 mg/kg; Fe: 19.87±0.01 mg/kg; Mn: 3.17±0.06 mg/kg; Ca: 347.76±5. 27 mg/kg; Mg: 289.92±2.47 mg/kg; K: 688.59±3.02 mg/kg; Cu: 3.29±0.02 mg/kg and Ni: 0.316±0.007 mg/kg) and was 2 times richer in mineral substances than the control sample (MC). BF also significantly improved the phytochemical properties of the muffins. Between MC and BWM3, there was an increase of 31.74 mg/100g, 124.43 mg/100g, and 286.97% respectively in total polyphenol, total flavonoid, and antioxidant activity. Among the samples with BF, the one with 10% was the most appreciated by the evaluators. Not only was it the most appreciated, but it also obtained values close to MC. Our analyses and the literature suggest that BF can be used as an ingredient in bakery products to improve nutritional and phytochemical composition. However, due to its acrid taste, it is recommended that its level of substitution in muffins should not exceed 10%, to obtain a product acceptable to consumers.

**Keywords:** Nutritional, Muffins, Phytochemical and Baobab Pulp Flour.

## 1. INTRODUCTION

Muffins are a widely popular baked product due to their sensory attributes, convenience, taste, texture, and long shelf life [1, 2, 3, 4]. Traditional ingredients for muffin recipes include wheat flour (Triticum vulgare). Despite the unique baking qualities that this flour imparts (such as extensibility and viscoelastic structure due to its gluten content) and the essential carbohydrates and protein it provides, it does not contain enough minerals, particularly calcium, zinc, and iron [5, 6]. Consequently, although muffins are

popular amongst consumers, they may not meet their nutritional requirements, especially for those who are malnourished. Considering the numerous alternative flours available that possess superior nutritional characteristics to wheat flour, Baobab flour included, muffins can be readily enhanced to meet specific dietary or consumer requirements [7, 8].

The baobab is a substantial plant that is indigenous to the wooded savannas of sub-Saharan Africa. It holds immense cultural significance and is a physically magnificent tree. This tree is commonly referred to as "the little medicine tree" or "the pharmacy tree" due to its versatile applications in traditional medicine, nutrition, and even cosmetics [9, 10, 11]. All components of the baobab, namely the bark, leaves, fruit pulp, and seeds, have practical applications across diverse fields and provide sustenance to rural communities in Africa [12, 13]. Multiple research studies conducted on various parts of the baobab indicate that it possesses antiviral, antibacterial, and anti-inflammatory properties, which justify its traditional use [14, 15, 16]. Among the numerous parts of the baobab, the pulp of its fruit has received significant attention over the years due to its nutritional importance [17]. Fruit pulp is a valuable source of calcium, with 100g providing anywhere from 116 to 370mg, depending on the source. This amount falls slightly below the recommended daily allowance of 500mg/day. The iron content in fruit pulp ranges from 1.7 to 9.1mg, which is significantly lower than the recommended daily allowance of 18mg/day, as per Alercia, 2013 [18]. Baobab fruit pulp has been identified as a natural dietary supplement or ingredient in a variety of value-added products, such as bread, cookies, cakes, and some traditional foods. Technical terms are consistently used and explained, and the language is clear, objective, and free from ornamental language. Several studies have shown that it is abundant in vitamin C, minerals, including calcium, phosphorus, and magnesium, and dietary fiber [8, 19, 20, 21, 22, 23, 24, 25].

Baobab pulp includes organic acids, phenolic compounds and flavonoids [26, 27, 28, 29]. These constituents allow baobab pulp to function as a carrier for creating functional foods. Accordingly, the objective of this study is to determine the nutritional, phytochemical and organoleptic potential of muffins with varying degrees of replacement of wheat flour with baobab pulp flour.

## 2. MATERIALS AND METHODS

### 2.1. Ingredients and preparation of the composite flours

Baobab flour (BF) was purchased in Northern Benin, and wheat flour (WF) type 650 was bought from the Profil supermarket in Timisoara, Romania. Muffin production involved substituting three levels of baobab flour, as determined by Górecka et al. 2010 [30]. The resulting three types of baobab composite flour were: BWF 1 comprised of 10% BF and 90% WF. BWF 2 included 20% BF and 80% WF, while BWF 3 consisted of 30% BF and 70% WF. The remaining ingredients used were egg, sugar, honey, milk powder, oil, and baking powder which were all purchased from Auchan supermarket in Timisoara, Romania. For the milk powder, 130g was mixed with 900ml of warm water. Table 1 highlights the exact amount of each ingredient used.

Table 1. Recipe for muffin with composite baobab flour

| Samples | Ingredients | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Baobab flour (g) | Wheat flour type 650 (g) | Baking powder (g) | Salt (g) | Honey (g) | Oil (ml) | Eggs (ml) | Milk (ml) | Sugar (g) |
| MC | - | 500 | 14 | 2 | 200 | 400 | 300 | 140 | 200 |
| BWM1 | 50 | 450 | 14 | 2 | 200 | 400 | 300 | 140 | 200 |
| BWM2 | 100 | 400 | 14 | 2 | 200 | 400 | 300 | 140 | 200 |
| BWM3 | 150 | 350 | 14 | 2 | 200 | 400 | 300 | 140 | 200 |

### 2.2. Preparation of the muffins

The muffins were prepared following the methodology of Białek et al. (2016) [31] with slight modifications. A control muffin (MC - made with 100% wheat flour) and three additional muffin variants with varying levels of Baobab flour substitution (BWM1 - featuring 10% Baobab flour and 90% wheat flour, BWM2 - featuring 20% Baobab flour and 80% wheat flour, and BWB3 - consisting of 30% Baobab flour and a 70% Baobab/wheat flour blend) were prepared using the previously derived compound flours. Following the mixing of butter and caster sugar, the resulting mixture was aerated for ten minutes. The milk, honey, oil, and eggs were combined and mixed for two minutes. Then, the appropriate flour (wheat flour or compound flour), salt, and baking powder were added and mixed. The mixture was chilled in the fridge for an hour at a temperature between 4 and 5°C. Afterward, the dough was poured into molds lined with greaseproof paper and baked for 17 minutes at 190°C. Once cooled to room temperature, the final product was obtained. The technological scheme for obtaining muffins is shown in figure 1, and the muffins obtained are shown in Figure 2.

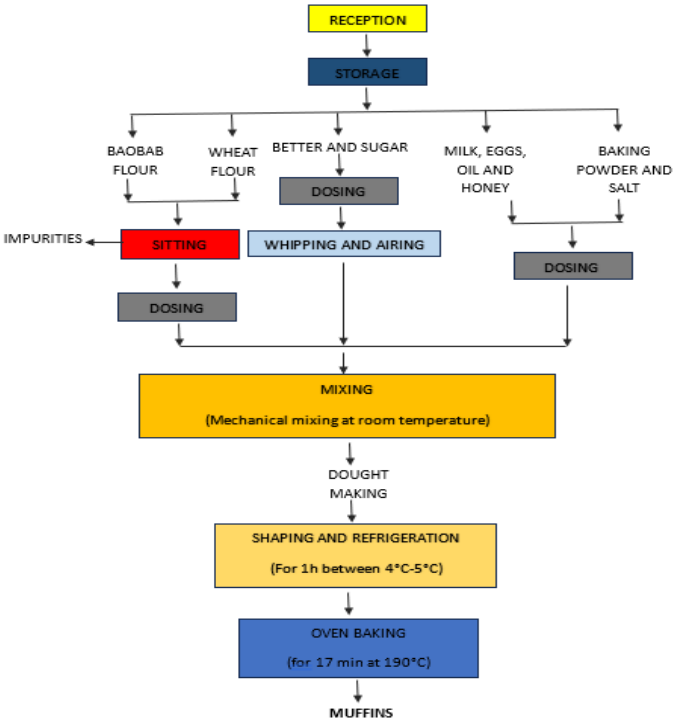



**Figure 1.** The technological scheme for obtaining muffins




**Figure 2.** Muffins made with wheat flour (WF) enriched with different proportions of Baobab flour (FB)

### 2.3. Approximative composition of muffins

In order to determine the approximate composition of the muffins, several methods were utilized. The ash content was determined using the ISO no. 2171/2007 method. The fat and protein content were analyzed using the standard method of the Association of Official Analytical Chemists (AOAC 2000) [32]. The moisture content was determined using the ICC (2003) standard method [33]. To calculate the total carbohydrate content and energy value, the method described by [34] was followed.

### 2.4. Macro and microelements

The macro- and micro-element content (mg/kg) was determined in triplicate using the method described by Plustea et al. 2022 [34].

### 2.5. Phytochemical profile of different muffins

The alcoholic extracts were prepared following the method by [8]. The total phenolic content was determined using the Folin-Ciocalteu method as described by Poiana et al. (2023), and reported as mg GAE/100g. The total flavonoid content (in mg quercetin [QE] per 100g) was determined using the method presented by [35]. The antioxidant activity (%) of the various muffin samples was determined through Ciulca et al., 2021 method [36].

### 2.6. Sensory Analysis

The sensory analysis for this study follows the procedures outlined by Poiana et al. (2023) [37]. A group of 23 assessors willingly participated in the trial, consisting of 7 males and 16 females, ranging in age from 19 to 41 years.

### 2.7. Statistical Analysis

The results presented represent the mean ± standard deviation (SD) of three independent experiments. We conducted a one-way analysis of variance (ANOVA), accompanied by t-test for equal variances, to determine the statistical significance of differences between formulations. Differences were considered statistically significant at a probability of less than 0.05 ($p < 0.05$).

## 3. RESULTS AND DISCUSSION

### 3.1. Approximate composition of muffins

The approximate composition of the different muffins has been determined. This includes their moisture content, mineral substances, proteins, lipids, carbohydrates, and the energy value they provide. The data resulting from these assorted analyses are summarized in the table provided below.

Table 2. Approximate composition of muffins

| Samples | Nutritional Characteristics | | | | | |
|---|---|---|---|---|---|---|
| | Moisture (%) | Mineral Content (%) | Proteins (%) | Lipids (%) | Carbohydrates (g/100 g) | Energy Values (kcal/100 g) |
| | | | Muffins | | | |
| **MC** | 18.56 ± 0.02$^{a}$ | 0.92 ± 0.07$^{a}$ | 7.27 ± 0.02$^{c}$ | 18.72 ± 0.12$^{a}$ | 54.53 ± 0.07$^{d}$ | 415.69 ± 0.80$^{c}$ |
| **BWM1** | 23.35 ± 0.05$^{b}$ | 1.10 ± 0.20$^{a}$ | 6.51 ± 0.03$^{b}$ | 21.12 ± 0.23$^{b}$ | 47.91 ± 0.10$^{c}$ | 407.77 ± 1.73$^{b}$ |
| **BWM2** | 24.41 ± 0.17$^{c}$ | 1.47 ± 0.15$^{b}$ | 6.32 ± 0.02$^{b}$ | 21.99 ± 0.11$^{b,c}$ | 45.81 ± 0.13$^{b}$ | 406.42 ± 1.38$^{a,b}$ |
| **BWM3** | 25.80 ± 0.03$^{d}$ | 1.97 ± 0.03$^{c}$ | 5.79 ± 0.08$^{a}$ | 22.86 ± 0.02$^{c}$ | 43.57 ± 0.14$^{a}$ | 403.18 ± 0.29$^{a}$ |

Values of the table represent the mean of three determinations ± standard deviation (SD). Different letters (a-d) in the same column of each sample category represent statistically significant differences ($p < 0.05$) recorded using a t-test.

Analysis of the data obtained reveals that the control muffin (MC) is less rich in minerals, lipids, and water than other muffins with different percentages of baobab. Nevertheless, it is richer than the other muffins in terms of protein, carbohydrates, and energy values provided.

As for moisture, the higher the baobab content in the muffins' composition, the more abundant it is. From 18.56 ± 0.02% in MC, moisture rose to 25.80 ± 0.03%. This increase can be explained by the fact that the moisture content of baobab flour is higher than that of wheat flour [8, 19]. They obtained respectively 13.79 ± 0.01% and 13.28 ± 0.26% for baobab and 10.80 ± 0.04% and 11.70 ± 0.59% for wheat flour. Also, their various studies were in agreement with the present one on the fact that the moisture content increases with the baobab concentration of the finished products.

MC is less rich in minerals than the other samples. We also note an increase in the mineral content with increasing baobab content in the different muffins. There were 0.92 ± 0.07%, 1.10 ± 0.20%, 1.47 ± 0.15% and 1.97 ± 0.03% respectively for MC, BWM1, BWM2 and BWM3. [8] and [19] reported the same finding. The increase in mineral content with the abundance of Baobab in the composition of muffins can be explained by the fact that Baobab flour is more than 12 times rich in minerals than

wheat flour [8]. It can therefore be deduced that the partial substitution of wheat flour by baobab flour resulted in muffins with a high mineral content.

Like minerals, lipids are more abundant in muffin samples with different percentages of baobab than in the control sample. Lipid abundance also increased with increasing baobab content in the different samples. Lipid composition also varied significantly from one sample to another. We had 18.72 ± 0.12%, 21.12 ± 0.23%, 21.99 ± 0.11% and 22.86 ± 0.02% respectively for MC, BWM1, BWM2 and BWM3. Here, however, our results, although consistent with those of [8] and [20], are not consistent with those of [19]. In the studies by Barakat, 2021, lipid content did not vary significantly from one cake sample to another. On the other hand, in the studies by Dossa et al., 2023 [8] and Mounjouenpou et al., 2018 [20], there was an increase in lipid content from one sample to another, as is the case in the present study. From the analysis of these results, it can be said that enriching wheat muffins with baobab flour would improve the lipid intake of the muffin.

In contrast to lipids, the control sample is richer in protein than the other samples. This is because wheat is richer in protein than baobab [8]. Moreover, the more baobab in the muffins, the lower the protein content. Indeed, from 7.27 ± 0.02% for the control sample, the protein level dropped to 5.79 ± 0.08% for the sample with 30% baobab. Similar results were reported by Dossa et al., 2023 [8] and Mounjouenpou et al., 2018 [20] in their various studies. Carbohydrate levels followed the same pattern as protein levels in the different samples. Thus, we had respectively 54.53 ± 0.07 g/100g, 47.91 ± 0.10 g/100g, 45.81 ± 0.13g/100g, and 43.57 ± 0.14g/100g for MC, BWM1, BWM2, and BWM3. These results lead us to conclude that wheat provides more carbohydrates and protein in muffins than baobab.

The energy value provided by MC (415.69 ± 0.80 kcal/100 g) is higher than those provided by the other muffins with different proportions of baobab (respectively 407.77 ± 1.73 kcal/100 g, 406.42 ± 1.38 kcal/100 g and 403.18 ± 0.29kcal/100 g for BWM1, BWM2 and BWM3). Our results thus reveal that baobab flour would provide a lower source of energy than wheat flour [8, 19, 20]. We thus deduce that the partial substitution of wheat flour for baobab flour results in muffins providing less energy value.

### 3.2. Macro- and Microelements

To determine the macro- and micro-nutrient potential of baobab/wheat muffins and control muffins, their composition in terms of copper (Cu), nickel (Ni), zinc (Zn), iron (Fe), manganese (Mn), calcium (Ca), magnesium and potassium (K) was determined. The results of these various analyses are presented in the table below.

**Table 3. Macro- and Microelements Contents of muffins**

| Samples | Macro- and Microelements Contents (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cu | Ni | Zn | Fe | Mn | Ca | Mg | K |
| Muffins | | | | | | | | |
| MC | 1.59±0.02$^{a}$ | 0.038±0.02$^{a}$ | 5.20±0.09$^{a}$ | 7.47±0.04$^{a}$ | 4.60±0.09$^{c}$ | 199.94±1.84$^{a}$ | 234.00±3.68$^{a}$ | 566.50±4.10$^{a}$ |
| BWM1 | 2.24±0.07$^{b}$ | 0.188±0.005$^{b}$ | 6.44±0.25$^{b}$ | 7.69±0.17$^{a}$ | 3.70±0.16$^{b}$ | 215.51±8.48$^{b}$ | 260.32±1.82$^{b}$ | 592.36±2.31$^{b}$ |
| BWM2 | 2.58±0.02$^{b}$ | 0.229±0.009$^{c}$ | 7.71±0.13$^{c}$ | 18.64±0.34$^{b}$ | 3.43±0.07$^{a,b}$ | 261.30±4.90$^{c}$ | 267.00±1.96$^{b,c}$ | 647.01±12.74$^{c}$ |
| BWM3 | 3.29±0.02$^{c}$ | 0.316±0.007$^{d}$ | 11.12±0.12$^{d}$ | 19.87±0.01$^{c}$ | 3.17±0.06$^{a}$ | 347.76±5.27$^{d}$ | 289.92±2.47$^{c}$ | 688.59±3.02$^{d}$ |

Values of the table represent the mean of three determinations ± standard deviation (SD). Different letters (a-d) in the same column of each sample category represent statistically significant differences ($p < 0.05$) recorded using a t-test.

Analysis of the results from this table reveals that, apart from Mn, MC is significantly less rich than all the other muffins with different proportions of baobab (BWM1, BWM2, and BWM3) in terms of the macro- and microelements studied in the present study. Similar results were obtained by [8] and [19]. On the other hand, in the work by [20], the control sample is also less rich in Mn than the sample with 20% baobab. This could be explained by the fact that in their study, they had used rice as the basic ingredient, whereas in our study wheat flour was used as the basic ingredient. We can therefore deduce that Baobab contributes significantly to improving the mineral content of muffins.

Potassium (K) was the most abundant macroelement in both MC and muffins with different percentages of baobab. Similar results were obtained by [8, 19, 20]. It is therefore the most abundant mineral in baobab pulp. Various studies by [8, 19, 22, 38] confirm that potassium is the most abundant macroelement in baobab. In the present study, K was significantly higher in muffins with different proportions of baobab than in MC. Also, as baobab content in muffins increased, so did K content. The figures were 566.5±4.1, 592.36±2.31, 647.01±12.74 and 688.59±3.02 mg/kg for MC, BWM1, BWM2 and BWM3 respectively. This suggests that baobab flour is richer than wheat flour in terms of K content [8, 19], and that incorporating baobab flour into wheat muffins significantly improves K content (whatever the level of substitution).

Calcium (Ca) and magnesium (Mg) content followed the same pattern as K content. These results are in line with those of [8] and [19]. For Ca content, there were 199.94±1.84, 215.51±8.48, 261.30±4.90 and 347.76±5.27 mg/kg respectively for MC, BWM1, BWM2 and BWM3. On the other hand, Mg levels were 234±3.68, 260.32±1.82, 267±1.96 and 289.92±2.47 mg/kg for MC, BWM1, BWM2 and BWM3 respectively. Here too, we deduce that baobab flour is richer in Ca and Mg than wheat flour [8, 19]. We also deduce that baobab contributes to Ca and Mg abundance in muffins.

MC was less rich in iron (Fe) than BWM1, BWM2 and BWM3. Moreover, the iron content of muffins is abundant when the proportion of baobab in them is high. From 7.47±0.04 mg/kg in MC, Fe content rose to 19.87±0.01 mg/kg in BWM3. Zinc content was more abundant in muffins with different levels of baobab than in the control muffin. Also, the more baobab in the muffins, the more zinc they contained. The levels were 5.20±0.09, 6.44±0.25, 7.71±0.13 and 11.12±0.12 mg/kg respectively for MC, BWM1, BWM2, and BWM3. These results are in agreement with those obtained by [8,

19] and allow us to conclude that partial substitution of wheat flour by baobab flour improves the Fe and Zn composition of muffins.

In contrast to Zn, manganese (Mn) content was more abundant in MC than in muffins with different proportions of baobab. In addition, Mn content was less abundant when baobab content in muffins was high. It was 4.6±0.09, 3.70±0.16, 3.43±0.07 and 3.17±0.06 mg/kg for MC, BWM1, BWM2 and BWM3, respectively. Baobab flour would therefore be less rich in Mn than wheat flour [8]. We also deduce that the partial replacement of wheat flour with baobab flour would be the origin of the reduction in the Mn composition of the muffins.

### 3.3. Phytochemical profile

To establish the phytochemical profile of the various muffins, their total polyphenol content (mg/100g), total flavonoid content (mg/100g), and antioxidant activity (%) were determined. The results are presented in Table 4.

**Table 4. Phytochemical profile of muffins**

| Samples | Total polyphenols content (mg/100g) | Total flavonoids content (mg/100g) | Antioxidant activity, DPPH (%) |
|---|---|---|---|
| | | *Muffin* | |
| CM | *113.6±0.2*[a] | *2.54±0.16*[a] | *29.17±0.09*[a] |
| BWM1 | *118.4±0.85*[b] | *89.34±2.74*[b] | *79.13±0.09*[b,c] |
| BWM2 | *124.51±0.06*[c] | *107.44±6.34*[c] | *81.66±0.01*[c,d] |
| BWM3 | 145.34±0.35[d] | 126.97±3.22[d] | 83.71±0.07[d] |

Values of the table represent the mean of three determinations ± standard deviation (SD). Different letters (a-d) in the same column of each sample category represent statistically significant differences ($p < 0.05$) recorded using a t-test.

From the analysis of all the results, the muffins with different proportions of baobab (BWM1, BWM2 and BWM3) are significantly richer than the control muffin (MC) in terms of phytochemical profile. Similar results were obtained by [8] and [19].

Total polyphenol content was not only significantly higher in muffins with different proportions of baobab but also increased as a function of baobab abundance in the recipe. From 113.6±0.2 for MC, total polyphenol content rose to 145.34±0.35 for BWM 3. Total polyphenol content had adopted a similar pattern in the studies by Dossa et al., 2023 [8] and Barakat, 2021 [19]. This finding can be explained by the fact that baobab flour is a good source of polyphenols compared with wheat flour. [8], reported that the total polyphenol content of baobab flour was 629.7 ± 0.35 mg/100g, compared with 176.7 ± 0.69 mg/100g for wheat flour. Based on the above information, we deduce that the partial substitution of wheat flour for baobab flour contributes to the abundance of total polyphenols in muffins.

Total flavonoid content was over 35 times more abundant in BWM1 than in MC. This being said, there is a significant difference in total flavonoid content between MC and other muffins with different levels of BF substitution. There was also an increase in total flavonoid content as the level of baobab in the muffin composition increased. Similar results were obtained by Dossa et al. 2023 [8]. We deduce that baobab is a good source of flavonoids and that its substitution could contribute to improving the total flavonoid content of bakery products.

Antioxidant activity followed the same pattern as total polyphenol and total flavonoid content. Thus, we had 29.17±0.09; 79.13±0.09; 81.66±0.01 and 83.71±0.07 respectively for MC, BWM1, BWM2 and BWM3. Similar results were reported in studies by [8, 19]. Baobab, while richer than wheat in terms of antioxidant activity [8], also contributes to the abundance of these compounds in muffins.

### 3.4. Sensory analysis

In order to determine the impact of baobab on the organoleptic characteristics of the muffins, a tasting test was carried out on each of the muffins produced as part of this study. The organoleptic characteristics evaluated in this study were: appearance, color, texture, taste, flavour and overall acceptability of the cookies. The results of this evaluation are illustrated in the figure below.

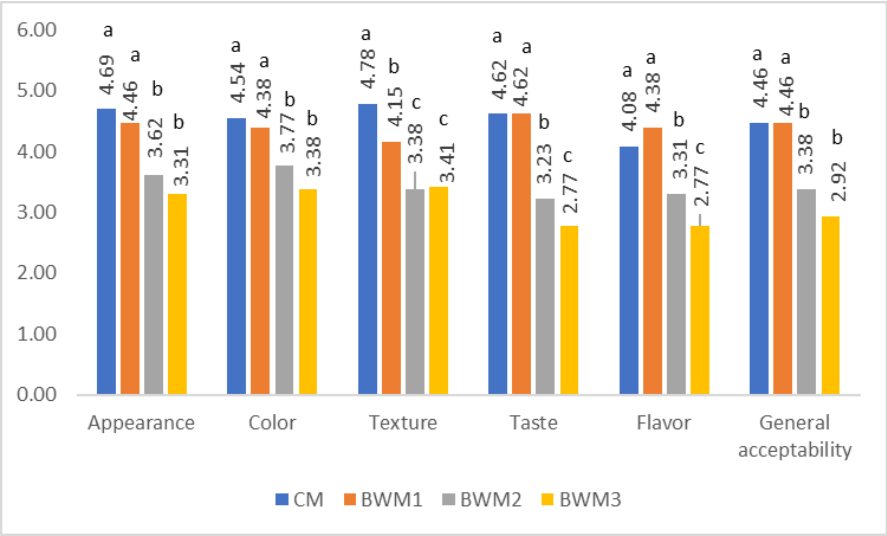



**Figure 3.** Sensorial anlysis of muffins

Values of the table represent the mean of three determinations ± standard deviation (SD). Different letters (a-c) in the same column of each sample category represent statistically significant differences ($p < 0.05$) recorded using a t-test.

Overall analysis of the data from this figure reveals that MC was the most popular muffin sample in terms of appearance, color, and texture. On the other hand, the sample with 10% baobab (BWM1) was the most appreciated for its flavor. In terms of taste and overall acceptability, MC and BWM1 came out on top with equal scores. We can therefore deduce that among the samples with different proportions of Baobab, BWM1 was the most appreciated for all organoleptic characteristics.

In terms of appearance, MC obtained the highest score (4.69), followed by BWM1, BWM2 and BWM3 with 4.46, 3.62 and 3.31 respectively. The more baobab in the muffins, the lower the score. Similar observations were made for color and texture. These results are in line with those of [8, 19]. We therefore deduce that baobab had a reducing effect on consumer acceptance of muffins in terms of appearance, color and texture.

In terms of flavor, BWM1 was the most popular muffin, including MC. It scored 4.38. It was followed respectively by MC (4.08), BWM2 (3.31) and BWM3 (2.77). It can be

seen that at 10% baobab, baobab provided a good flavor to the muffins, but above 10%, panelists began to like the muffins less and less in terms of flavor.

Panelists appreciated MC and BWM1 equally for their taste and overall acceptability. For these same characteristics, from 20% baobab onwards, panelists liked the muffins less and less. We can therefore deduce that substituting baobab flour for wheat flour up to 10% did not affect the taste or overall acceptability of the muffins.

Based on all the organoleptic results obtained in this study, and on the results of previous studies, we recommend a substitution of wheat flour for baobab flour of no more than 10% for muffin production.

## CONCLUSION

In this study, it was examined to improve the nutritional quality of the muffin by substituting wheat flour with different proportions of baobab flour. The results obtained from the approximate composition analyses reveal that baobab flour improved the muffins' composition in terms of minerals, lipids, and moisture. Nevertheless, it resulted in muffins that were lower in protein and carbohydrates and provided consumers with less energy. Regarding the macro and micro-element composition of muffins, the study concluded that, apart from Mn, Baobab made a significant contribution to improving the mineral content of muffins. It also concluded that muffins with different proportions of baobab were significantly richer than the control muffin in terms of phytochemical profile. This means that baobab improved muffin composition in terms of total polyphenol, total flavonoid, and antioxidant activity. Based on the data obtained for organoleptic characteristics in this study and on the data and conclusions of previous studies, we suggest a substitution of wheat flour with baobab flour not exceeding 10% for muffin production. Baobab is a superfood ingredient that can undoubtedly be used to improve the nutritional quality of baked goods.

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# NUTRITIONAL AND SENSORIAL CHARACTERISATION OF RAW – DRY SALAMI WITH ADDITION OF GOJI AND NUTS

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## ABSTRACT

Goji fruits (Lycium barbarum) are known for their very high content of antioxidants, supplemented by a high intake of vitamins, (including vitamins A, C and vitamins from the B complex), minerals (calcium, magnesium, selenium), proteins, fibers and amino acids. Thus, goji fruits have a very high nutritional potential, which is why their inclusion in the daily menu or their use for therapeutic purposes have proven extremely beneficial for the human body. Hazelnuts (Corylus avelana) and pistachios (Pistachia vera) are a great source of phytosterols, fibers and protein, also containing vitamins and minerals such as magnesium and vitamin E.

The purpose of this study was to obtain a raw-dry salami with addition of goji fruits, hazelnut and pistachio, and to characterize our new formula in comparison with two types of salami from the local market, from the nutritional and sensory point of view.

The total contents of fats, proteins, carbohydrates, mineral content, sodium chloride and moisture were determined. The results show that, compared to the salami sold on the market, our new formula - raw – dry salami with goji, hazelnut and pistachio has higher nutritional values. Regarding the sensory analysis, the tasters' preference was demonstrated for the salami with hazelnuts, pistachios and goji, this obtaining the highest scores in terms of texture, color, smell, taste and general acceptability. Consequently, we can observe that addition of goji berries, hazelnuts and pistachios when obtaining dry raw salami can optimize its nutritional and sensory quality.

**Keywords:** raw-dry salami, goji, pistachio, hazelnut, nutritional profile

## INTRODUCTION

Nowadays, in response to the growing interest in having a healthy diet, there is a tendency to reformulate the classic recipes for obtaining food products by adding biologically active compounds with beneficial effects on the nutritional value. [1]

Fruits of Lycium species, also named as wolfberry or goji berries, have become popular in the global market as a "superfood", initially being known for their use in traditional medicine, in Asian countries [2]. Goji berries are fruits with attractive colors (yellow, orange to red (L. barbarum) or black (L. ruthenicum), with sweet flavor. Based on their high nutritive properties (hypoglycemic effects, anticancer, lipid-lowering, antioxidant, immunomodulatory) and high content of bioactive compounds (polysaccharides, fatty acids, carotenoids, polyphenols, micronutrients, including vitamins, such as thiamin, riboflavin, and vitamin C) they are considered as a potential functional food with beneficial effects on the human body. [3]

Nuts are dry fruits, with high density of nutrients, widely consumed in daily diets all over the world. They contain the best nutritional density and possess high quantities of protein and fat, especially unsaturated fatty acids, alongside with minerals, vitamins, dietary fibers, and various bioactive compounds (polyphenols, and flavonoids) [4].

Hazelnut, (Corylus avellana L.), are dried fruits containing high levels of fat, (the most representative being oleic acid), proteins, (amino acids: arginine, glutamic acid, aspartic acid, and alanine) and carbohydrates, dried fibers. The major amino acids found in hazelnuts are arginine, glutamic acid, aspartic acid, and alanine, together with vitamin E (α-tocopherol) and polyphenols, the latter being known for their antioxidant action. There are data confirming that consumption of hazelnuts contributes to the prevention of cardiovascular diseases and to the reduction of specific symptoms of Alzheimer's disease [5,6].
Pistachio nuts (Pistacia vera) have a high fat content, being also a good source of protein, carbohydrates and total dietary fiber. Pistachio skin is a great source of anti-inflammatory and phenolic compounds, including flavonoids and gallotannins, [7,8]. Hazelnut and pistachio are plant-based protein sources rich in antioxidants, minerals, and healthy fatty acids, whose consumption is encouraged by dietary recommendations.

From these considerations presented above, the purpose of our study was the obtainment of a food product (raw - dried salami) with addition of dried goji, pistachio and hazelnut, and compare the characteristics of these new formula with two other similar varieties on the market, obtained without the addition of fruits and nuts.

## MATERIALS AND METHODS

### Materials

The raw and auxiliary materials used to obtain the salami were: fresh pork meat, lard, raw hazelnuts, roasted pistachios and dehydrated goji, and as spices the following were used: salt, mosaic pepper and granulated garlic.

Fresh meat and lard were purchased from a local producer (Timisoara, Timis County, Romania). Dehydrated goji berries, hazelnuts and pistachios (Sanovita, Romania), and ingredients such as ground mosaic pepper (Fuchs Condimente RO SRL, Romania), granulated garlic (Fuchs Condimente RO SRL, Romania) and salt (Salrom, Romania) were purchased from a local supermarket. The reagents used for the chemical analyses were purchased from Sigma-Aldrich (St Louis, MO, USA) and Chimreactiv (Bucharest, Romania) and were of analytical purity.

## Manufacture of Salami Formulas

The salami samples were prepared in the production department of a local manufacturer. Chilled pork meat (70/30) and lard were cut into cubes and minced using a mincer up to ø 4 mm (Maxima Holland MMM 12-229, Mijdrecht, The Netherlands). The ingredients weighed according to the recipes shown in table 1 were mixed using the meat mixer (La Felsinea 10M, Piazzola sul Brenta PD, Italy), the total mixing time was 10 minutes and the final temperature of the mixture was below 12°C.

Table 1. The recipe used to obtain salami formula

| Ingredient | Quantities | Ingredient | Quantities |
|---|---|---|---|
| Pork meat (g) | 720 | Granulated garlic (g) | 3 |
| Lard (g) | 180 | Hazelnut (g) | 9 |
| Salt (g) | 22.5 | Pistachio (g) | 9 |
| Mosaic pepper (g) | 3 | Dehydrated goji (g) | 9 |

The resulting mixture was filled into ø 43 mm fibrous cellulosic membrane using a salami filler (MSFA15L, Maxima, Mijdrecht, The Netherlands), resulting in 15 cm long pieces. The technological parameters used in the technology of obtaining salami with hazelnuts, pistachios and goji are presented in Table 2.

Table 2. The technological parameters used in the technology of obtaining salami with hazelnuts, pistachios and goji

| **Technological operation** | **Technological parameters** |
|---|---|
| Maturation | At a temperature of 23°C, humidity of 85% for 24h |
| Smoking | 2 hours at a maximum temperature of 20°C |
| Drying | It is done for 15 days at a temperature of 13° C, humidity of 75 % and a ventilation of 65 $m^3/h$ |

The resulting salami was stored at 4°C until analysis.

## Proximate Composition of Salami samples

The salami with hazelnuts, pistachio and goji prepared for this study was analyzed alongside with two other types of salami present on the market (Dried salami and Banățean salami).

To determine the proximate composition of the analysed salami samples, the moisture and total dry wheight (according to SR ISO 1442:2010), mineral content [9], total protein (according to SR ISO 936:2009), total lipids (according to SR ISO 1443:2008)

and sodium chloride (according to SR ISO 91-2007) was determined. The amount of carbohydrates was calculated according to the relationship shown in equation below:

Carbohydrates (%) =100 - [lipids (%) + proteins (%) + ash (%) + moisture (%)]

### Sensory analysis

Sensory evaluation of salami samples was carried out according to the method described by [9]. The samples were evaluated by 20 trained panellists (10 men and 10 women, aged 20-25 years) at the Faculty of Food Engineering (Timisoara, Romania), in natural light (daylight) and at room temperature. Salami samples were prepared on plates 5 minutes before the evaluation. The characteristics evaluated by each panellist were: color, odour, taste, texture and general acceptability. A nine-point hedonic scale was used to analyse each characteristic as follows: Like extremely (9); Like very much (8); Like moderately (7); Like slightly (6); Neither like nor dislike (5); Dislike slightly (4); Dislike moderately (3); Dislike very much (2); Dislike extremely (1).

### Statistical Data Analysis

Results were expressed as the mean of three determinations ± standard deviation (SD). Statistical differences ($p < 0.05$) between samples were performed by t-test using Microsoft Excel 365 (Version 2208, Redmond, WA, USA).

For the sake of simplicity in our analysis, we used some abbreviations: (GNS) for goji and nuts salami - our new formula, (DS) for dry salami, (BS) for Banatean salami.

## RESULTS

The experimental data obtained are presented in the figures below.

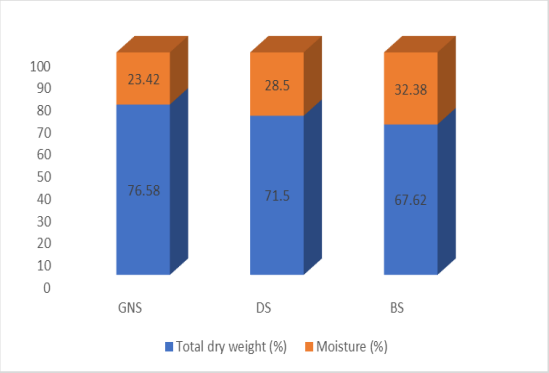



**Figure 1. Moisture and total dry weight of salami samples**

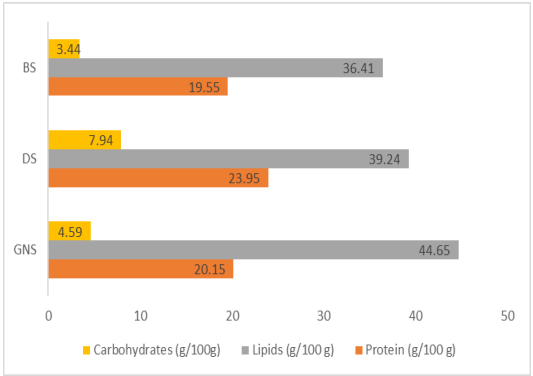



**Fig. 2 Proximal composition of analyzed samples**

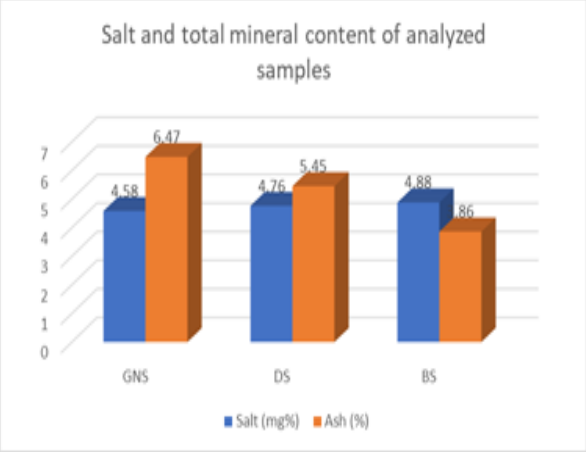



**Fig. 3 Sodium chloride and total mineral content of analyzed samples**

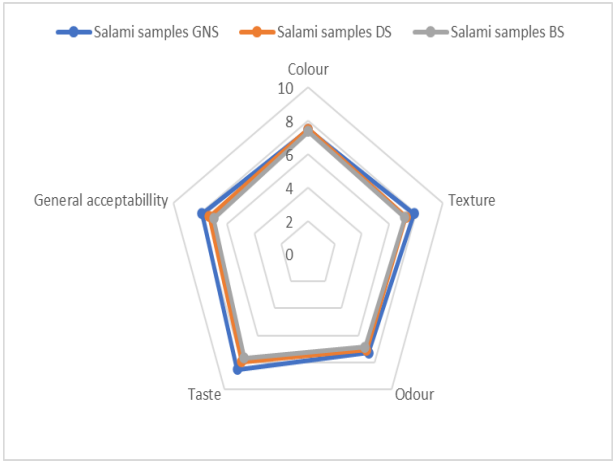



**Figure 4. Sensory analysis of the salami samples**

## DISCUSSION

### Proximate analysis of the salami samples

Proximate composition is important because it provides information about the quality of the raw materials used and determines the finished products' nutritional value [1]. The main physico-chemical characteristics monitored in the salami samples (fig.1-4) were: moisture content (%), total dry content, (%), ash (%), sodium chloride (%), total protein (%), total lipids (%) and carbohydrates (%).

The salami samples were analysed from a physico-chemical point of view using the analysis methods regulated by the current legislation.

The moisture of meat and meat products is inversely proportional to the fat and protein content. The moisture content of salami samples ranges from 23.42-32.38%. Accordingly, to these values, total dry weight content recorded for the same salami samples ranged from 67.62-76.58 % (figure 1). The analysis of the values obtained shows that the nuts and dehydrated goji content increases the total dry weight content in the salami samples. The recorded values agree with literature data showing a water content in dry salami between 30.5 – 48.62% [10].

The protein content in meat products is of particular importance because this quality parameter provides information regarding nutritional value of the product, ensuring that the metabolic processes occurring in human nutrition run smoothly.

According to literature data, the protein content varies according to species and fat cover, ranging from 15.5-31.3% in dry salami [11]. In the analysed salami samples, the protein content was found to be in the range of 19.55-20.15% (figure 2), with the high protein percentage in case of DS, salami with addition of nuts and goji samples being between the other two types of dry salami analysed.

Regarding the fat content of the analysed salami samples, following extraction with the Soxhlet extractor, the values recorded for the samples analysed ranged from 36.41-44.65%, with the highest fat content registered for the GNS sample and the lowest value for the BS salami sample. From the analysis of the experimental data, it can be seen that

the fat percentage increases with addition of nuts and goji, the addition of the mix of nuts and dried fruits, bringing an increased supply of lipids to the product. [3,4].

The carbohydrate content of the studied sausage samples was in the range of 3.44-4.59%, the lowest value being recorded for BS and the highest value for DS (figure 2).

The natrium chloride content (%) of the samples analysed ranged from 4.58-4.88 mg % (figure 3) and was not influenced by the addition of nuts and dehydrated goji. A content between 3.8 – 5.50 (%) was also reported for raw-dried salami [11]. The mineral content ranged between 3.86 – 6.47 %, the highest value was observed in case of our new formula, and it can be explained by the addition of the mix of nuts and dried fruits, bringing an increased supply of fiber to the product, the obtained values being similar to those provided by the literature data [12]. From a statistical point of view, for all determined parameters, there were statistically significant differences ($p < 0.05$) between the three salami samples analysed.

**Sensory analysis**

The sensory analysis looked at the influence of added nuts and goji in raw - dry salami on the sensory characteristics: texture, colour, taste, smell and general acceptability. The results of the sensory analyses in case of salami samples are shown in figure 4.

For texture, the highest score was recorded for GNS salami (7.87), followed by DS (7.29) and the lowest scores were recorded for BS (7.18). For evaluation of the color, the highest value was recorded for GNS (7.49), followed by DS (7.49), and the lowest value was recorded for BS (7.37). The evaluation of the taste of the salami samples recorded the following values: the highest for GNS (8.53), followed by DS (7.95) and the lowest value was recorded for BS (7.69). Also, in the sensory analysis of smell, the highest value was recorded for GNS (7.29) compared to the other samples analysed, which recorded in descending order the following values: 7.07 for DS and 6.87 for BS.

In the general acceptability analysis, the highest value was also recorded for GNS (7.89), followed by DS (7.35) and the lowest values were recorded for BS (7.02).

As a result of the sensory analysis carried out for the three salami samples, the highest total and individual scores for each characteristic were obtained for our new formula – raw-dry salami with goji and nuts addition. In conclusion, from a sensory point of view, addition of pistachio, hazelnut and dehydrated goji improve the characteristics of the product to which they are added.

## CONCLUSION

The increasing interest of consumers for foods with improved nutritional value has led the food industry to a wave of research on the use of ingredients rich in active principles as additions to obtain food products designed to meet these requirements.

Through this study, the nutritional implications of the addition of hazelnut, pistachio and goji in the technology of raw - dry salami were followed, as well as the obtaining and characterization of this new formula with the addition of hazelnut, pistachio and goji. The physico-chemical analysis concluded that addition of nuts (pistachio and hazelnuts) and dehydrated goji improves nutritional characteristics of the new obtained

formula, based on high nutritional value of the added compounds. By adding nuts and goji increased the amount of total dry weight, ash and fats and decreased the moisture,

protein and carbohydrate content. The sodium chloride content was not influenced by adding nuts and goji to salami formula.

From the data obtained for moisture, dry matter and ash, it is observing that our product - dry salami with the addition of dehydrated goji, hazelnuts and pistachios has the highest amount of dry matter, correlated with the highest value of mineral substances (ash), followed by of the other two samples used.

At the same time, following the sensory analysis, the tasters' preference was demonstrated for the salami with hazelnuts, pistachios and goji, obtaining the highest scores in terms of all the analyzed characteristics.

Thus, the data obtained from this study demonstrate the technological and commercial potential of the salami with the addition of hazelnuts, pistachios and goji, which stands out for its original recipe and for the health benefits it brings.

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# OPTIMIZING A PROTOCOL FOR INDUCING AND SYNCHRONIZING ESTRUS IN SHEEP DURING NON-BREEDING SEASON

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## ABSTRACT

Induction and synchronization of estrus during non-breeding season is the most frequently used biotechnological method of assisted reproduction in sheep. Synchronization of estrus in sheep is a very important breeding technique as it can effectively improve the reproductive performance of sheep, minimize the effects of seasonal factors on sheep reproduction, extend the breeding season of sheep, shorten the estrus interval, increase the gestation rate, increase the birth rate, it can also lead to the shortening of the time between generations. The study proposes a method of inducing and synchronizing estrus during non-breeding season in Turcan sheep, which uses vaginal sponges with FGA (20 mg), supplemented by the administration of PGF2α (0.20 mg) and GnRH (7 μg). The analyzed parameters were: sponge loss rate, vaginitis onset rate, total percentage of ewes in estrus and gestation rate. The results obtained for the induction rate and synchronization of estrus during non-breeding, the reproductive period in sheep from the Turcana breed of 88.88%, respectively for the rate of pregnancy deception of 81.25% indicate that the method used was an efficient one.

**Keywords:** sheep, estrus, non-breeding season

## INTRODUCTION

Productivity at the farm level is influenced by the reproductive capacity of the animals on the farm. Biotechnological methods of assisted reproduction are applied to ensure the increase in the reproductive capacity of farm animals and productivity. In the case of sheep, the induction and synchronization of estrus with the main objective of increasing reproductive performance in this group of animals [1]. Synchronization of oestrus in sheep is achieved by managing the aspects related to their physiology, namely:

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photoperiod and melatonin secretion, the early introduction of rams into the flock, but especially the application of different hormonal treatment schemes [2,3].

There are studies that have highlighted the favorable effects of photoperiod manipulation and the early introduction of sexually active rams in the non-breeding season [4,5]. The use of the ram effect during the breeding season and in the non-breeding season to induce and synchronize estrus in sheep without administering a hormonal treatment scheme is a concern of many research groups [4-6]. It has been shown that the effect of the ram is to modulate the secretion of LH in sheep that are not in the ovulatory period, the results of these studies indicate that regardless of the stage of the estrus cycle, the sheep responded by increasing the concentration of LH.

Also, it was established that adult rams determine a rate of mounts and a more obvious estrus response compared to young rams. The ram effect in sheep subjected to hormonal treatment schemes based on involving PGF2α, progesterone and eCG has been analysed by different groups of researchers. The introduction of the ram into the flock after the application of the treatment scheme determined an estrus induction response comparable to that of the breeding season in non-breeding season ewes. In another study, repeated administration of eCG was found to improve the effectiveness of the ram effect in the non-breading season [6].

In many geographical areas, very differently positioned around the globe, following hormonal treatments that involve the administration of progesterone for a long or short time in combination with gonadotropins seems to be the most effective way to induce and synchronize oestrus in sheep [7].

Among the exogenous hormones used for the purpose of inducing and synchronizing estrus in sheep flocks on farms, in the breeding season but also in the non-breeding season, we mention: prostaglandins, progesterone, equine chorionic gonadotropin (eCG), melatonin and human chorionic gonadotropin (hCG) [2,3].

The use of some hormonal treatment schemes for the induction and synchronization of estrus, which involves the use of intravaginal sponges, is quite frequent and associated with injectable treatment with other progestogenic substances. In recent years, several attempts have been made to optimize the length of time that intravaginal sponges are kept in the vagina of sheep. The introduction of intravaginal sponges can be done for a short (7 days) or long (12 days) period of time both during the breeding season and in the non-breeding season, obtaining a high rate of pregnancy establishment [8]. The use of intravaginal sponges can also have less favorable effects on the sheep in the sense of changing the vaginal flora and the risk of vaginal discharge and vaginitis. These sponges influence the vaginal secretions and can promote the development of pathogenic microorganisms, the species *Staphilococcus aureus* being the main agent of vaginitis. Extending the period of application of intravaginal sponges increases the risk of vaginitis occurring and setting in, which can decrease the fertility rate [2,9].

The aim of the study was to optimize a method for inducing and synchronizing oestrus in the off-season in Turcana sheep, respectively to assess the efficiency of the method based on some reproductive parameters.

## MATERIALS AND METHODS

### Animals taken in the study

The study was carried out between April and July 2023 in a sheep microfarm in the western part of Romania. Sheep from the Turcana breed in number of 20 (n=20) with a weight of 43.75 ± 2.27 kg represented the experimental group. The choice of ewes was made randomly from the entire flock in which there were ewes after the second or third lambing, the lambings took place normally, without dystocia and without dead lambs. Ewes in this study were separated from the rest of the flock and subjected to the oestrus induction and timing protocol scheme. The rams belonging to the same breed, with a weight of 76.42 ± 3.78 kg, were introduced into the pen together with the ewes for natural lambing after the hormonal treatment.

All the experimental procedures applied in the present study received the approval of the ethics committee of the University of Life Sciences "King Mihai I" from Timisoara.

### Estrus synchronization protocol

All sheep were treated with FGA (20 mg) vaginal sponge (SYNCRITE-20 Vaginal Sponge, Animal Health Supplies, 37 Charles St., Ascot Vale, VIC, Australia). Before using FGA vaginal sponges, in order to prevent vaginitis in sheep, they were placed in a container with a solution of 160 IU of penicillin sodium, and the contents of the container were shaken for 5 minutes so that the antibiotic spread evenly on the surface of the sponges.

The sponges were inserted into the vagina using the Chronogest CR applicator device (Intervet International, Boxmeer, Netherlands), the day of sponge insertion being day 1 of the estrus synchronization and induction protocol. The sponges were removed on day 14. PGF2α (0.20 mg) was injected intramuscularly on the 12th day. Sheep were given 7 μg of GnRH by intramuscular injection 36 hours after sponge withdrawal.

### Estrus detection and monitoring

Identification of estrus in hormone-treated ewes was determined to be established when they exhibited a standing reflex in response to the ram. The number of ewes in estrus was determined 24, 36, 48, 60 and 72 h after administration of the synchronization treatment. The percentage of ewes in estrus during the different time periods mentioned in relation to the total number of ewes in estrus was then calculated.

During the administration of the treatment scheme, the sheep were followed, if there was loss of the sponge and if there were cases of vaginitis. In addition, the onset of vaginitis was also analysed when the intravaginal sponge was withdrawn. Sponge placement and removal, evaluation of sponge loss, and identification of vaginitis were always performed by the same person. The foul-smelling condition and purulent discharge from the sheep's vagina was the symptom that highlighted the onset of vaginitis.

After about 60 days from delivery, with the aid of the MiniTube Sonograf (Minitüb GmbH, Germany) the establishment of pregnancy was followed.

All the information was centralized and based on them the following parameters were calculated: sponge loss rate, vaginitis installation rate, total percentage of ewes in estrus and gestation rate.

Sponge loss rate (%) = number of ewes that lost sponge/number of ewes treated with intravaginal sponge × 100,

Vaginitis rate (%) = number of ewes showing vaginitis/number of ewes treated with intravaginal sponge × 100,

Percentage of ewes in estrus (%) = number of ewes in heat/number of ewes in heat × 100,

Gestation rate (%) = number of pregnant ewes/number of mated ewes × 100.

## RESULTS

Based on the observations and determinations made in the barn for the sheep in the experimental group, the following parameters of interest were determined: the rate of loss of sponges, the rate of installation of vaginitis, the total percentage of ewes in estrus and the gestation rate. The obtained values were represented graphically Fig. 1 - Fig. 2.

Analyzing the dynamics of coming into heat of the flock of sheep in the experimental group, we find that their establishment was mainly achieved in the time interval 24-36h, respectively 36-48h after the injectable administration of GnRH. From the total of 20 sheep in the experimental group, 50% showed heat in the 36-48h interval, respectively 20% in the 24-36h interval (Fig. 1). Estrus induction was identified in 88.80% of all sheep in the experimental group.

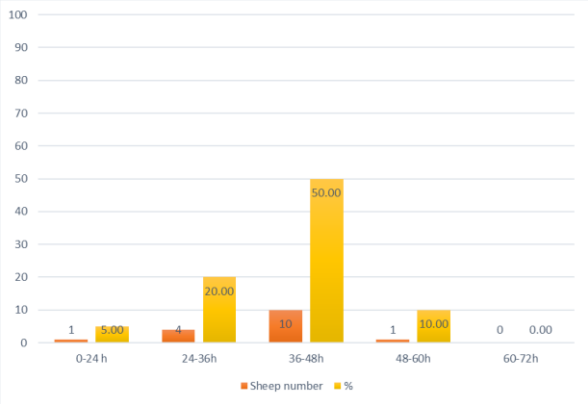



**Figure 1.** *Percentage of estrus ewes in different time periods (%)*

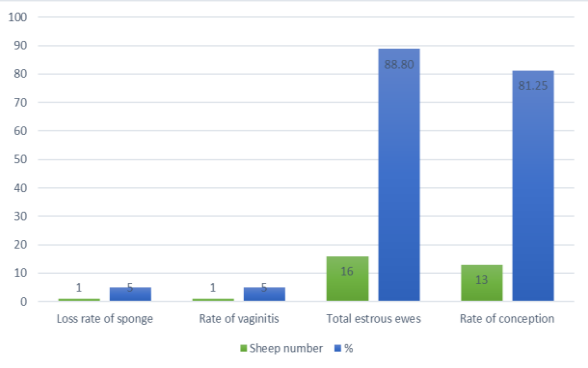



**Figure 2.** *Reproductive performance of Turcana ewes with the estrus synchronization protocol (%)*

Vaginal sponges impregnated with FGA remained intravaginal throughout the duration of the experiment in most sheep, only one sheep (5%) lost its vaginal sponge during this period. Regarding the onset of vaginitis as a result of the application of intravaginal sponges, following the analysis of the sheep, only one sheep (5%) was identified that showed symptoms of vaginitis, a slight purulent discharge (Fig. 2).

After approximately 60 days from the realization of the natural mount, the pregnancy rate was determined and it was found that it has an incidence of 81.25% (Fig. 2), a relatively high percentage.

## DISCUSSION

The administration of progesterone to sheep in hormonal treatment schemes for the induction and synchronization of estrus is quite frequent both in the breeding and non-breeding season. Administration of progesterone is done in different ways: with devices that determine the controlled internal release of this, in the form of injectable treatment or with intravaginal sponges as presented in the present study. The maintenance of intravaginal sponges in sheep is done at different intervals depending on the treatment scheme proposed by the researchers, and the most frequently used progesterone-based preparations: medroxyprogesterone acetate (MPA) and fluorogestone acetate (FGA) are in different concentrations [10,11]. It has been established that the application of intravaginal sponges and the extension of the maintenance period inside the vagina in sheep can cause the blocking of vaginal secretions with the stimulation of the development and installation of pathogenic microorganisms that lead to the installation of vaginitis [12].

There were studies that followed the effect of sponges treated with FGA and administered intravaginally for several days to Merino sheep both during the breeding and non-breeding season, the results obtained indicated a good estrus inducing response and installation of pregnancy. However, if the sponges treated with FGA are associated with other hormonal treatments, for example eCG, better responses to the induction and synchronization of estrus are obtained for the treated sheep [13].

In other studies, the application of sponges treated with FGA was associated with the additional administration of homonal preparations from the group of prostaglandins, known for their effects to determine estrous synchronization [14 ,15]. The effects of prostaglandins are more evident during the reproductive period due to the presence of the corpus luteum, for example in sheep the response to PGF2α occurs on day 3 after ovulation considered day 0 [16].

The administration of PGF2α can be done in a single dose or in a double dose at different time intervals, these aspects are related to the treatment scheme to which the sheep flock is subjected. Establishing the moment when the administration of PGF2α takes place is very important to be able to induce estrus, as a result, double injections are recommended for synchronization. The time interval differs from 7-16 days, but it is considered that the 7-day interval is optimal for obtaining the efficient estrous synchronization in sheep in the breeding season [14]. In those outside the breeding season, as in the case of the present study, the need to administer PGF2α after

withdrawing the intravaginal sponges with FGA is more than necessary for the induction and synchronization of estrus.

The administration of different types of prostaglandins for the induction and synchronization of estrus allowed the establishment of some differences regarding the efficiency in inducing estrus but also in shaping the gestation rate. Thus, a comparative study on the effect of 2 substances from the PGF2α group (dinoprost tromethamine and d-cloprostenol) was carried out to highlight the number of doses, the time interval between doses and the rate of estrus induction. Although d-cloprostenol had a greater response than dinoprost tromethamine to the induction of estrus during the breeding season, the pregnancy establishment rate was the same for both hormonal preparations tested. If a single hormonal preparation was tested but in a double dose, the effect produced was to induce estrus with the same rate, but increasing the dose determined a much higher pregnancy rate [17].

Completing treatment schemes with gonadotropins to obtain a more efficient estrus synchronization is frequently applied. It is known that gonadotropins are responsible for synchronizing follicular growth and maturation, as well as ovulation. The most common gonadotropins used in hormonal treatment schemes for estrus synchronization both in the breeding season and in the non-breeding season are equine chorionic gonadotropin (eCG), GnRH, follicle-stimulating hormone (FSH). The administration of even small doses of eCG or GnRH successfully induces and synchronizes estrus in sheep both in the breeding season and in the non-breeding season [1].

## CONCLUSION

The increase in the gestation rate, the increase in the birth rate, the short period between calvings, the increase in the production of lamb meat, so an economic increase at the level of sheep microfarms can only be achieved by applying reproductive biotechniques, which presuppose the application of hormone treatment schemes outside breeding season. Finding effective and low-cost hormonal treatment schemes is a permanent concern for different groups of researchers in the area of sheep breeding, considering sheep breeds and their characteristics from a reproductive point of view. The rate of installation of vaginitis in the sheep in the experimental group is relatively low, the condition was detected in only one sheep (5%). Also, the loss rate of the sponge was low, probably due to the shorter time of maintaining it intravaginally, only 1 sheep lost its sponge treated with FGA (5%). The use of hormonal treatment schemes that ensure the combination of the application of vaginal sponges with FGA with the injectable administration of PGF2α and GnRH determined an estrus induction rate outside the reproductive period in Turcana sheep of 88.80%, respectively a rate of gestation of 81.25%. The method of induction and synchronization of estrus proposed for sheep of the Turcana breed proved to be effective, the analysed parameters having increased values.

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# OXIDATIVE STABILITY OF COCONUT OIL AND WALNUT OIL BY PHYSICO-CHEMICAL ANALYSIS AND FTIR SPECTROSCOPY

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## ABSTRACT

The indicator of the quality of edible oils is its oxidative stability. The oxidative reactions can be influenced by several factors (light, heat, oxygen reaction with unsaturated lipids) and by chemical and enzymatic mechanisms (autoxidation, photo-oxidation and lipoxygenases). These factors can accelerate lipid oxidation, decrease oxidative stability and cause significant modification on sensory properties, what lead to nutritional depreciation of edible oil and decrease in the shelf life. The aims of this study are to evaluate the oxidative stability of coconut oil and walnut oil during storage (12 month) because this has a significant influence on degree of oil freshness. The lipid oxidation gives rise to the existence of toxic compounds in the food products and contribute to the development of heart disease, cancer and atherosclerosis. The progress of lipid oxidation was assessed by measuring peroxide value (PV), p-anisidine value (AV) and total oxidation value (TOTOX). The low peroxide value signifies a high oxidative stability. The Totox value gives clear overall data analysis of the freshness of the oil; the lower the Totox value, the better the quality of oils. FTIR spectral data were used to determine the bands, which can be considered as the fingerprints of the oxidation. The results suggest that walnut oil quickly go rancid but the coconut oil keeps its good chemical properties during storage.

**Keywords:** coconut oil, walnut oil, oxidative stability

## INTRODUCTION

Walnut is characterized as seeds from the walnut tree with genus Juglans (*Juglans regia*). The Romanian walnut fruits are rich in nutritional compounds, with a high lipid content (55-76%), proteins (11-25%) and carbohydrates (16%) [11]. The walnut oil contains large amounts of vitamin E, which provides stability in time [6]. The coconut oil that comes from the fruit of the coconut tree (*Cocos nucifera* L) and is an edible oil derived from the kernels, meat and milk of the coconut palm fruit and has a pale yellow coloration with a coconut odor [15].

The quality of edible oils depends on their qualitative and quantitative chemical composition. The oxidative stability of oils is one of the most important indicators of shelf life [8]. Oxidation of edible oils is influenced by light or heat, fatty acid composition, types of oxygen and other compounds (free fatty acids, phospholipids, mono- and diacylglycerols, pigments, antioxidants). In order to improve the oxidative stability of vegetable oils, many studies have been carried out on these factors. The peroxide value (PV) of oil is a measurement of the primary oxidation of hydroxyl groups of unsaturated fat into hydroperoxides and peroxides [13]. According by AOCS [4] standard methods, PV of fresh oils should be less than 10 mEq $O_2$/kg oil, while the PV above 30 mEq $O_2$/kg indicate a rancid oil. The p-anisidine value was used to assess the secondary oxidation of the oil or fat, which is mainly due to aldehydes and ketones; represent an indicator of oxidative rancidity in oils and fatty foods.

FTIR spectroscopy is used for the oxidation process of coconut oil and walnut oil and provides information about the different functional groups present in a sample, not just one type of compound [5].

## MATERIALS AND METHODS

The oil samples were acquired from local supermarkets. The walnut oil (WO) is obtained from walnut kernels by cold pressed and were made in Romania and coconut oil (CO) is made from West Africa Countries. The coconut oil traditionally produced in West Africa is made by crushing and pressing copra to extract the oil [3]. The determination of primary and secondary oxidation products (peroxide value and p-anisidine value) were determined periodically (at 3 months). Oxidative stability by FTIR spectroscopy was made on non-oxidized (fresh) oil and after storage at room temperature condition (25°C) during 12 months stored in dark colored bottles; so, the oil is protected from the deteriorating effects of light.

**Physico-chemical analysis**

*The peroxide value (PV)* was determined according to standard iodometric methods for the edible oil and the results were expressed in milliequivalents $O_2$/kg (mEq $O_2$/kg).

*The p-anisidine value (p-AV)* is a measurement of carbonyl content in the oils and fats. According to AOCS [2], p-AV standard method is based on the reactiveness of the aldehyde carbonyl bond on the p-anisidine amine group and the formation of a Schiff base that absorbs at 350 nm.

Brief, 2 g (w) of oils sample were dissolved in 25 mL isooctane and the absorbance (A1) were measured at 350 nm. A blank was prepared with isooctane. An aliquot (5 mL) of this solution, respectively 5 mL of isooctane (as blank) was transferred to each of two test tubes of 10 mL and was added, to each, 1 mL anisidine solution (0.25% g/v glacial acetic acid). After 10 minutes, the absorbance (A2) was measured, also at the same wavelength, against isooctane containing p-anisidine. p-AV was calculated according to the formula (1):

$$\text{p-AV} = 25 \times \frac{1.2 \times A_2 - A_1}{w} \qquad (1)$$

*The total oxidation value (TOTOX)* was used to estimate the oxidative deterioration of lipids. TOTOX value were calculated according to the formula (2) as the sum of both values (PV and p-AV) to total oxidation [12]:

$$\text{TOTOX value} = 2\text{PV} + \text{p-AV} \quad (2)$$

All determinations were carried out in triplicates. All reagents and solvents used in this study were of analytical reagent grade.

*FTIR spectroscopy.* Absorption spectra of oil samples were measured on a Fourier Transform Infrared Spectrophotometer - FTIR-8400S by Shimadzu (Japan) using a high sensitivity pyroelectric detector DLATGS (L-alanine-doped deuterated triglycerine sulphate) element. The detector relies upon the temperature-dependent pyroelectric effect created on the crystal surface by spontaneous ferroelectric polarizarion. All FTIR spectra were recorded in the wavenumber range from 4000-600 $cm^{-1}$. BKG scan a sample were sequentially measured at 4 $cm^{-1}$ resolution, and 2.8 mm/s mirror speed with 45 accumulations (1 minute scan). Background measurements were made against air. The spectrometer was connected to a computer using Windows XP Professional software to manipulate the spectra.

***Statistical analysis***

Simple linear regression analysis was applied using Origin 8.0 software to obtain some correlations between the PV, p-AV and TOTOX values of the two oils and storage time.

## RESULT AND DISCUSSION

Oil oxidation refers to chemical reactions involving oxygen that degrades oil quality. The primary oxidation products, lipid hydroperoxides, are relatively stable at room temperature. The time taken for secondary products from the primary oxidation product, hydroperoxide, varies with different oils. Oils with high peroxide values are unstable and easily become rancid. It is known that the rate of peroxide formation in coconut oil depends on the processing method, storage time of copra and storage time of the oil. In addition, CO obtained by the traditional method is considered to be the most stable of all extraction methods [3]. However, most of the degradation products of hydroperoxides are responsible for the off-flavors in oxidized edible oils, especially aliphatic carbonyl compounds which have a greater influence on the off-flavor of oxidized oils.

PV increased from month to month from 0.06 to 0.42 mEq $O_2$/kg, but after 9 months the values decreased up to 0.40 mEq $O_2$/kg when the secondary oxidation products appear. This indicates a high oxidative stability for coconut oil. The p-anisidine value ranged from 0.10 to 0.68; a lower p-anisidine value is considered a indicators of oils quality [8]. As the CO samples analyzed in this study has anisidine values of less than 1, the oil was considered the good quality. Some authors [11] have found that oils with a high content of polyunsaturated fatty acids have a higher p-AV, even when fresh. Is not the case with us. Also, the lower the TOTOX value, the better the quality of oil. However, the TOTOX value is an empirical parameter because corresponds to the addition of two parameters with different units [6]. The initial PV of fresh WO is very low (0.4 mEq $O_2$/kg) in according with Leahu et al. [6]. Six months of

storage induced an increase of peroxide value [14]. Our analyses showed that in WO the PV increases slowly up to six months of storage and very rapidly in the last six months.

In Figure 1 and 2 were illustrated the modifications of PV, p-AV and TOTOX value after oils storage in room temperature. All equations revealed a good relationship (with the coefficients of determination $R^2 > 0.9019$) between the physico-chemical parameters of the oils and storage time.

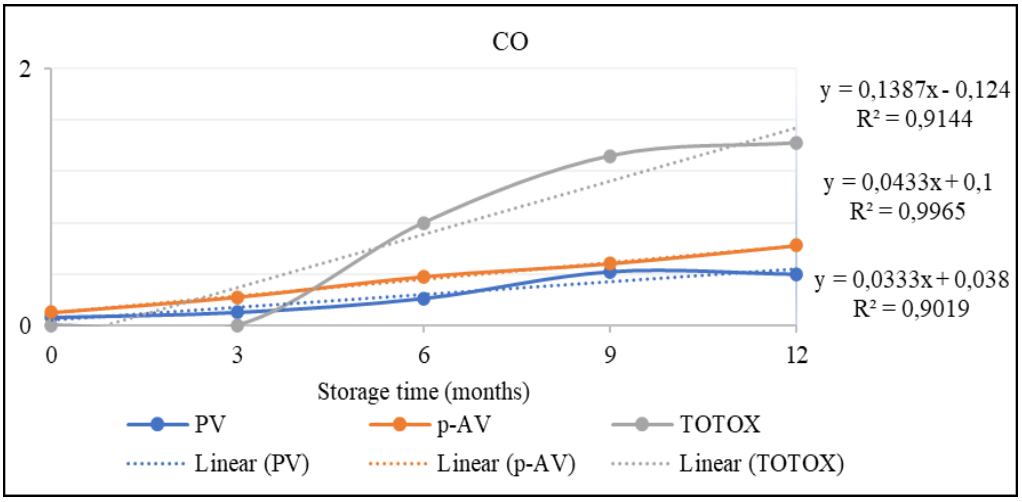



Figure 1. Linear regression analysis between physico-chemical parameters of coconut oil and storage

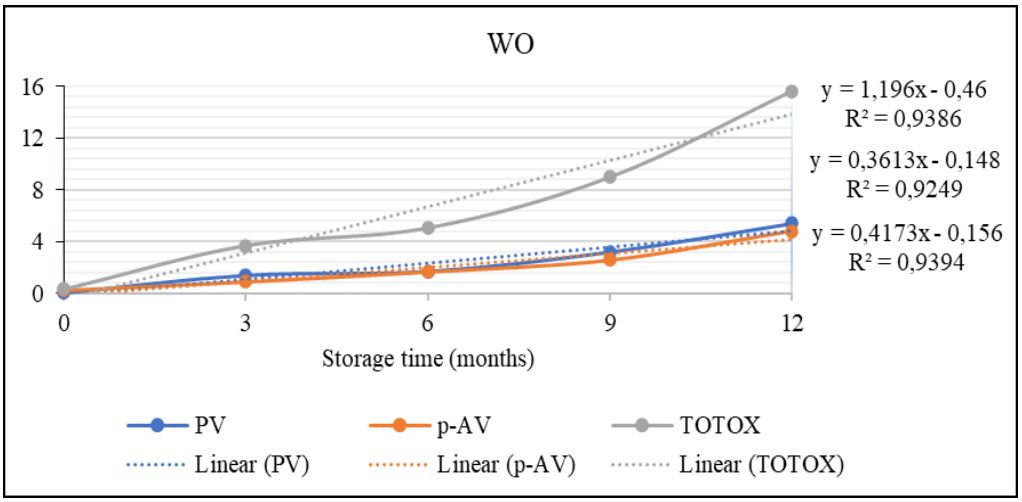



Figure 2. Linear regression analysis between physico-chemical parameters of walnut oil and storage time

Some regions of vegetables oil FTIR spectra present a high signal/noise ratio, which corresponds to various types of vibration characteristic to different types of atoms [1]. The results showed different functional groups of the coconut oil (Table 1) and walnut oil (Table 2).

Table 1. FTIR absorption for coconut oil

| Oil sample | Frequency ($cm^{-1}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2953 | 2922 | 2852 | 1742 | 1450 | 1417 | 1377 | 1151 | 1111 | 962 | 721 |
| Fresh oil | 0.49 | 1.12 | 0.86 | 1.26 | 0.39 | 0.27 | 0.38 | 1.17 | 0.85 | 0.24 | 0.44 |
| After storage | 0.56 | 1.22 | 0.55 | 1.36 | 0.38 | 0.27 | 0.39 | 1.18 | 0.85 | 0.22 | 0.41 |

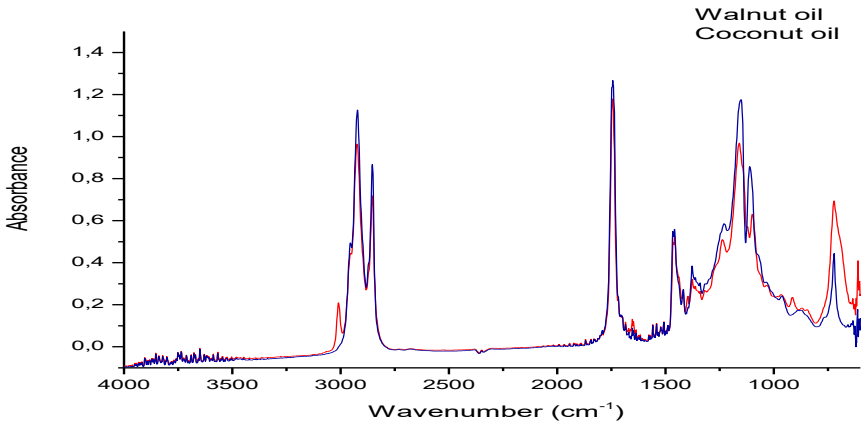
Table 2. FTIR absorption for walnut oil

| Oil sample | Frequency ($cm^{-1}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3006 | 2924 | 2853 | 1743 | 1464 | 1456 | 1377 | 1236 | 1159 | 1099 | 721 |
| Fresh oil | 0.20 | 0.96 | 0.72 | 1.17 | 0.50 | 0.54 | 0.32 | 0.50 | 0.97 | 0.63 | 0.69 |
| After storage | 0.25 | 1.07 | 0.80 | 1.23 | 0.50 | 0.54 | 0.33 | 0.51 | 0.97 | 0.62 | 0.64 |

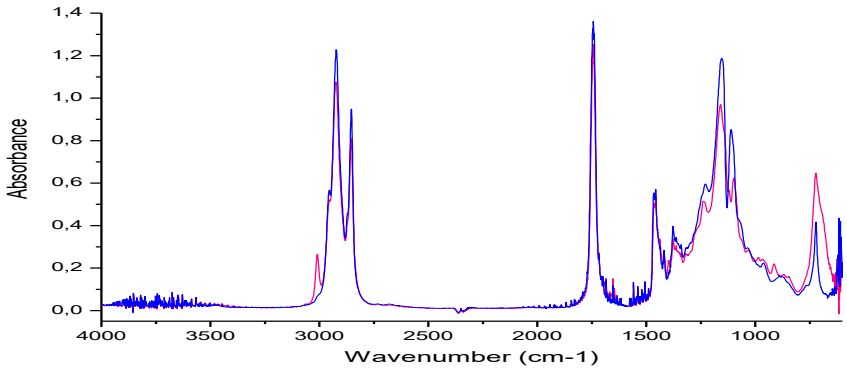
Comparative FTIR spectra for CO and WO, fresh and after storage, are shown in Figure 3. WO is more susceptible to oxidation due to the high content of unsaturated fatty acids in its composition (90%) of which the linoleic acid (47.4%) and the linolenic acid (15.8%) [9]. So, the band corresponding to the cis double-bond (=CH) are due to presence of the oleic and linoleic acids in WO and was to stretching vibration of 3006 $cm^{-1}$[1, 10]. The spectrum showed that the band of C-H stretching of the $CH_2$ groups with wavenumber of 3000-2800 cm indicates the presence of alkanes groups in the oil [12]. The absorption peaks are the same for both oil samples where the C-H stretching absorption occurs at wavelength 2922 $cm^{-1}$ and 2924 $cm^{-1}$ for CO and WO, respectively. For WO, we observed two peaks at 1743 $cm^{-1}$ and 1159 $cm^{-1}$ wich are due to stretching absorption of aldehyde (C=O) and esters (C-O). For CO at at frequency 1742 $cm^{-1}$ can be seen a strong intensity due to the great difference of electronegativity between carbon and hydrogen atoms [7]. The spectral differences have been observed between non-oxidized coconut oil (1.26) and after storage (1.36), where the oil had a high absorbance.

The intensity of the bands at 1464 to 1456 $cm^{-1}$ remains unaltered in non oxidized oils and during storage; high oxidative stability of the oils tends to be achieved. For WO, the peak which is attributed the bending absorption of methylene ($CH_2$) group appear at 1464 $cm^{-1}$. For CO, two alkanes peaks which is attributed the bending absorption of methylene ($CH_2$) and methyl ($CH_3$) groups appears at 1465 and 1377 $cm^{-1}$ respectively. An absorption band at 1417 $cm^{-1}$ is attributed from rocking vibrations of CH bonds of alkenes with *cis*-disubstituted. An absorption band at 1111 $cm^{-1}$ is attributed from -CH bending and –CH deformation vibration of fatty acids; have the same value (0.85) for CO non-oxidized and during storage.

A bending vibration of cis-disubstituted olefinic groups is associated with an absorption band at a frequency of 962 $cm^{-1}$. The absorption peak at frequency 721 $cm^{-1}$ results from the rocking vibration of the methylene group, which overlaps with the bending vibration on the out-of-plane mode of the cis-disubstituted alkenes [5].




a




b

Figure 3. Comparative FTIR spectra for coconut oil (blue) and walnut oil (red): a - fresh oil, b - after storage

Our data analysis suggest that walnut oil quickly go rancid but the coconut oil keeps its good chemical properties during 12 months on storage.

## CONCLUSIONS

The walnut oil is stable for six months when stored in dark bottles at room temperature. Compared to walnut oil, which is lower in saturated fatty acids but higher in unsaturated fatty acids, coconut oil's high content of saturated fatty acids makes it more stable. It is important to select the most adequate methods for assessing the primary and secondary oxidation products, depending on the state of oxidation. In conclusion, is a high correlation positive linear correlation between peroxide value (PV), p-anisidine value (p-AV), total oxidation value (TOTOX) of coconut oil and walnut oil and storage time.

Fourier transform infrared (FTIR) spectroscopy was used to monitor the peak changes as effect of oxidation during storage. The changes observed in the frequency values of the bands of the spectra provide in a simple way accurate information about the oxidation process. The storage conditions have not changed the peak intensities of coconut oil at studied frequencies that prove the high stability of this oil after storage

time investigated. FTIR spectral data can be considered as the fingerprints of the oxidation.

Studies have shown that the addition of synthetic antioxidants in walnut oil stored in dark warehouses and at room temperature increases its shelf life and reduces lipid oxidation.

## ACKNOWLEDGEMENTS

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# POSSIBILITIES FOR IMPROVING THE PRODUCTION AND CONSUMPTION OF MEAT AND MEAT PREPARATIONS IN TIMIȘ COUNTY, ROMANIA

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## ABSTRACT

To improve individual and total meat production, it is necessary to implement control models of anthropogenic influence on meat production, i.e., the readjustment and diversification of mammal and poultry meat productions to the needs of the processing industry and the regional market trend. Depending on the evolution of herds and productions, the implementation of measures to improve organizational management is aimed at ensuring, in addition to nutrition, environmental control and favourable maintenance conditions, obtaining maximum growth in all phases of the technological flow and good production of meat, in the context of an efficient use of feed and other inputs necessary for the expression of the species' biological characteristics at optimal parameters. Direct or indirect intervention through integrated management along the supply chain contributes to the stimulation and increase of individual productions, based on the attitude and level of knowledge, and on appropriate solutions, procedures, techniques, and operational facilities to reduce weight losses, exits from the herd, individual and total meat productions. The analysis of the evolution of the content and structure of the rural market depending on the differences between exports and imports requires the implementation of integrated management solutions to stimulate local production.

**Keywords:** mammals, birds, productions, livestock, poultry, consumption

## INTRODUCTION

The production systems implemented in professional animal farms should rely on general principles of maintenance and nutrition according to genetic value and condition. [1, 6] The measures that the production systems impose to obtain meat with valuable nutritional properties refer to ensuring the most optimal level of welfare of animal species according to the general criteria, to which add specific criteria for managing the production of the species. [9, 13] Effectiveness and yields of animal species for meat vary with resources. The implemented farm management, the consumption needs and the improvement of technological conditions impact the increase of individual productions and their quality. [8] Management measures to

control nutrition, maintenance, and improvement of welfare [5] can increase productivity in the meat industry. The best managerial solutions for improving individual productions and obtaining quality carcasses and meat are the application of biotechnologies and genetic selection to increase the degree of adaptability to stress, control the exploitation environment, reduce the gap between old developments in exploitation technologies and existing knowledge, manage the weight and optimal number of animals to be transported to slaughter, and work with the transporter to deliver live animals fit for slaughter [4].
Stress caused by loading/unloading animals at processing facilities and in the slaughter aisle is due to mixing the animals from different holding areas, to fighting to establish group hierarchies on boarding ramps and means of long-distance transport, to deprivation of food for more than 18-20 h, which is unacceptable from an animal welfare point of view, or to the lack of rest of at least 12 h before slaughter, which causes the deterioration of quantitative production. [2, 3] Due to the multiple interactions between environmental factors and other factors that, in turn, influence the energy exchange, the intensity of these potential stressors is worrying from the perspective of the adaptive capacities of animals because exposure to a type of stressor can lead to a change in resistance and to a reduction of individual productions. Innovations in the field of electronic systems capabilities could be exploited to improve animal environments, resulting in improved economic use of environmental measures, climate change mitigation strategies, coverage with meat of the needs of the market according to the trend of consumer preferences by perfecting the technologies of production, processing, and valorisation of meat. [7, 11] There is still much potential for the application of need-based electronic animal identification management, improved sensors, and expert systems. Continued progress should be closely linked to the needs of animals based on rational criteria and include further recognition of health criteria for animal caretakers as well. The ability of animal target tissues to respond to perturbations of normal physiological circadian rhythms can be an important indicator of stress. [10, 12] The importance of more careful measurement of stress is increasing; however, including and weighting multiple factors (endocrine function, immune function, health status, vocalizations) is not an easy task in developing integrated stress measures that lead to high individual performance through improved modelling of physiological systems as the knowledge base expands and can assist the manufacturing integration process. [14]

## MATERIALS AND METHODS

The increasing interest in breeding meat animals results from the trends of society, e.g., the interest in the consumption of quality raw materials, which means viable solutions for individual and total meat productions. The purpose of this scientific approach was to find the most effective methods of diversifying production systems and to implement high-performance technologies for the exploitation of meat animals, forcing the farms in the researched area to produce efficiently for the market. The use of the good growth capacity and precocity of breeds and hybrids of animals can be, through the management solutions proposed following the studies carried out in the researched area, a decisive factor in the profitability of reproduction, growth, and exploitation, expressed mainly by the average daily mass gains, feed conversion indices, and modelling of individual body parts with the most valuable meat parts. To reach the proposed

objectives, specific economic methods of analysing the evolution of animals and of production were used starting from to the analysis of the evolution of the herds and productions of the main species of animals in the period 2018-2021 exploited in Timiș County.

## RESULTS

Analysis of the trend of livestock and production of animals in Timiș County.

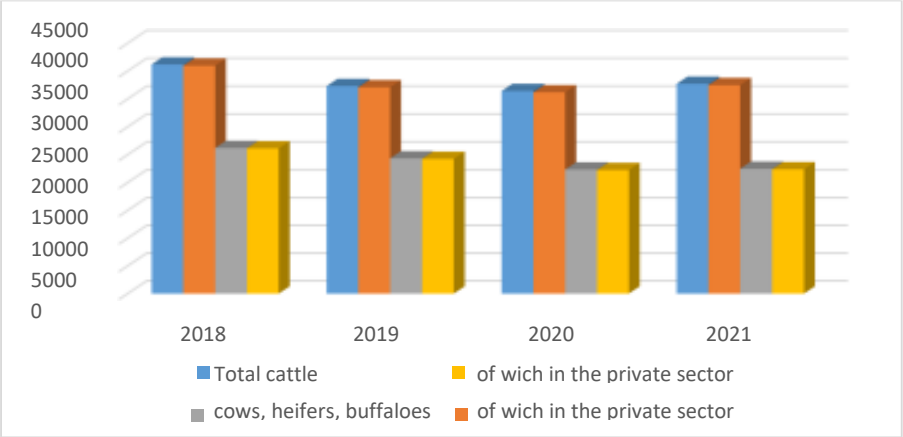



Figure 1. Evolution of cattle herds in the period 2018-2021 (Timiș County)

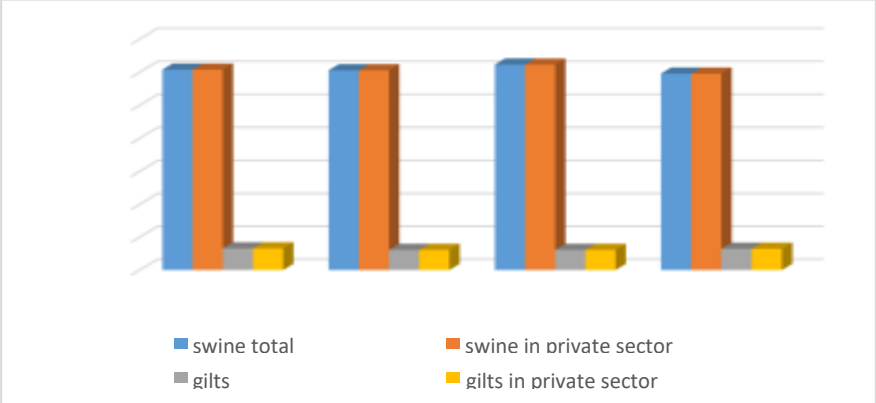



Figure 2. Evolution of swine herds in the period 2028-2021 (Timiș County)

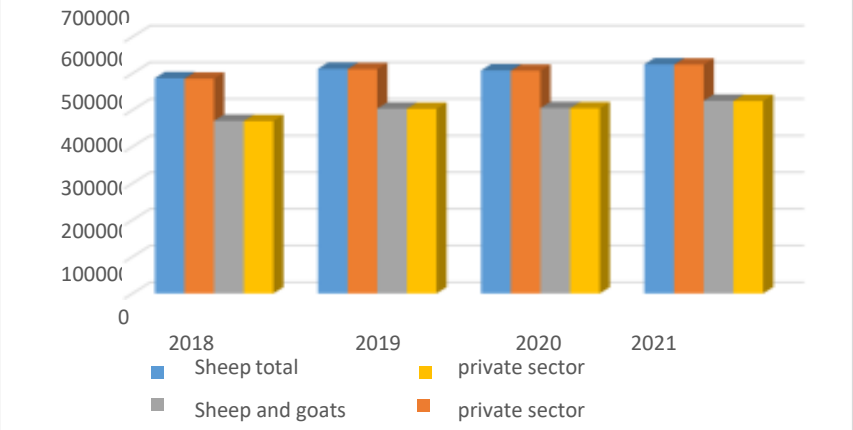



Figura 3. Analysis of the evolution of sheep flocks exploited in Timiș County in the period 2018-2021

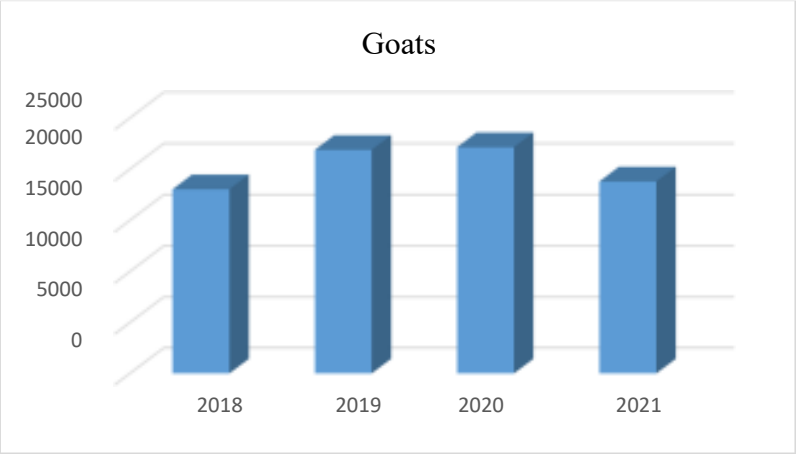



Figure 4. Evolution of goat herds in the period 2018-2021 (Timiș County)

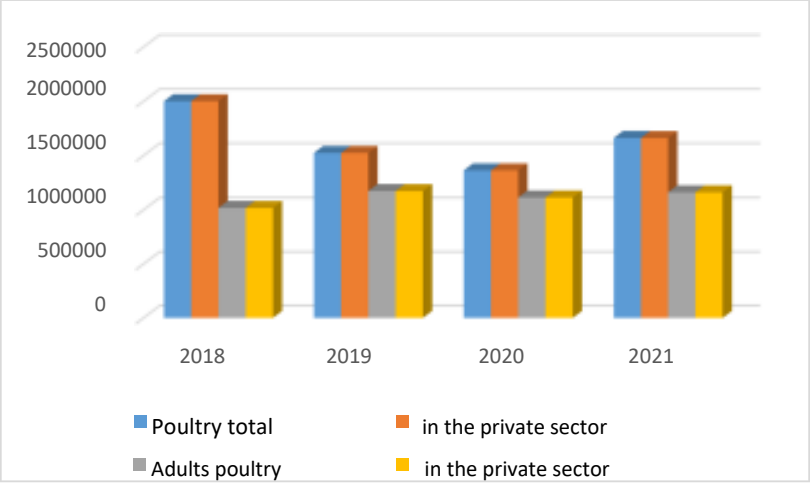



Figure 5. Analysis of poultry flocks in the period 2018-2021 (Timiș County)

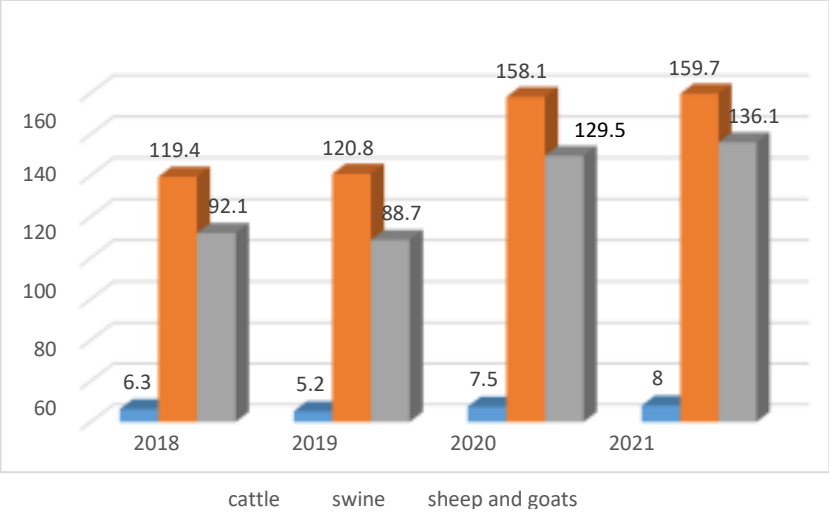



Figure 6. Analysis of animals returning to 100 ha in Timiș County

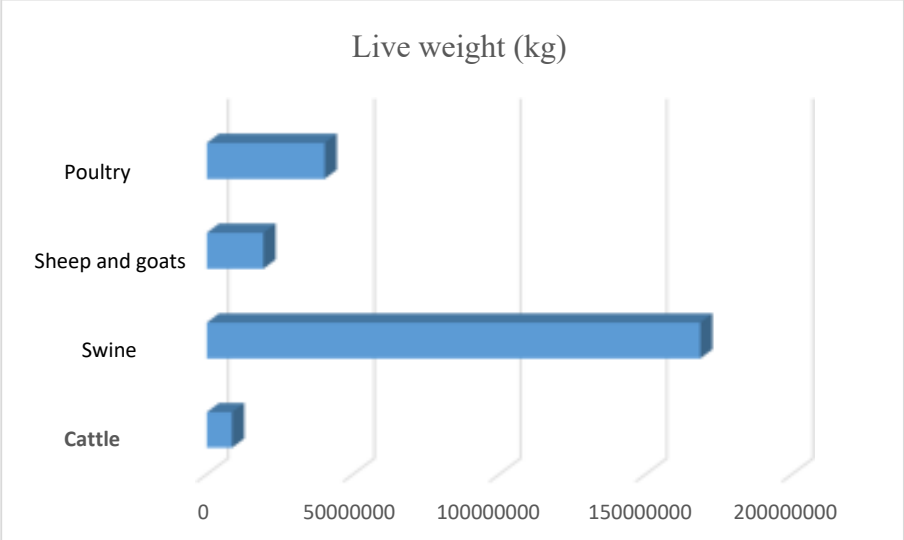



Figure 7. Meat production (live weight) in 2021 in the Western area

## DISCUSSION

Ways to improve individual productions:

a. In cattle, good weight gains according to sex, age, nutrition, and technology, indicate the degree of adaptation of a breed and commercial meat hybrid to the specific exploitation conditions, the genetic basis of an individual influenced by the final weight when processing to obtain the casing. Genetic improvement of meat production performance depends on breeding programs that exploit genetic variability between and within breeds; breeding and handling conditions of animals can influence their live weight gain because breeding efficiency is always the result factors that determine the cost of cattle for fattening, their genetic value, and the ability to provide welfare conditions for its expression, and young cattle selling price. It is recommended to use specialized breeds or mestizos obtained from the crossing of mixed breeds of cattle with meat bulls. The viable solutions proposed through the organizational management of the farm refer to the level of nutrition, meat production technologies, and maintenance system. Controlling these aspects through the best fattening management, on needs to increase individual meat production nutrition models and feed administration solutions to obtain genetic material for fattening, and vigorous calves with good calving weights, by controlling the nutrition of cows in last part of gestation for:

- Calves with high calving weight and expected maximum production (EMP);
- Production permitted by basic ration (PPBR) = PMA – PPBR.

The administration of concentrated feed to cows in the last period of gestation should be done according to PEMP (maximum expected milk production) and the difference between PEMP and PAR (production allowed by the ration), to obtain milk quantities to determine an optimal average daily gain for feeding levels.

For the growth and development of mixed-breed calves, it is recommended to use rations that stimulate consumption and produce good growth gains to shorten the fattening period:

- Introducing concentrates into the rations progressively according to the responses by increasing the daily accumulated body mass;

- Increasing the feed amount between the 3rd and 4th week after calving;
- Reaching the maximum weight according to the predicted meat production through the average daily gain.

The nutrition management plan for good meat production according to resources will include:

- A good rate of distribution identical to that in lactation for concentrates;
- Supplying 3 kg of concentrates in the first 7 days after parturition as in the 3rd week of pregnancy;
- Increasing the amount of concentrate feed by 1 kg weekly if the young respond with higher amounts of growth up to the 3rd week of lactation;
- Reducing the amount to 3 kg for maximum production up to 12 weeks.

b. Technical ways to increase individual sheep and goat meat productions in alternative systems and reduce the risks of environmental contamination need:

b1. An intensively managed system consisting in:

- Regulating the number of youth per area depending on the productive value of the meadow;
- Controlling the response through body mass accumulated daily through the provided fodder rations.

b2. A system of good practices of the sustainable management of resources for the increase of individual productions in young sheep and goats by:

- Controlling the conversion of food into body mass;
- Managing the use of pasture resources;
- Balancing the biological potential by organizing the meat-dairy selection;
- Adapting the youth to exploitation for meat on landscaped pastures;
- Using alternative, concentrated feeds to balance rations.

These contribute to the improvement of allometric growth coefficients by linking body and carcass fat, and muscle and bone to live weight, carcass weight, fat-free carcass weight, together with estimates of carcass composition. From the perspective of modern consumers' demand for lean meat, young male carcasses may have some advantages over females and even castrated males: it is necessary to study the progeny of many males slaughtered at commercial weights to obtain information on the relative performance of different breeds and crosses for quantity and quality production in different meat production systems.

c. In broiler chicken, technical measures can influence the level of growth and the effects of dietary supplementation of α-amylase on digestibility of feed nutrients, conversion of food into meat, secretion of feedback enzymes in broilers, growth performance, and nutrient digestibility of diets. It improves the apparent ileal digestibility of starch, the viscosity of the digestive bolus from the jejunum, the activity of intestinal amylase, and it increases the resistance of broilers to diseases and stress caused by exploitation.

For the protection of meat broilers and the expression of their biological value one needs to avoid feed contamination, delimit the administrative area from the production area, reduce contamination agents, and monitor meat production.

d. Pig productions, especially the technical indicators that represent growth performance, differ depending on the exploitation systems and techniques:
- In natural conditions, with freedom of movement, pigs achieve small increases in growth with high food consumption to achieve meat production because part of the energy from the feed is consumed for movement;
- In the conventional system, where the exploitation technique is at optimal parameters, good increases in body mass accumulation (1 kg of body mass) are obtained with 3.4-3.7 kg of combined feed.

## CONCLUSION

From the analysis and comparison of the official data, it follows that the trend of the herds of meat-producing animals was:

- In 2018, the largest number of cattle was in Timiș County, 41,184 heads, of which only 204 were found in the state sector, in some research stations. In the next 3 years, cattle herds were approximately constant, with small differences of about 800 heads from one year to another;

- In 2020, the largest number of pigs exploited in Timiș County was registered, 622,965 heads, of which 622,945 were exploited in the private sector, while in 2021, 595,850 were registered;

- In 2021, the largest number of exploited sheep was in Timiș County, 624,923 heads, while in 2018, only 586,990 were registered;

- In 2018, 2,001,486 poultry were in Timiș County, and in 2021, 1,662,637 were registered;

- At the level of the Western area, the production of pork was the highest in 2021, with 168,701,216 kg live, followed by poultry with 40,314,663 kg live.

The technical exploitation factors have a major influence on the quantitative and qualitative meat productions because feed administration technologies (on a regular basis or ad libitum), balancing the rations, constantly supplying water and controlling the environment determine the manifestation of heredity at optimal parameters depending on the provision of well-being conditions, breed, hybrid, morpho-productive type, weight, age, the functioning of the digestive system, and state of health. Considering the specialization of the commercial bull breed or hybrid, it is recommended, to obtain quality carcasses and meat, to keep the young bulls in the stable for fattening, maintaining an optimal level of rations and temperatures.

In young sheep, when exploitation for meat production is done in the classic system, in stables on sections, the most effective technical solution for increasing individual quantitative and qualitative meat productions is the one that uses microorganisms that favour fermentation, increase digestibility and the achievement of larger amounts of meat

In poultry broilers, technical measures can influence the level of growth and the effects of dietary supplementation with amylases, improving the apparent ileal digestibility of starch, the viscosity of the digestive bolus from the jejunum, the activity of intestinal amylase and increasing the resistance of broilers to diseases and stress caused by exploitation, thus increasing individual productions of meat, and decreasing the period of growth and development. For the protection of meat broilers and the expression of

their biological value according to the nutritional intake through increased individual meat production, several models of production techniques for the protection of the vulnerability of broilers, efficient feed management, the implementation of technologies efficient meat production, close to the natural environment and the automation of technological processes are recommended.
Carcass quality from fattened pigs in different production systems can be improved by controlling nutrition, maintenance, and welfare, implementing the most efficient integrated management in the meat supply chain.

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# POWER OF NANOPORE ANALYSIS FOR SUSTAINABLE AND EFFICIENT DIAGNOSTICS

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## ABSTRACT

Nanopore technology has become widely used because of its ability to analyze molecules at an individual level, so we are seeing a dynamic development in the use of nanopores to analyze biological molecules. In 2014, the first commercial nanopore-based device capable of sequencing long strands of DNA or RNA. While the system appears to be working well, signal analysis still has barriers. The main advantage of nanopore sequencing is that it can study the structure of a single molecule and generate reads many times longer than genotyping methods. The nanopore analysis technique is low-energy and consumables are minimal, as samples in the order of tens of microliters are used, significantly reducing reagent requirements compared to traditional methods. In addition, the technique is targeted at the single molecule level, which can make it an accurate diagnostic method. Another advantage of nanopore analysis is its portability and the variety of samples that can be analyzed, making it a versatile technique for different areas of research. Using nanopores as a diagnostic tool can provide rapid diagnosis at the point of patient care without requiring significant material resources. This method is also environmentally sustainable, as it uses low levels of electricity and material waste, and could help reduce medical laboratories' environmental impact. Clinical laboratories are a factor that negatively impacts the planet's ecology, using equipment with a significant amount of electricity, chemical reagents, and disposable containers. Approaches to solving the problems are minimal, probably because it is difficult to do without certain facilities when it comes to health. We aim to highlight the importance of reducing energy and consumables consumption in medical laboratories by introducing an innovative solution, the nanopore analysis technique which has multiple benefits in terms of both medical and energy efficiency.

**Keywords:** molecular analysis, portability, minimal consumables, new technology

## INTRODUCTION

In biomedical sciences and ecology, the analysis of molecules at the individual level is essential. Nanopore analysis technology is a revolutionary solution, allowing researchers

to analyze individual molecules such as DNA, RNA, and proteins with high sensitivity and in a short time compared to conventional analysis methods. Nanopore analysis techniques have become increasingly studied, especially with the development of commercial devices such as those from Oxford Nanopore Technologies (ONT). Historically, the first DNA sequencing experiments on biological alpha-hemolysin (α-HL) nanopores were performed in 1996 [1]. Nanopores have been implemented in molecular analysis to provide more accurate medical diagnoses. This technique allows the identification of genetic variants, bacteria or viruses, proteins involved in body dysfunctions [2]. Another important point is using this technique for wastewater analysis, which helps identify the species of organisms and their spread. Medicine is looking forward to the widespread use of nanopores as there are numerous articles proving the diagnostic and treatment benefits of using these techniques. On the other hand, nanopores can also be used in the food and energy industries, making the technique versatile and of current interest. In the context of increasing disease complexity, climate change, and environmental degradation, the ability to monitor different parameters or biomarkers in real time and assess their impact on humans is essential.

## THEORETICAL BACKGROUND

Nanopores are very small pores of nanometre order that have essential properties in the analysis of various molecules such as DNA molecules, or proteins. Pores were first discovered as self-sustaining structures in cell membranes, facilitating intra- and extracellular transport. Later it was observed that they can be used in artificial lipid membranes such as biological nanopores or synthetic membranes by etching them to form solid-state nanopores. The principle of operation of nanopores is based on the measurement of the intensity of the electric current generated when molecules pass through these nanopores. Analysis of changes in the intensity of the electric current resulting from the passage of molecules through nanopores provides information about the molecular composition and other characteristics of the molecule. This data can be used to identify DNA sequences and analyze proteins or other biological molecules.

Two categories of nanopores are used in sequencing technology and molecular analysis. Biological nanopores, which are proteins secreted by various bacteria such as α-hemolysin secreted by Staphylococcus aureus with a diameter of about 1.4 nm. They use their chemical properties to allow molecules to pass through and can also have a higher degree of selectivity, as in the case of aerolysin nanopores [3]. Solid or synthetic nanopores are fabricated by various techniques such as electron beam, controlled breakdown, and electrochemical reaction. Typically, these nanopores are constructed from solid materials such as silicon or polyimide membranes [4]. Nanopores have several characteristics that make them suitable for medical diagnostics, they can detect and analyze individual molecules such as DNA, RNA, or protein. The main use of nanopores in diagnostics is DNA sequencing. This technique allows the identification of genetic sequences associated with different diseases or genetic predispositions, thus facilitating early diagnosis and the use of personalized treatments. The existence of wearable nanopore (MinION$^{TM}$) technologies may revolutionize medical diagnostics, enabling point-of-care or home testing. The technique has reached the point where a relatively small device weighing about 90 g can be used outside the laboratory. The device called MinION™ uses the same principle, measuring changes in electrical conductivity as a DNA molecule passes through the pore. The performance of this device is given by a

large number of channels, more precisely in a single cell, 512 independent channels allow the identification of 512 DNA molecules. The sequencing process is relatively simple for the operator, once the samples are taken, the device connects via a USB port to a computer running MinKNOW specific software. The software performs both data acquisition and analysis, providing real-time feedback. Another important factor in the nanopore sequencing process is library preparation. This process is quite complex, requiring coding each nucleic acid structure to arrive at DNA structures. It is well known that making libraries is one of the most expensive steps due to expensive reagents and long working time. In recent years, many techniques have been developed that seem to simplify the process of creating libraries, and there is even a trend toward automated models. For example, algorithmic error correction methods have been introduced, the IsONCorrect platform can correct with an accuracy between 98.9 - 99.6%. The principle of the IsONcorrect platform is to divide reading into smaller intervals and then analyze and correct each interval according to a specific algorithm. The Oxford Nanopore Technique continues to improve its features making the device increasingly reliable for clinical analysis. The fourth generation of gene sequencing technology, ONT has an accuracy of about 99.5% and a short analysis time of about 2-6 hours [5].

The specificity of the technique brings multiple benefits in personalized medicine, DNA analysis allows tailoring the treatment to the genetic specificity of each individual and thus treatments are tailored specifically to each patient according to their genetic profile. The discovery of significant new biomarkers for various diseases requires rapid quantification methods for early diagnosis and monitoring of disease progression. Certain nanopore-based sequencing technologies have the potential to be less costly and more efficient compared to traditional sequencing technologies, making them more affordable.

All these properties make the nanopore analysis technique a powerful tool that supports classical biomarker analysis methods by offering outstanding possibilities for the detection and diagnosis of diseases in an accurate, fast, and personalized way. Even though the technique has a huge potential, it is still under continuous development and optimization for large-scale use in the medical field.

The following picture schematically illustrates the advantages and disadvantages of nanopore technology. It is obvious that although the technique is not yet fully developed, it deserves investment and efforts from researchers to maximize its potential in medical and scientific applications.

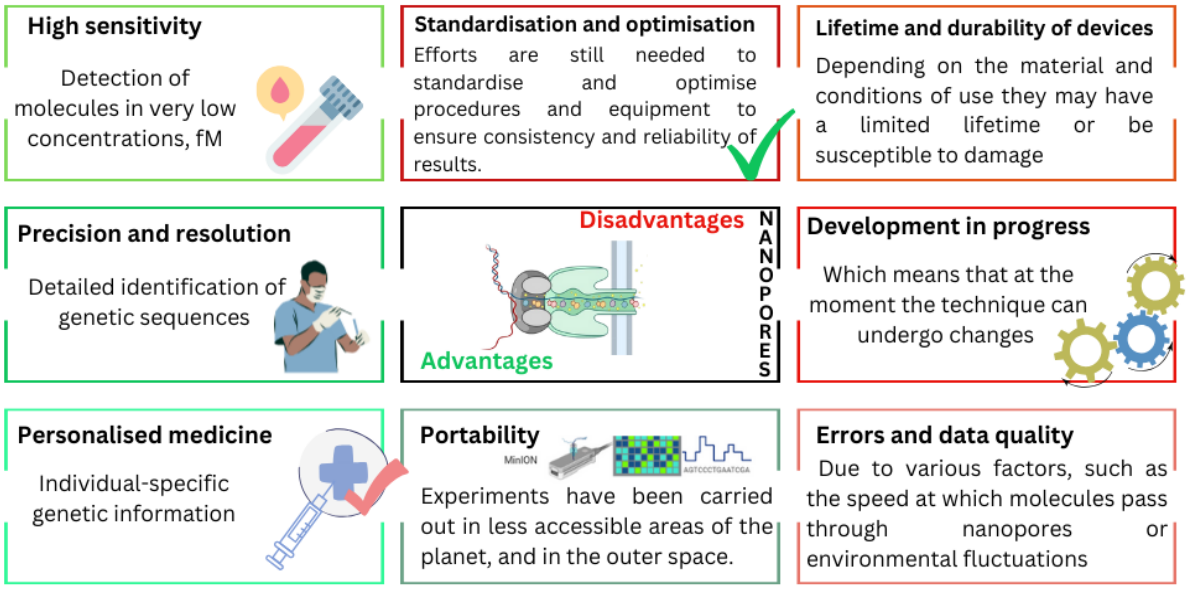



Figure 1. Main advantages (green rectangular shape) and disadvantages (red rectangular shape) of the nanopore analysis technique.

## APPLICATIONS OF NANOPORE USE IN MEDICAL DIAGNOSTICS

In the context of evolving medical technologies, the development of nanopore assay technology promises to analyze various molecules at high sensitivity without affecting the structures of biological molecules. In addition to the fact that diagnosis must be accurate, it must also be rapid because often the detection of diseases in their early stages makes the difference between life and death. Nanopore sensors can use small amounts of substance at low concentrations (even ordinal fM). DNA sequencing is the most researched area in nanopore analysis, as significant gains have been recorded. The technique works by reading nucleotide-by-nucleotide sequences as the molecule passes through the nanopore. Compared to classical methods that involve DNA strand segmentation, amplification, and synthesis, the nanopore analysis technique offers long reads. As far as protein analysis is concerned, proteins are essential in cellular processes but are often difficult to identify at an early stage, often correlating changes in either protein structure or quantity with symptoms, such as Alzheimer's neurological disease [6]. Protein analysis is less well developed, and there are currently studies in which proteins of about 30 amino acids have been analyzed [6], [7].

Identification of molecular changes for correct diagnosis requires complex analyses such as Ion Torrent Sequencing, Illumina, ELISA, and mass spectrometry. All these techniques are time and energy consuming which means there is potential for improvement. The device developed by Oxford Nanopore Technologies (ONT) is now comparable to classical methods, for example for the analysis of bacteria, although the Illumina analysis technique produces short readings with good accuracy and the ONT technique produces readings of months but with lower accuracy. However, the results can be compared, as both technologies detect almost identical sets of genetic markers [8]. Despite the fact that the nanopore analysis technique may have errors compared to classical methods, innovation continues both on the medical side, with advances in the analysis of cancer

biomarkers, viruses, bacteria, metabolic markers, proteins, etc. [2], but also in environmental research analyzing various pollutant compounds[9].

As an example, let's consider the analysis of pathogens that are currently a cause of mortality out of the top 10 analyzed by the World Health Organization. Respiratory samples taken in case of infection with certain pathogens are complex, with a large amount of human DNA in addition to the pathogen content. For this reason, the analysis of the pathogen involved in respiratory infections is challenging [10]. While Ion Torrent or Illumina techniques require sequencing to be completed before analysis and molecule identification can begin, ONT sequencing allows real-time analysis. ONT sequencing was tested on 40 samples from infectious respiratory disease patients. Initially, ONT sequencing was used on samples without removing human DNA, but most of the reads were of human origin, and only two reads were of pathogens. The protocol includes a host DNA removal step for the microbial DNA to be extracted and prepared for sequencing. The host DNA removal step can be shortened by introducing bead-beating or adding an enzyme cocktail to improve microbial lysis [11]. Through nanopore sequencing, specific genes that could not be grown on culture media can be identified. In the case of a 63-year-old patient hospitalized for treatment of chronic lymphocytic leukemia developed a fever during hospitalization. He received a clinical diagnosis of severe pneumonia. Considering the medical history and the symptoms that occurred, the medical team concluded that it was necessary to take a sample of the lung tissue to analyze the cause of these symptoms through various tests. Although symptoms seemed to resolve after treatment with antibiotics, fever and cough recurred, A tissue sample was sent to a laboratory for metagenomic sequencing and also to confirm the culture tests performed in the hospital laboratory. DNA was extracted from the lung tissue sample using a special kit for metagenomic sequencing using nanopores. Then, a sample of only 16.05 ng of DNA was loaded into the Flow Cell and sequenced on the MinION instrument. In parallel, sequencing was performed on the BGISEQ-500 and Illumina platforms. In only 12.14 hours, 79.23 MB of data was generated with a total of 34,831 reads [12]. Bacterial sequences were found in a proportion of 1.883%, i.e., 656 sequences, of which 421 were specific sequences of Klebsiella pneumonia, and the other 235 were compatible with other bacteria. Furthermore, in this case, given the early administration of antibiotic treatments, genomic analysis of antibiotic resistance was necessary to optimize treatment [13]. The importance of real-time sequencing is evidenced by the accuracy and short response time of nanopore sequencing compared to other methods where the analysis time was up to 200 hours. The effectiveness of these methods in clinical diagnostics seems to meet the current needs, however, in the future, it is necessary to optimize the sample preparation methods in terms of extraction and bioinformatics.

Almost all cancers develop long before symptoms appear. Early detection of cancer can save lives - in 2020 it was estimated that the disease led to 10 million deaths. This calls for the use of ultra-sensitive detection methods for biomarkers, be they DNA molecules, RNA, proteins, enzymes, or others. For example, circulating tumor cells (CTCs) have a low concentration in the blood, making it difficult to detect them. The nanopore analysis method has been used to detect cancer cells [14]. miRNA plays an essential role in regulating gene expression, cell proliferation. Each cancer type is thought to have a specific miRNA expression profile, making microRNA analysis valuable information about tumor specificity. Nanopores have been used as label-free miRNA pattern recognition systems. The study was performed on plasma samples with a very low

concentration ($10^{-16}$fM) of miRNA from bile duct cancer patients. The results were satisfactory and comparable to classical methods using higher concentrations of miRNA in samples [15].

## SUSTAINABILITY ASPECTS

Nanopore technology is an essential tool in the medical field. However, durability is a critical point in the long-term use of this technology. The structural strength of the devices depends on the components of which the nanopore system is composed. In the case of biological nanopores, it depends on the stability of the molecular bonds under different conditions, and in the case of solid nanopores, it depends on the mechanical strength of the material under different forces. Solid nanopores are considered to be more durable. In the case of the nanopore matrix in ONT devices, the durability is about 3 months under controlled conditions of 2-8°C.

Another essential aspect of sustainability is data accuracy. Fluctuations and errors in nanopore measurements affect the accuracy of the data and implicitly the associated clinical analyses. Also, the environmental impact of these technologies involves the manufacturing processes of the nanopores, the reagents, and the materials used. For example, choosing biodegradable or recyclable materials could reduce the impact on the environment, such as attempting to manufacture polymer films with nanopores. In addition to the experimental setup, it is necessary to pay attention to how waste is managed, such as those resulting from the following uses of chemical substances, and disposable tubes. The ONT company has already implemented a flow cell recycling system, otherwise, they want their devices to be sustainable. Currently, there are no clear strategies for maintaining sustainability in this field, so it is necessary to develop some strategies to ensure the elimination of associated waste in optimal and ecological conditions.

The durability of nanopore technology is not only limited to the structure of the devices and their lifespan, but also to aspects related to precision in analysis, environmental impact, and, by extension, the costs involved in using this technology. An overview of all aspects (clinical accuracy, execution time, precision, sensitivity, reduced consumables, environmental impact) will open new horizons in medicine, research, and related fields.

## CONCLUSION

Nanopore analysis technology is extremely useful because of its ability to analyze individual molecules, and there is currently a dynamic development in the analysis of various molecules, DNA, RNA, proteins, enzymes, and small molecules, all having the function of markers in various pathologies. Although the system appears to be able to analyze various molecules in low concentrations, it is not widely available and is still hampered in terms of accuracy and signal interpretation. The main advantage for which the technique is recognized is that it analyses molecules at the individual level and generates longer readings than conventional methods. This will help enormously in identifying diseases at an early stage which will lead to timely targeted treatments, thus increasing life expectancy for serious diseases. In today's human health environment, given the external factors (pollution, nutrition, stress), continuous monitoring at the molecular level is needed precisely to predict possible processes that may be triggered by the increase of a biomarker in very low concentrations. The method is also energy-

efficient and requires few consumables because samples of the order of tens of microlitres are used, thus significantly reducing the volume of reagents used compared to traditional methods of analyzing various molecules. The technique also stands out for its portability, which makes it versatile for different fields of research besides medicine, the method can also be used in the food industry, energy systems, ecosystems, environment, etc. This area focuses on both the molecular capture mode, which involves elements of molecular biology and molecular interaction mechanisms, and the interpretation of the resulting signal, for which knowledge of bioinformatics is required. Thus, solving all the problems in this case requires an interdisciplinary vision that can be achieved through a good collaboration of researchers. In conclusion, the nanopore analysis method has great importance in the medical field, with high potential in optimizing the diagnostic and resource efficiency of medical laboratories, the method contributes to sustainable development in this field.

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# PRELIMINARY RESEARCH REGARDING THE IMPACT OF MOISTURE CONTENT ON NUTRITIONAL PARAMETERS OF NETTLE POWDER

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## ABSTRACT

Nettle leaf powder is an excellent source of nutritional compounds (proteins, minerals, fibers, carbohydrates) and biologically active compounds: terpenoids, carotenoids, fatty acids, various polyphenolic compounds, essential amino acids, chlorophyll, vitamins, etc., essential for the health of the human body. Therefore, nettle powder could be used as a nutritional supplement. The nutritional quality of the nettle powder can be influenced by the drying technique of the nettle leaves, respectively by the water content of the final product. This study aims to evaluate the nutritional content of nettle powder obtained by drying nettle leaves through three drying processes. Basically, the variation of moisture, ash, protein, fiber and carbohydrate content of nettle leaf powder obtained by drying was observed in case of fresh leaves of wild nettle (Urtica dioica L.) from the spontaneous flora, using three drying methods: shade drying (SHD), sun drying (SD) and oven drying (OD). The obtained results show that the nettle leaf powder obtained under the conditions of this experiment contains important amounts of nutritional compounds. This research provides evidence that nettle leaf powder contains greatly increased amounts of nutrients compared to fresh leaves.

**Keywords:** nettle leaf powder, moisture, nutritional parameters, drying methods

## INTRODUCTION

The Stinging nettle (Urtica dioica L.) is a perennial herbaceous plant of the Urticaceae family that grows mainly wild almost worldwide, with a greater presence in Europe, North America, North Africa and some regions of Asia [1, 2]. The leaves of this edible plant are very nutritious, easy to digest and contain a large amount of protein, amino acids, minerals (calcium, potassium, magnesium, phosphorus and iron), vitamin C, provitamin A and carotenoids [3]. A comprehensive proximate analysis showed the shoots harvested from Stinging nettle showed close to 90% moisture and rests are

proteins (3.7%), fat (0.6%), ash (2.1%), dietary fiber (6.4%), total carbohydrate (7.1%), and total calories (45.7 kcal/100 g) (wet basis); besides, Stinging nettle (shoot) contains vitamin A, vitamin C, calcium, iron, sodium, and rich fatty acid profile [4]. Also, the leaves and young shoots are a rich source of polysaccharides, vitamin (β-carotene, C vitamin), iron, potassium, manganese, calcium protein, silicon, and phosphate [5].

Stinging nettle leaves contain important amounts of biologically significant classes of compounds such as fatty acids (especially the dominant cis-9,12 linoleic and α-linolenic acids), carotenoids, polyphenolic compounds and minerals (especially Na, K, Ca, Mg, Fe, Mn and Zn), and we can also notice the absence of toxic heavy metals [6,7]. Chlorophyll, carotenoids, vitamins, proteins, lipids, carbohydrates, organic acids, minerals and trace elements are abundant in nettle leaves [8,9]. Regarding the nutritional qualities of nettle leave Said et al. (2015) reported that nettle leaves are rich in protein, fat, carbohydrates, vitamins, minerals and trace elements and cites the following values (fresh samples): 65 - 90% water, 4.3 - 18.9% proteins, 3.4 - 18.9% ashes, 7.1 - 16.5% carbohydrates, 0.7 - 2.0 lipids, 3.6 - 5.3% fibers, 57 - 99.7 kcal/100g calories. According to these authors, the concentrations of mineral and trace elements (mg/100 g of dry matter) show the following values: 113.2 - 5090 Ca, 2200 - 3560 Mg, 29 - 75 P, 532 - 917.2 K, 5.5 - 16 Na, 0.0084 - 0.018 Co, 0.52 - 1.747 Cu, 3.4 - 30.30 Fe, 0.768 - 5.784 Mn, 0.4265 Mo, 0.0732 Ni, 0.0027 - 0.0074 Se, 0.9 - 3.033 Zn [9]. Being rich in proteins, fats, carbohydrates, vitamins, minerals and trace elements [9], fresh nettle leaves are frequently used as an additional vegetable, similar to spinach, in the form of side dishes, soups, salads, etc. [1,10].

The increased content of nutritional compounds and bioactive components of nettle powder (NP) obtained by drying of stinging nettle leaves recommends the use of nettle powder as an ingredient/additive to enrich food products [11].

Nettle leaves of good quality are available in abundance only in a certain season [12]. Therefore, it is necessary to harvest the plants during the growing season when the leaves are tender and raw green in color. For the use of nettle leaves as a source of nutritional and biologically active compounds for a longer period, it is very important to choose an appropriate drying process. The drying technique used to dry nettle leaves is very important to keep the nutritional components intact in the fresh leaf as much as possible. Drying is the most rational way of preservation, because microbiological processes slowdown in dried products, and the composition of nutrients and biologically valuable substances remains close to that of the fresh product [13]. The drying process can produce changes in the dried product both biochemically (i.e. Maillard reactions, vitamin degradation, fat oxidation, denaturation of thermally unstable proteins, enzymes, etc.) and physically [12].

The consulted literature records data obtained by researchers in the field who were interested in obtaining dried nettle leaves using different techniques for drying fresh leaves: shade drying [14], sun drying [14], solar tunnel-dried [12], freeze-dried [10, 11, 15], oven-dried [10,15], microwave-dried [14,16], convective drying [11], heat pump drying [15], also highlights the disadvantages of each method. The results obtained from the analysis of nutritional and bioactive compounds show that the values differ according to the chemical composition and the pretreatments performed on the plants before drying, the drying technique used as well as the pretreatments performed and the nature of the analyzed compounds [11].

Results for the proximate composition of the freeze-dried and oven-dried samples obtained from Stinging nettle young and tender shoots show the following values: 3.4% moisture, 19.2% ash, 3.4% fats, 18.9% available carbohydrates, 26.3% fibers, 28.8% proteins - in oven dried, respectively 6.4% moisture, 19.5 ash, 3.5% fats, 14.7% available carbohydrates, 27.5% fibers, 28.3% proteins - in freeze dried [10]. According to Pant (2019) the protein content is less affected by the drying method: 29.53%, 28.37% and 29.61% protein - by drying in the sun, in the shade, respectively in the microwave [14].

## MATERIALS AND METHODS

### Materials

#### *Raw material collection and powders reparation*.

We used stinging nettle plants (Urtica dioica L.) grown in the spontaneous flora in a plain area in Timis county (Romania). The nettle samples were collected in the spring of 2023 during the vegetation period specific to the appearance of young and tender leaves. The collected samples were transported to the laboratory, where young leaves were selected and cleaned by washing to remove foreign particles. Washing was carried out under running tap water, and rinsing with distilled water. The leaves were then drained to remove excess water, after which four homogenous work samples (samples) of 200g each were formed. The first group of fresh nettle leaves was used to determine the nutritional parameters of fresh nettle, and the rest were used to obtain nettle leaf powder. Nettle leaves were dried using three drying methods: shade drying (SHD), sun drying (SD) and oven drying (OD), until a crispy texture was observed. The dried leaves were ground in a coffee grinder and sieved when a fine dark green powder was obtained. Until the time of the analysis, the NPs were transferred into hermetically sealed glass containers and kept in places away from heat and light.

### *2.2. Analysis*

The determination of the nutritional composition for the studied nettle leaves samples was performed according to AOAC Official Methods of Analysis, 2000 [17] and according to the recommendations of Rada et al., 2019 [18]. Moisture was determined by the oven drying method (100-102°C) until to constant weight. The mineral substances (ash) were determined by the calcination method at 550°C. The protein content was determined by the Kjeldahl method, using a conversion factor for nitrogen of 6.25. The crude fat was determined using the Soxhlet method with hexane as solvent. The carbohydrate content was obtained by difference: 100 - (% moisture +% ashes +% crude nitrogen +% total lipids).

### *2.3. Statistical analysis*

Results were expressed as the mean of three determinations ± standard deviation (SD). Statistical differences ($p < 0.05$) between samples were performed by t-test using Microsoft Excel 365 (Version 2208, Redmond, WA, USA).

## RESULTS

The experimental results obtained from the analysis of the nutritional parameters of fresh nettle leaves (Urtica dioica) and NPs obtained by three drying methods: SHD, SD, and OD, are graphically illustrated in figure 1.

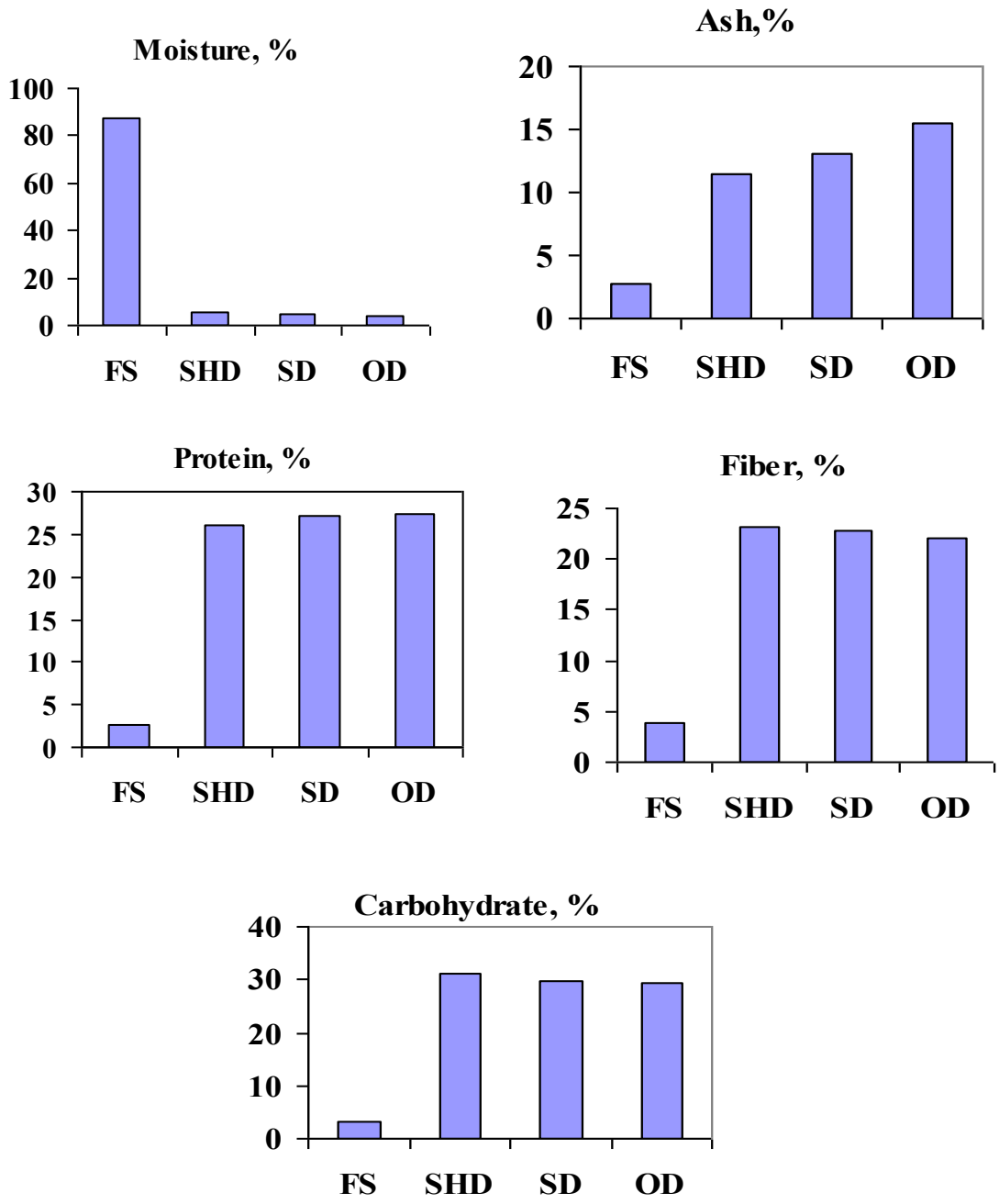



**Figure 1.** The concentration of some nutritional parameters in fresh nettle leaves (FS) and in nettle powders (NP) obtained from Urtica dioica by different drying methods: in the shade (SHD), in the sun (SD) and oven drying (OD).

## DISCUSSIONS

The leaves of stinging nettle (Urtica dioica L.), especially nettle leaf powder contain important amounts of nutritional compounds, so this plant is an excellent source of nutrients. Compared to fresh leaves, with the exception of moisture content, NPs present much higher contents of nutritional compounds. The moisture content in the analyzed NPs shows different values, depending on the drying method. The highest value was determined in the samples dried in the shade. Lower values of humidity by 23% and 33% were determined in the samples dried in the sun and in the oven. Therefore, nettle powder obtained by drying in the oven is richer in dry matter than those dried in the sun, respectively in the shade. The results obtained when determining the ash content also show that dry leaves are much richer in minerals compared to fresh leaves. Regarding the ash contents determined in the analyzed NPs, it can be seen that they differ depending on the drying technique. The dried nettle powder sample in OD has the highest ash content, with values lower by 16%, and 26% in the SD and SHD samples, respectively. The protein content of the fresh sample and the dry samples reveals that NPs have increased protein content, compared to fresh leaves. Regarding the protein content in NPs obtained by OD, SD and SHD, the differences are much smaller. Slightly higher and similar contents (0.8% difference between OD and SD) were determined in dry NP at OD and SD, respectively. Proteins from the NP obtained by SHD are 4% lower compared to those degraded in OD.

The fiber content determined in fresh leaves has much lower values compared to dried leaves. Comparing the fiber content of the NPs, it can be seen that the fibers are best represented in the NP obtained by SHD and SD, which show close values. The difference between the fiber content between NP obtained by SHD and SD is only 1.4%. NP obtained by OD shows lower values by 4.3% compared to SHD. Regarding the carbohydrate content in this case too, the values obtained are much lower in fresh leaves. Better represented in carbohydrates is NP obtained by SHD. Concentrations, lower by 5% and 6% of carbohydrates, were determined in NP obtained by drying in the sun, respectively in the oven. It is observed that the NPs obtained by SD and OD contain very close amounts, the difference between the determined values being 1%. Comparing the results obtained from the analysis of the nutritional parameters of fresh leaves and NPs, it can be appreciated that they fall within the range of values reported in the works of some researchers in the field [9,10, 11,14]. The differences can be justified by the nature of the fresh nettle leaves and the pretreatments before drying, the drying techniques, etc. [11].

## CONCLUSION

Nettle leaf powders obtained by drying stinging nettle leaves (Urtica dioica L.) contain increased amounts of dry matter, minerals, proteins, fibers and carbohydrates, compared to fresh leaves. The concentrations of the analyzed parameters of nettle powder are significantly influenced by the techniques used to dry the nettle leaves: in the shade, in the sun or in the oven. Choosing a drying method that produces the highest concentration of nutrients is relatively difficult, because the amount of nutrients left in the dried powder is also influenced by the nature of the compound of each nutrient compound. Under the conditions of the present experiment, by drying in the oven, the minerals, proteins and dry substance are best preserved. Fibers and carbohydrates show

higher values in the case of drying in the shade. In conclusion, it can be stated that nettle powders obtained by drying nettle leaves are good sources of nutritional compounds, whose nutritional values are significantly influenced by the drying method used.

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# QUALITY EVALUATION OF FUNCTIONAL BREAD DEVELOPED FROM WHEAT FLOUR AND MALTED BARLEY FLOUR MIXTURES

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## ABSTRACT

The use of some non-wheat raw materials in the form of flours, extracts, and malts has always been of interest to food producers. The objective of this study was to evaluate the nutritional potential of some types of malted barley flour (MBF) to develop a range of breads with improved sensory, nutritional, and technological qualities. Malted barley flour (MBF) contains health-promoting compounds that enhance the nutritional value of bakery products when combined with wheat flour (WF). Two types of MBF (*Amber* and *Chocolate*) were used to replace WF in amounts of 10, 20, and 30%. Bread with 100% WF serves as a reference sample. Proximate composition, physical characteristics, sensory characteristics, total polyphenol content (TPC) and antioxidant capacity (AA) of bread samples were evaluated using standard laboratory procedures. Following the evaluation, it can be observed that with decreasing the percentage of WF and increasing MBF percentage, the studied bread samples showed changes in the analyzed characteristics. Centralizing the results obtained regarding the sensory analysis of the bread samples, it can be seen that the bread sample with 20% *Chocolate* MBF was the most appreciated by the evaluators (flavor - 7.62 and taste - 7.45, overall acceptance - 7.87). The results also showed an improvement in the nutritional profile of the breads proportional to the increase in the proportion of MBF (30%) in the composite flour blends in terms of protein, fiber and mineral content, as well as a significant increase in functional attributes such as TPC (12.04mg GAE/g) and AA (92.24 mg TE/g). Therefore, MBF can be considered a wholesome raw material in bakery production.

**Keywords:** malted barley flour, functional bread, quality evaluation

## INTRODUCTION

Barley, classified under the *Poaceae* family, *Hordeum* genus, is one of the oldest and most important cereals cultivated in the world, approximately 2% is used directly in food products [1]. The increased interest in barley as a human food ingredient is the result of many studies showing that barley is an excellent source of dietary fiber (11-34% compared to wheat's range of 11.5-15.5%), β-glucans (5- 10% in comparison to wheat which contains only 1% β-glucans), minerals (predominantly P, K, Ca and Mg), B-complex vitamins (tocotrienols, tocopherols) and proteins abundant in essential amino acids (predominantly lysine, threonine, methionine and tryptophan), phenolic

compounds [2]. Thus, due to its high fiber content and the presence of bioactive compounds, barley flour can be used in food products for the prevention of certain diseases and multiple health benefits (reduces the risk of coronary heart disease, reduces the glycaemic index of products) [3]. Due to its superior nutritional potential and multiple benefits on human health, barley could be an excellent raw material for the production of functional flour products such as bread, pastries, biscuits, pasta, extruded products by partially replacing wheat flour (WF). Barley is the most widely used cereal for the production of malt, malt extract and malt flour [4]. Malting process involves numerous steps, including steeping, germination, drying and cleaning. These malted grains are steam-dried, hulled, then ground and sifted to obtain malt flour, which could be added to WF, to give it higher technological characteristics for processing and obtaining bakery products, especially bread [5]. Malted barley flour (MBF) being rich in α–amylase, is added in WF to form composite flours for bread production. Although previous studies have focused on increasing the nutritional value of MBF-enriched bakery products, there is little information available on the techno–functional behaviors of MBF-incorporated composite flours, dough and bread obtained, which are the final parameters on which bread quality depends. Replacing WF with MBF at an adequate level could minimize the use of synthetic bread improvers and produce breads with extraordinary characteristics. MBF are relatively rich sources of maltose, minerals, soluble proteins, amylolytic and proteolytic enzymes, and flavoring compounds that encourage yeast activity, accelerate dough conditioning, and give bakery products a unique taste and aroma [6]. The high diastatic activity of MBF is excellent for improving bread made from flour that lacks amylase [7, 8]. Enzymatically inactive MBF, due to its maltose and glucose content, increases the gas-forming capacity of the dough, reduces fermentation time, increases the volume, porosity, and elasticity of the crumb, imparts a more pleasant taste and aroma, a darker specific color and helps to maintain freshness over time [8]. Based on these observations, the aim of this study was to obtain an assortment of bread with superior nutritional properties conferred by the additions of malted barley flour *Amber* (MBFA) and malted barley flour *Chocolate* (MBFC) and to investigate the effects of MBF supplementation on the physico-chemical, phytochemical and sensory attributes of the bread.

## MATERIALS AND METHODS

### Raw Material and Ingredients

The two types of MBF (*Amber* and *Chocolate*) were purchased from Brews Brothers, Bucharest, Romania, while wheat flour (WF) 72% extraction, yeast and salt were purchased from Auchan supermarket in Timisoara, Romania. Based on WF and BMF, three six of composite flour were prepared as follows: M1 (90% WF: 10% MBF *Amber*, MBFA); M2 (80% WF: 20% MBF *Amber*, MBFA); M3 (70% WF: 30% MBF *Amber*, MBFA); M4 (90% WF: 10% MBF *Chocolate*, MBFC); M5 (80% WF: 20% MBF *Chocolate*, MBFC); M6 (70% WF: 30% MBF *Chocolate*, MBFC) and a control sample CS (100% WF + 0% BMF).

### Technological process of obtaining bread

The bread formulas are shown in *Table 1*. Bread samples were prepared using a modified method as described by Belcar *et al.* (2022) [4]. The technological process used to obtain bread was the classic one (direct method). Six bread samples were

obtained from the obtained flour mixtures and control bread (CB: 100% WF:0%MBF**)** were obtained, with water - 56%, yeast - 3%, salt - 2% added to each sample.

**Table 1. Recipes for composite malted barley flours bread**

| Ingredients | Bread samples | | | | | | |
|---|---|---|---|---|---|---|---|
| | **CB** | **B10MBFA** | **B20MBFA** | **B30MBFA** | **B10MBFC** | **B20MBFC** | **B30MBFC** |
| Wheat flour (g) | 500 | 450 | 400 | 350 | 450 | 400 | 350 |
| Malted barley flours *Amber* (g) | 0 | 50 | 100 | 150 | 0 | 0 | 0 |
| Malted barley flours *Chocolate* (g) | 0 | 0 | 0 | 0 | 50 | 100 | 150 |
| Salt (g) | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Yeast (g) | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Water (mL) | 650 | 650 | 650 | 650 | 650 | 650 | 650 |

**WF** - wheat flour; **MBFA** - malted barley flour *Amber;* **MBFC** - malted barley flour *Chocolate;* **CB** – Control bread (100% wheat flour (WF): 0% malted barley flour (MBF); **B10MBFA** - Bread sample with 90% wheat flour (WF): 10% malted barley flour *Amber* (MBFA); **B20MBFA** - Bread sample with 80% wheat flour (WF): 20% malted barley flour *Amber* (MBFA); **B30MBFA** - Bread sample with 70% wheat flour (WF): 30% malted barley flour *Amber* (MBFA); **B10MBFC** - Bread sample with 90% wheat flour (WF): 10% malted barley flour *Chocolate* (MBFC); **B20MBFC** - Bread sample with 80% wheat flour (WF): 20% malted barley flour *Chocolate* (MBFC); **B30MBFC** - Bread sample with 70% wheat flour (WF): 30% malted barley flour *Chocolate* (MBFC)

Each flour mixture was added to the bowl of the Hauser DM601 mixer and homogenized at speed 1 for 5 minutes, then salt, yeast previously emulsified with warm water and the rest of water were added and mixing continued at speed 1 for 5 minutes and then at speed 2 for 10 minutes until a homogeneous dough was obtained. The dough thus obtained was divided into 250 g pieces, moulded, placed in trays and then left to rest at 37°C for 60 minutes and relative humidity of 80%. After resting, the dough trays were placed in the oven and baked at 220°C for 55 minutes. After baking, the bread loaves were cooled at room temperature, then packed in cardboard boxes and stored at temperatures of 12ºC [4].

**Proximate composition of flours and samples bread**

The proximate composition of flours and bread samples was determined according to the Association of Analytical Chemistry Officials (AOAC 2000) standard method guidelines [9]. The total carbohydrate content (%) was calculated by difference (carbohydrate content = 100 − moisture content – protein content − lipids content – ash content). All samples were measured in triplicate. The energy value of the products was determined considering the following correlations: 1 g lipids = 9 kcal, 1 g carbohydrates = 4 kcal, and 1 g proteins = 4 kcal.

$$Energy\ value\ (kcal/100\ g) = (9 \times lipids) + (4 \times carbohydrates) + (4 \times proteins) \quad (1)$$

**Physical properties of bread samples**

The quality parameters such as volume, porosity, springiness (core elasticity), and the height/diameter ratio were determined according to SR 91:2007 [10]. The results are expressed as the arithmetic mean of the two parallel determinations.

**Phytochemical profile of bread samples**

The total phenolics content (TPC) in flour and bread samples were determined with Folin-Ciocalteu reagent using the method described by Borrelli *et al.* [11] with some modifications. Extracts were prepared by weighing 2 g sample and mixed with 5 mL of acidified methanol (HCl/methanol, 1:100, *v/v*). The mixture was homogenised by vortexing for 2 min, placed in a Sonorex Digitec ultrasonic bath (RK 510 H Model;

Germany) at room temperature and sonicated for 1 hour. It was then centrifuged at 9000 rpm for 5 min at 4°C on a centrifuge (Universal 320R; Hettich, Germany). Total polyphenol content (TPC) was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g). The calibration curve was established in the range of concentrations of 2.5–250 µg/mL. The antioxidant activity was determined using the DPPH method. DPPH radical scavenging capacity was determined according to Borrelli *et al.* [11], using a Trolox calibration curve, and measuring the absorbance at 517 nm. Data were expressed as mg Trolox equivalents (TE) g DM. All assays were conducted in triplicate.

**Sensory evaluation of bread samples**

Bread samples were evaluated by a panel consisting in 25 untrained assessors (10 males and 15 females) aged between 19 and 40 years. The slices of bread, with the crust, were presented (1 cm thick) on cardboard plates, encoded, and served in random order under normal lighting conditions and at room temperature. A five-point hedonic scale was used to assess consumer's acceptance, with the following ratings: 1 = extremely unpleasant; 2 = slightly unpleasant; 3 = neither pleasant nor unpleasant; 4 = slightly pleasant; and 5 = extremely pleasant. Panel members rated sensory attributes of the samples, including appearance, texture, aroma, taste, and overall acceptability [11, 12].

**Data statistical analysis**

All tests were performed in triplicate and results were expressed as mean values ± standard deviation (SD). Analysis of variance (one-way ANOVA) was used to statistically process the data. Tukey's post-hoc comparison of means and Levene's test for equal variance were both included to estimate the statistical significance of variances between the means.

## RESULTS AND DISCUSSION

**Proximate composition of flours and samples bread**

The results presented in *Table 2* illustrate the proximate composition of flour and bread samples. The moisture content was lower in the BMF sample (8.493% in BMFA and 8.320% in BMFC) compared to WF (12.677%). BMFA and MBFC are characterized by a protein content (12.674 % in BMFA, respectively 12.541% in BMFC) compared to WF (10.767%), lipid content (2.767% in BMFA and 2.331% in BMFC to 1.747% WF), fibre content (7.592% in BMFA and 7.337% in BMFC to 2.549% WF) and macro and microelements intake (2.796% in BMFA and 2.708% in BMFC to 0.538% WF), which recommends BMF as a functional matrix in baking [6, 7]. The carbohydrate content was lower in the BMF sample (65.678% in BMFA and 66.763% in BMFC) compared to WF (71.722%), contributing to the decrease of carbohydrate content in bread samples. The results obtained from this study are similar to those obtained by Aslam *et al.* (2023) [6] which noted that as the level of BMF substitution increased, all compounds increased. The obtained bread formulas (B10MBFA, B20MBFA, B30MBFA, B10MBFC, B20MBFC, B30MBFC) have a superior nutritional profile compared to CB, a profile correlated with the percentage of MBF compared to WF. The results show that the addition of MBF significantly ($p<0.05$) affected the proximal composition of the bread samples. The moisture content of bread samples was higher than that of CB (36.588%), ranging from 37.160% in B10MBFC to 39.586% in B30MBFA, which is higher than

the average content reported by Aslam *et al.* (2023) [6], who reported bread samples moisture values between 6.97% to 8.86%.

**Table 2. Proximate composition of flours and bread samples**

| Samples | Chemical Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Moisture (%) | Fat (%) | Protein (%) | Fiber (%) | Ash (%) | CRB* (%) | Energy value (kcal/ 100 g) |
| **Flours** | | | | | | | |
| WF | 12.677±0.224 [a] | 1.747±0.071 [c] | 10.767±0.203 [b] | 2.549±0.025 [b] | 0.538±0.014 [b] | 71.722 | 345.679 |
| MBFA | 8.493±0.143 [b] | 2.767±0.112 [a] | 12.674±0.226 [a] | 7.592±0.248 [a] | 2.796±0.115 [a] | 65.678 | 338.671 |
| MBFC | 8.320±0.080 [b] | 2.331±0.114 [b] | 12.541±0.151 [a] | 7.337±0.142 [a] | 2.708±0.134 [a] | 66.763 | 338.195 |
| **Bread samples** | | | | | | | |
| CB | 36.588±0.815 [d] | 1.821±0.033 [d] | 9.935±0.084 [e] | 2.324±0.040 [d] | 1.318±0.062 [d] | 48.014 | 248.185 |
| B10MBFA | 37.560±0.271 [b] | 1.935±0.084 [d] | 10.574±0.175 [d] | 3.129±0.049 [c] | 2.614±0.045 [b] | 44.188 | 236.463 |
| B20MBFA | 38.717±0.358 [a] | 2.441±0.037 [b] | 11.811±0.130 [b] | 3.485±0.053 [b] | 2.793±0.052 [a] | 40.753 | 232.225 |
| B30MBFA | 39.586±0.540 [a] | 2.811±0.130 [a] | 12.475±0.158 [a] | 3.769±0.065 [a] | 2.910±0.057 [a] | 38.449 | 228.995 |
| B10MBFC | 37.160±0.179 [c] | 1.875±0.074 [d] | 10.141±0.133 [e] | 3.077±0.039 [c] | 2.350±0.054 [c] | 45.397 | 239.027 |
| B20MBFC | 38.484±0.505 [a] | 2.231±0.053 [c] | 11.198±0.128 [c] | 3.417±0.043 [b] | 2.566±0.042 [b] | 42.104 | 233.287 |
| B30MBFC | 39.120±0.921 [a] | 2.678±0.041 [a] | 12.075±0.074 [b] | 3.654±0.034 [a] | 2.857±0.062 [a] | 39.616 | 230.866 |

*CRB – carbohydrates. a-e different letters between samples signify statistical differences according to One-way ANOVA test. The results represent the mean of three independent determinations ± standard deviation (SD). Data with different superscripts in a column are statistically different (one-way ANOVA, $p < 0.05$).

The moisture content of bread formulations tended to increase due to the high fiber content of BMF, whose property is to increase water absorption capacity. Bread samples with the addition of BMF are distinguished by higher contents of proteins, fibers, lipids and mineral substances compared to CB, these increased with the proportion of BMF added to WF. The bread formulas obtained in this research proved to be a good source of protein, thus the protein content ranged from 10.574% in B10MBFA to 12.475% in B30MBFA, and from 10.141% in B10MBFC to 12.075% in B30MBFC compared to 9.935% in BC. The same upward trend was recorded in the case of fiber and ash content, which ranged from 3.129% to 3.769%, respectively form 2.350% to 2.910%, compared to 2.324% and 1.318% in BC, which can be considered products with high potential, being important sources of fiber and mineral substances [6, 7]. However, the carbohydrate content is significantly lower in composite flour bread. The energy value calculated from the lipid, protein and carbohydrate intake provided by the constituent raw materials varies in the following order CB> B10MBFC > B10MBFA >B20MBFC> B20MBFA> B30MBFC> B30MBFA. Increasing the percentage of BMF in the samples decreased the energy value from 248.185 kcal/100 g in CB to 228.995 kcal/100 g in B30MBFA. Thus, the use of BMF in the bread formulations allowed obtaining a less caloric product. The results obtained from this study are similar to those obtained by Vasyukova *et al.* (2021) [13], Lukinac *et al.* (2022) [1], and Aslam *et al.* (2023) [6] which noted that as the level of BMF substitution increased, all compounds increased.

## Physical properties of bread samples

After baking, the volume, porosity, elasticity, and height/diameter ratio (H/D) were determined for all samples according to SR 91:2007 [10]. The experimental results obtained are shown in *Table 3*. The results of the evaluation of physical characteristics of the bread formulas indicate that there were significant differences ($p$<0.05) between the control sample (CB) and samples with BMF. The CB registered the lowest values regarding volume (400 cm3/100 g), porosity (65.250 %), elasticity (63.183%) and H/D ratio (0.459) compared to the samples with BMF, which registered high values for these parameters. In the case of bread samples with added BMF, the volume ranged from 406 $cm^3$/100 g in sample B10MBFA to 411 $cm^3$/100 g in sample B30MBFC, the porosity

ranged from 68.253% in sample B10MBFA to 78.829% for sample B30MBFC, elasticity varied from 68.153% for sample B10MBFA to 78.629% for sample B30MBFC3 and H/D ratio varied from 0.482 for sample B10MBFA to 0.497 for sample B30MBFC. The increase in the values of the physical characteristics of the bread formulas could be due to the activity of amylolytic enzymes that favour the retention of a large amount of gas in the dough, causing a decrease in the viscosity of the dough and increase the volume of the product. The results are comparable with other studies [4, 8, 14], according to which, the physical properties of bread samples with added BMF increased proportionally with the ratio of WF to BMF.

**Table 3. Bread samples quality indicators**

| Bread samples | Bread quality indicators | | | |
|---|---|---|---|---|
| | Volume ($cm^3$/100 g) | Porosity (%) | Elasticity (%) | Ratio between high and diameter (H/D) |
| CB | 400±0.037 $^{g}$ | 65.250±0.136 $^{g}$ | 63.183±0.071 $^{g}$ | 0.459±0.015 $^{b}$ |
| B10MBFA | 406±0.034 $^{f}$ | 68.253±0.132 $^{f}$ | 68.153±0.055 $^{f}$ | 0.482±0.010 $^{a}$ |
| B20MBFA | 408±0.050 $^{d}$ | 72.389±0.140 $^{d}$ | 70.323±0.047 $^{d}$ | 0.487±0.015 $^{a}$ |
| B30MBFA | 410±0.047 $^{b}$ | 76.429±0.116 $^{b}$ | 72.283±0.029 $^{b}$ | 0.495±0.012 $^{a}$ |
| B10MBFC | 407±0.019 $^{e}$ | 70.434±0.105 $^{e}$ | 69.367±0.031 $^{e}$ | 0.485±0.010 $^{a}$ |
| B20MBFC | 409±0.012 $^{c}$ | 74.539±0.151 $^{c}$ | 71.406±0.076 $^{c}$ | 0.494±0.015 $^{a}$ |
| B30MBFC | 411±0.012 $^{a}$ | 78.829±0.115 $^{a}$ | 73.629±0.058 $^{a}$ | 0.497±0.010 $^{a}$ |

a-g different letters between samples signify statistical differences according to One-way ANOVA test. The results represent the mean of three independent determinations ± standard deviation (SD). Data with different superscripts in a column are statistically different (one-way ANOVA, $p < 0.05$).

## Phytochemical profile of bread samples

Phytochemical profiles of bread samples (TPC and AA) are presented in the *Table 4*.

**Table 4. Phytochemical profile of bread samples**

| Bread samples | Phytochemical Parameters | |
|---|---|---|
| | Total phenolic compounds (mg GAE/g ) | Antioxidant activity (mg TE/g) |
| CB | 10.470±0.091 $^{c}$ | 75.217±0.092 $^{g}$ |
| B10MBFA | 11.403±0.032 $^{b}$ | 81.187±0.035 $^{f}$ |
| B20MBFA | 11.875±0.096 $^{a}$ | 87.356±0.087 $^{d}$ |
| B30MBFA | 12.042±0.045 $^{a}$ | 92.241±0.097 $^{b}$ |
| B10MBFC | 11.437±0.069 $^{b}$ | 82.187±0.035 $^{e}$ |
| B20MBFC | 11.942±0.084 $^{a}$ | 87.923±0.068 $^{c}$ |
| B30MBFC | 12.081±0.082 $^{a}$ | 92.444±0.055 $^{a}$ |

a-g different letters between samples signify statistical differences according to One-way ANOVA test. The results represent the mean of three independent determinations ± standard deviation (SD). Data with different superscripts in a column are statistically different (one-way ANOVA, $p < 0.05$).

The obtained results shown that the content of TPC and AA increased with the level of BMF added in the bread samples. It is also worth noting that the TPC content in BMF-added bread samples is higher than that in CB. Thus, in the case of bread samples the highest TPC content was found in B30MBFC (12.081 mg GAE/g), while the lowest value was recorded for the CB control sample (10.470 mg GAE/g). The same trend was maintained for antioxidant activity (AA), thus, in the case of bread samples, the highest AA was recorded for B30MBFC (92.444 mg TE/g), while the lowest value was recorded for the control sample CB (75.217 mg TE/g) [11, 15]. These results are consistent with those obtained by El-Taib *et al.* (2018) [3] who reported a TPC content of 12.58 mg GAE/g and an AA of 97.34 mg TE/g in barley flour.

**Sensory evaluation of bread samples**

Sensory scores of bread samples supplemented with different levels of BMF are presented in *Table 5*.

**Table 5. Quality attributes scored in sensory assessment of bread samples**

| Bread samples | Sensory evaluation | | | | |
|---|---|---|---|---|---|
| | Appearance | Taste | Flavor | Texture | Overall acceptability |
| CB | 4.094±0.029 $^{d}$ | 4.071±0.065 $^{e}$ | 4.038±0.022 $^{c}$ | 4.301±0.139 $^{a}$ | 4.101±0.019 $^{d}$ |
| B10MBFA | 4.153±0.035 $^{d}$ | 4.220±0.077 $^{d}$ | 4.053±0.080 $^{c}$ | 4.220±0.118 $^{a}$ | 4.160±0.035 $^{c}$ |
| B20MBFA | 4.289±0.047 $^{c}$ | 4.356±0.087 $^{c}$ | 4.189±0.068 $^{a}$ | 4.169±0.165 $^{a}$ | 4.196±0.026 $^{b}$ |
| B30MBFA | 4.463±0.034 $^{b}$ | 4.529±0.057 $^{b}$ | 4.329±0.064 $^{a}$ | 4.146±0.105 $^{a}$ | 4.263±0.020 $^{a}$ |
| B10MBFC | 4.267±0.031 $^{c}$ | 4.367±0.031 $^{c}$ | 4.167±0.072 $^{b}$ | 4.214±0.143 $^{a}$ | 4.167±0.043 $^{c}$ |
| B20MBFC | 4.339±0.035 $^{c}$ | 4.606±0.044 $^{b}$ | 4.273±0.088 $^{a}$ | 4.196±0.101 $^{a}$ | 4.239±0.040 $^{a}$ |
| B30MBFC | 4.562±0.031 $^{a}$ | 4.829±0.063 $^{a}$ | 4.367±0.046 $^{a}$ | 4.163±0.121 $^{a}$ | 4.292±0.025 $^{a}$ |

a-e different letters between samples signify statistical differences according to One-way ANOVA test. The results represent the mean of three independent determinations ± standard deviation (SD). Data with different superscripts in a column are statistically different (one-way ANOVA, $p < 0.05$).

The sensory evaluation of bread samples for appearance shows that the CB sample was rated the lowest (4.094), followed by the B10MBFA sample (4.153) while the B30MBFC sample was rated the highest (4.562). Variations in scores were also recorded for the flavour of bread samples, with scores increasing proportionally with the percentage of substitution of WF with BMF. Variations in scores were also recorded for the flavour of bread samples, with scores increasing proportionally with the percentage of substitution of WF with BMF. Thus, the scores recorded for taste and flavour varied from CB (4.071 respectively 4.038) to 4.829 respectively 4.367 in B30MBFC. The texture of the bread samples with added BMF was well accepted by the panel members, as sample B10MBFA received a score of 4.220 and sample B30MBFC received a score of 4.163 compared to 4.301 in CB. The same increasing trend proportional to the increasing substitution rate of WF with BMF was also recorded for the overall acceptance scores for bread formulas compared to the CB sample, as sample B30MBFC received a score of 4.292 compared to 4.101 in CB. In earlier studies [3, 4, 6] observed similar increase in the sensory attributes of the bread samples when WF was substituted with increasing levels of MBF.

## CONCLUSION

The results obtained from this study show that the use of BMF (10%, 20%, 30%) in bread production results in a flour product with improved functionality and high nutritional value. Sensory evaluation of WF and BMF-based bread shows that the use of up to 30% BMFA or BMFC in the recipe, results in increased consumer acceptance. Increasing the BMF content in bread composition results in a decrease in carbohydrates and total energy value. Bread samples with the addition of BMF feature higher values of physical parameters, such as volume, porosity, elasticity, and high specific volume to diameter ratio compared to CB. The content of phenolic compounds as well as the antioxidant activity of the bread increased considerably. The overall nutritional, physical, phytochemical and sensory evaluation of the obtained bread showed that BMF can represent a valid vegetal source in baking for a sustainable economy.

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# RECOGNISING DAIRY COW'S BEHAVIOUR WITH LSTM MODEL TO IMPROVE FARM MANAGEMENT PRACTICES

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## ABSTRACT

This paper focuses on recognizing the activity of dairy cows using a non-invasive approach that monitors four key behaviors: licking, feeding, standing, and lying. The study used IoT devices with accelerometers and gyroscopes attached to the cow's neck to continuously monitor its movements. The data collection process aimed to capture the dynamic and static nature of dairy cow behaviors, providing a valuable data set for subsequent analysis. To efficiently process the raw data, we analyzed it and then used long short-term memory (LSTM) neural networks, a type of recurrent neural network (RNN) suitable for sequential data processing. The LSTM model was trained on the collected sensor data to recognize and classify the four target activities. The model achieved an accuracy of 96%, indicating its robust ability to accurately identify dairy cow activity. Furthermore, the model consistently maintained a low loss value hovering around 0.25, demonstrating its generalization and predictive performance. This research has important implications for dairy production and animal welfare. Accurate real-time recognition of dairy cow activities can help improve farm management practices, enabling timely interventions when needed.

**Keywords:** farm management, IoT (Internet of things), cow's behavior recognition, LSTM (long short-term memory) neural networks, accelerometer and gyroscope data

## INTRODUCTION

The aim of non-invasive monitoring of cow behavior is to make farm practices more sustainable and efficient. The concept of Internet of Living Things applies digital technologies to every segment of the food-production supply chain to improve environmental and economic sustainability [1]. IoT technology [2] gives an ability to measure and manage herd behavior and welfare using sensor technology already available on many farms. The development of new IoT-based approaches, along with integrations with other operational tools, can bring a new level of efficiency [3] to the livestock process. On the other hand, cows are living creatures that interact with their environment [4]. This interaction has influence on milk production and its quality. It is well known that one can only manage what one measures. IoT-based, non-invasive monitoring of cow behavior aims to help farmers maintain sustainable practices [5] and produce the best possible products — and maintaining the humane treatment of cows.

Let's look at the most important aspects of observing cow behavior:

**Animal welfare**: Monitoring dairy cow activity helps ensure animal welfare. By tracking their behavior, farmers can identify signs of health problems at an early stage. For example, changes in activity patterns that may signal illness, lameness or discomfort are addressed in [6], allowing for prompt veterinary care and intervention.

**Reproductive health**: Accurate activity data can provide insight into the reproductive health of dairy cows. Cows tend to be more active when the cow ready for insemination [7], which is a crucial indicator of successful reproduction. Recognizing the patterns can help optimize the overall reproductive performance of the herd.

**Nutritional management**: Tracking eating patterns is important to optimize nutritional management. Understanding when, how much and for what duration they eat can help farmers adjust their feeding strategies [8] to ensure cows are getting the right amount of nutrients for optimal milk production and overall health.

**Disease detection**: Changes in cow activity can be indicative of various diseases or conditions, such as mastitis, which is a common infection of the udder. Early detection by monitoring activity can prevent the spread of disease in the herd and reduce treatment costs.

**Productivity and efficiency**: Monitoring cow activity can contribute to increasing productivity and efficiency on the farm. It helps farmers identify and address factors that can affect milk production, such as stress, environmental conditions or feeding schedules, when it's time to move cows into new groups.

**Activity management**: can help determine the ideal time for cleaning, milking and other farm tasks, resulting in more efficient farm operations.

**Data-driven decision making**: This includes adjusting herd management practices, optimizing rearing and feeding schedules, and implementing preventative measures based on historical activity patterns.

This study aims to develop a long short-term memory (LSTM) neural network model to classify caw behaviour patterns. The model uses data from already mounted IoT devices on the cow's neck. IoT devices contain accelerometers and gyroscopes along with RFID tags for animal identification. The collected data are used for LSTM training on the TensorFlow framework. The classification performance of the model was evaluated.

## MATERIALS & METHODS

To monitor the behavior of the cows, a preliminary analysis and preprocessing of the data described in [9] was performed. Based on the collected accelerometer and gyroscope data from IoT devices attached to the cows' necks, new feature extraction was performed. These features are body and gravity components, Jerk signal, signal magnitude, and statistical measures that include minimum, maximum, and mean average values.

The calculations are created for each X, Y, and Z. The total number of variables is 85 of type *float64* and one feature of type *object* that serves as a target and contains records for four key behaviors: licking, feeding, standing, and lying.

Long short-term memory (LSTM) neural network [10, 11] is used to precisely classify the behavior of the cow. LSTM is a type of Recurrent Neural Network (RNN) and is designed to retain information over long periods of time. Each LSTM unit consists of

four main components that interact with each other: a forget gate, an input gate, a cell state, and an output gate. All of them are discussed in detail as follows:

***Forget gate*** - It plays an important role in managing information within an LSTM cell by deciding how much of the previous cell state to keep or discard. It is calculated according to the following formula:

$$f_t=\sigma(W_f\cdot[h_{t-1},x_t]+b_f) \quad (1)$$

where

$f_t$ is the activation of the forgetting loophole,

$\sigma$ is the sigmoid function,

$W_f$ are the weights of the forgetting loophole,

$h_{t-1}$ is the previous hidden state, $x_t$ is the current input,

$b_f$ is the displacement of the forgetting loophole.

***Input Gate*** - The main function of the input gate is to control how much of the newly received information should be added to the cell state. This allows the LSTM cell to update its state with new, relevant information.

Activation formula:

$$i_t=\sigma(W_i\cdot[h_{t-1},x_t]+b_i) \quad (2)$$

Generating candidate information:

$$\tilde{C}_t=tanh(W_C\cdot[h_{t-1},x_t]+b_C) \quad (3)$$

where

$i_t$ is the gateway activation,

$\tilde{C}_t$ is the cell state update candidate,

$W_i$ and $W_c$ are the weights,

$B_i$ and $b_c$ are the displacements.

***Cell Status Update*** - is a key mechanism that allows the network to retain information for long periods of time. It carries information throughout the sequence and is updated at each step of sequence processing.

$$C_t=f_t*C_{t-1}+i_t*\tilde{C}_t \quad (4)$$

where $C_t$ is the new cell state, $C_{t-1}$ the previous state.

***Output Gate*** – the primary function that regulates the flow of information from the LSTM cell to the next layer or the next timestep in the sequence. It filters the information from the cell state, deciding what is relevant to be passed on.

Sigmoid function for the gate:

$$O_t = \sigma\,(W_O\,[h_{t-1}\,,\,x_t] + b_O) \quad (5)$$

Final output of the LSTM cell:

$$h_t = O_t\ tanh(C_t) \quad (6)$$

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where

$O_t$ is the output of sigmoid function,

$W_O$ are the weights associated with the output gate,

$h_{t-1}$ is the previous hidden state,

$x_t$ is the current input,

$b_O$ is the bias term,

$C_t$ is the current cell state.

LSTM neural network training is done using the TensorFlow framework.

**RESULTS**

Our approach is to build a model that contains two fully connected and two LSTM layers with 64 units each. The number of classes is four.

We define a function *create_LSTM_model(input)* to build the model. It takes inputs as its parameter, which will be the data fed into the network.

**1. Building the model**

*Initializing weights and biases*

Initialize two sets of weights W[‘hidden’] and biases[‘output’] using random values. The weights and biases will be learned during the training process. W[‘hidden’] connects the input features to the hidden layer, while W[‘output’] connects the hidden layer to the output layer. Biases help the model to adjust regardless of the input data.

*Inputs preparation*

The inputs are transposed and reshaped to fit the requirements of the LSTM network. This is necessary for the network to process the data correctly.

*Hidden layer*

The hidden layer is created by applying a ReLU (Rectified Linear Unit) activation function to the linear transformation of the inputs. This adds non-linearity to the model, allowing it to learn more complex patterns.

Two LSTM layers are then constructed using *tf.contrib.rnn.BasicLSTMCell*, each with the specified number of hidden units. These layers are then combined into a multi-layer RNN using *tf.contrib.rnn.MultiRNNCell*.

*Inputs processing*

The LSTM layers process the input sequence (hidden) through *tf.contrib.rnn.static_rnn*. This function unrolls the LSTM cells and feeds the input through them, producing a sequence of outputs.

*Output extraction*

The last output of the LSTM sequence (outputs [-1]) is taken as the final output.

*Model output*

The final step in the model is a linear transformation of the last LSTM output, applying the output weights and adding the output biases. This produces the final output of the model, which corresponds to the class scores.

*Defining Placeholders*

Placeholders X and Y are defined for inputs and labels, respectively. X holds the input data of shape [None, N_TIME_STEPS, N_FEATURES], and Y holds the output labels of shape [None, N_CLASSES]. The "None" dimension indicates that the batch size can vary.

*LSTM Model creation*

The function *create_LSTM_model(X)* constructs the LSTM model using the input data X. The model's architecture is defined in this function.

*Applying Softmax and Defining Loss Function*

The *softmax* function is applied to the model's output *pred_Y*, converting raw logits to probabilities for classification.

The loss function includes cross-entropy for classification and L2 regularization = 0.0015 (to prevent overfitting), combined into a single loss term.

*Optimizer setups*

An Adam optimizer is used to minimize the loss function. The learning rate is set to 0.0025.

*Performance evaluation*

Two key metrics are used to evaluate performance: accuracy and loss. These metrics provide insights into how well the model is performing and are crucial for monitoring the training process and making decisions about model adjustments. Accuracy measures the proportion of correct predictions made by the model. It is calculated by comparing the predicted output of the model (e.g., the class with the highest probability) with the actual target values. Loss is a measure of how far the model's predictions are from the actual target values. The loss function takes the model's predictions and the actual target values as input and calculates a numerical value representing the error.

**2. Training the model**

The model is trained for several epochs with a batch size of 1024. In each epoch, the training set is divided into batches, and the optimizer is run on each batch.

After processing all batches, the training and test accuracy and loss are calculated and stored in history. Periodically (every 10 epochs), the training progress (accuracy and loss on test data) is printed.

**3. Evaluation**

After training, the final accuracy and loss on the test dataset are calculated and printed. A *tf.train.Saver()* is created for saving and retrieving variables of the TensorFlow model. Furthermore, an interactive TensorFlow session is initiated and used for executing the training and evaluation operations. Based on the obtained epoch results

(Figure 1) from the training of the LSTM neural network model, we observe the following:




Figure 1: Training progress, the x-axis represents the training epoch, y-axis represents accuracy and loss values on the training and testing dataset.

- Epoch 1: test accuracy: 77.41%, loss: 1.31.

At the first epoch, the model shows moderate accuracy and a relatively high loss. This is expected as the model is just beginning to learn from the data.

- Epoch 10: test accuracy: 92.32%, loss: 0.585.

By the 10th epoch, there's a significant improvement in accuracy and a substantial reduction in loss. This rapid improvement suggests that the model's architecture and hyperparameters are well-suited to the task.

- Epoch 20: test accuracy: 95.1%, loss: 0.399.

The model continues to improve, showing that it is still learning effectively from the training data. The diminishing returns in accuracy gains are typical as the model starts to converge.

- Epoch 30: test accuracy: 95.5%, loss: 0.32.

By this epoch, the model has achieved high accuracy, and the loss has decreased further. This shows a good generalization of the test data.

- Epoch 40: test accuracy: 96.4%, loss: 0.250.

The model shows a high accuracy of over 96% and a low loss of 0.250 from 200 time-step sequences, indicating strong learning and predictive capabilities.

These results demonstrate successful training of the LSTM model by following the described approach. The consistent improvement in test accuracy and reduction in loss are indicative of effective learning [12]. However, it is also important to monitor for

overfitting [13]. If the model performs significantly better on the training data compared to the test data, it might be overfitting.

The presented LSTM neural network lays a solid foundation for the next crucial steps. The next phase is to apply the model to actual data to recognize cow's behavior. This phase will test the robustness of the model and its ability to generalize from training data to real-world scenarios. It is important to keep in mind that real-world data can be more variable and less predictable [14] than the data used during training. Therefore, careful monitoring of the model's performance in this new context is essential to ensure its effectiveness and reliability.

## CONCLUSION

Tracking cow activity increases productivity and efficiency, enables resource allocation, contributes to sustainability by reducing environmental impact, supports data-driven decision-making, and helps farmers meet regulatory standards, ultimately encouraging responsible and efficient dairy farming practices. Early detection of non-normal behavior patterns through the application of automated technology can have a positive impact on farm management.

Recognition of cows' behavior is usually done using an accelerometer and gyroscope sensors. Thanks to the widespread availability of these sensors at a low cost, it allows their widespread use in various fields such as in animal husbandry, which contributes to the collection of a huge amount of data.

The construction, training and evaluation steps in building the proposed model are described in detail. Achieving an over 96% accuracy rate alongside a minimal loss rate of 0.25, the model demonstrates substantial robustness in its current form. However, there is potential for further refinement. By broadening the scope of behavioral patterns analyzed and prolonging the period of continuous data collection on cow behavior, we anticipate a higher degree of accuracy. Looking ahead, we aim to augment the model's capabilities by integrating semantic analysis derived from video observations of cow behavior.

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# ROSEHIP CHUTNEY - A VALUABLE SOURCE OF ANTIOXIDANTS

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## ABSTRACT

The new tendency of consumers and processors in the food industry is to use organic products. Because intensive crops involve the use of pesticides and fertilizers, a bio alternative to tomatoes can be the rosehip from spontaneous flora. In this study, four variants (control, sweet, spicy and hot) of rosehip chutney were prepared as an alternative for tomato ketchup. The sensory characteristics were evaluated (by hedonic scale). The vitamin C content (by titrimetric iodometric method), polyphenols content (by Folin Ciocalteu assay) and free radical inhibition activity (by DPPH assay) were determined. Our results did not show significant differences in vitamin C content between the analyzed variants (81.20±0.67 -81.80±0.34 mg/100 g). The highest polyphenols content was detected in the "hot" chutney version (56.90±3.11 mg GAE/100g DW) in direct correlation with DPPH free radical inhibition activity (91.61±0.22%). The degree of the rosehip chutney acceptability and its chemical composition confirm that rosehip chutney can be successfully used to replace the tomato ketchup.

**Keywords:** rosehip, chutney, vitamin C, polyphenols, antioxidant activity.

## INTRODUCTION

The benefits of tomato consumption are well-known (Collins et al., 2022)[2], also notable for their pleasant, specific taste and aroma. Tomatoes are the most frequently consumed vegetables, both raw and cooked/preserved. Intensive crops, the prevention and control of diseases and pests in tomato crops and the desire to obtain high yields involve, among other things, the use of pesticides and fertilizers, which remain in the fruits offered for consumption and/or processing with consequences on consumer health. Since the organic alternatives of pesticides and fertilizers are still few, and the management of the

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application of the classical variants is difficult to control, in these conditions, healthy alternatives for tomatoes with similar benefits are sought. In this context, from the point of view of chemical composition, rose hips can successfully substitute tomatoes in ketchup / chutney preparations, the great advantage of rose hips being that they can grow spontaneously, without the need for chemical treatments such as pesticides or fertilizers.

Rose hips (*Rosa* spp.) are notable for their high content of antioxidant compounds. The content of polyphenols, essential fatty acids, vitamins A, C and E, minerals such as Ca, Mg, K, S, Si, Se, Mn and Fe (Türkben *et al.*, 2010; Patel, 2017; Medveckienė *et al.*, 2021)[14,8,6], present in rose hips, are components with an important role in reducing the risk of cardiovascular diseases (Medveckienė *et al.*, 2021)[8,6], osteoarthritis and cancer (Patel, 2017; Medveckienė *et al.*, 2021)[8,6]. Also, rose hips are recognized for their effectiveness in strengthening the immune system, for their anti-inflammatory, anti-diabetic, neuroprotective properties, against obesity, kidney stones and kidney problems (Patel, 2017)[8]. Just as tomatoes can be processed into juice, broth, paste, ketchup and rosehips can be processed into tea, juice, syrup, jam, marmalade, chutney. Also, rose hips are noted for having the highest vitamin C content of any other commonly available fruit or vegetable, so the vitamin C content of rose hips (Nojavan *et al.*, 2008; Türkben *et al.*, 2010; Medveckienė *et al*., 2021)[7,14,6] far exceeds that of tomato (Tan *et al.*, 2021)[11]. Also, the lycopene content in rose hips (Türkben *et al.,* 2010)[14] is comparable to that found in tomatoes (Tan *et al.*, 2021; Medveckienė *et al.*, 2021)[11,6]. Phenolic compounds such as quercetin, ellagic acid, quercetin-glycosides, hydroxycinnamic acids, proanthocyanidin, aglycones, flavonoids, catechin have been detected in abundance in rose hips (Ghendov-Mosanu *et al.*, 2020; Medveckienė *et al.*, 2021; Taneva *et al.*, 2016)[5,6,12]. The presence of antioxidant compounds in rose hips also causes remarkable antioxidant activity (Georgieva and Boyadzhieva, 2014)[4].

## MATERIALS AND METHODS

Just like tomato ketchup, chutney can be made sweeter or spicier. In this work, the content of vitamin C, the content of total polyphenols, the antioxidant activity of 3 varieties of chutney were monitored and the sensory characteristics were evaluated.

*Making rosehip chutney*

Rose hips from the western part of Romania were used to prepare the chutney. They were processed by boiling, mashed and deseeded to obtain an aqueous extract. The other ingredients were then added: chopped onion, olive oil, lemon juice, erythritol, grated ginger, minced garlic, salt, coriander, white pepper and chili according to Table 1. Both the control variant and the three spicy variants they were boiled for about 45 minutes on low heat, after which they were placed in glass jars and hermetically closed with lids.

**Table 1** Recipes used to obtain the rosehip chutney

| Raw and auxiliary materials (%) | Sweet | Spicy | Hot |
|---|---|---|---|
| Rosehip extract | 78.964 | 78.939 | 78.914 |
| Onion | 8.154 | 8.151 | 8.149 |
| Olive oil | 3.262 | 3.261 | 3.259 |
| Lemon juice | 3.262 | 3.261 | 3.259 |

| | | | |
|---|---|---|---|
| Erytritol | 3.262 | 3.261 | 3.259 |
| Ginger | 1.631 | 1.630 | 1.630 |
| Garlic | 0.815 | 0.815 | 0.815 |
| Salt | 1.305 | 1.304 | 1.304 |
| Coriander | 0.033 | 0.033 | 0.033 |
| White peper | - | 0.033 | 0.033 |
| Chili | - | - | 0.033 |

*Assessment of vitamin C content*

The vitamin C content from our samples was determined by iodometric method in acording to the work protocol presented by Dumbrava *et al.* (2016)[3], the results being expressed in mg vitamin C/100 g. Assay was performed in triplicate.

*Assessment of total polyphenols content (TPC)*

To evaluation of TPC from rosehip chutney, we used the spectrophometric Folin-Ciocalteu assay presented by Dumbrava *et al*. (2016)[3]. Assay was performed in triplicate.

*Assessment of antioxidant activity*

In order to evaluate antioxidant activity of rosehip chutney we used the DPPH free radical scavenging activity (RSA) assay. They were prepared alchoolic extracts 1:10 of each sample using 70% (v:v) ethanol by keeping 2 hours in room temperature under continuous stirring. After that, these extracts were filtered through Whatman No 1 filter paper. A volume of 1 ml of each extract was put in a test tube and then added 2.5 ml of 0,1 mM DPPH solution in 70% (v:v) ethanol. After homogenized these mixtures were left in dark condition at room temperature for 30 minutes and then read its absorbance at 517nm against 70% ethanol as a reference. Assay was performed in triplicate. The DPPH RSA was calculated using the following equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \cdot 100$$

*Statistical analysis*

Microsoft Excel 2010 was used for statistical data processing. The results were expressed as mean values ± standard deviation (SD).

*Sensory evaluation*

To sensorial evaluation we used hedonic scale from 1 to 5 points. The sensory characteristics were evaluated by a panel of 18 people who rated: external appearance, color, consistency, smell and taste.

## RESULTS AND DISCUSSIONS

*Vitamin C content*

The results about vitamin C content of rosehip chutney is presented in figure 1. Our results show that the vitamin C content was in the range of 81.2±0.71 and 81.8±0.52 mg vitamin C / 100 g sample, the values being very close in all the chutney samples analyzed.

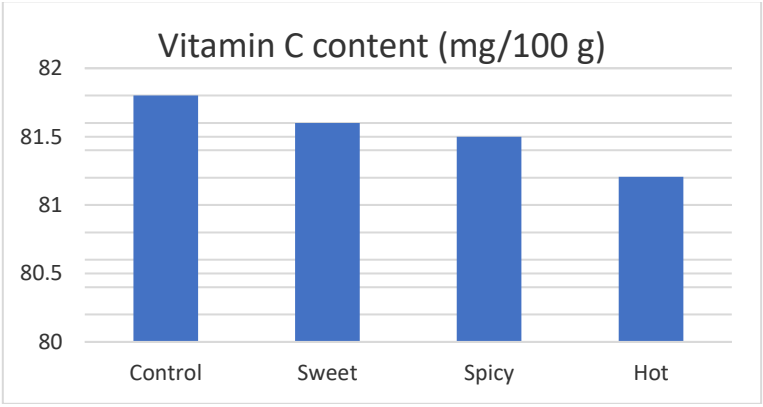



**Figure 1. – Vitamin C content of rosehip chutney samples**

Studies in the literature show that fresh rosehip fruits have a vitamin C content between 125-2650 mg/100 g (Medveckienė *et al.*, 2020)[6], respectively in mature fruits being reported values of 417 mg/100 g (Nojavan, *et al.*, 2008)[7], higher values than those reported for tomatoes 4.15 mg/g DW (Tan *et al.*, 2021)[11]. Although a good amount of vitamin C is lost through heat treatment, the values we found are three times higher than those reported by Türkben *et al.*, (2010)[14] in rosehip marmalade (which undergoes heat treatment similar to chutney) (26.71 mg / 100 g), respectively by Yildiz and Alpaslan, (2012)[15] (25.05 mg / 100 g), but they used another method of determination of vitamin C.

*Total polyphenols content*

Our results regarding the total polyphenols content of the rosehip chutney are presented in Figure 2. In acccording to fig. 2, the highest TPC was recorded in the hot chutney version (56.9 ± 0.18 mg GAE / g) which was 11.9% higher than in the sweet chutney version and 4.01% higher than the spicy chutney version. Compared to the base for chutney (control), the TPC content was higher in all chutney variants analyzed. This fact is due to the ingredients added in the chutney variants compared to the base.

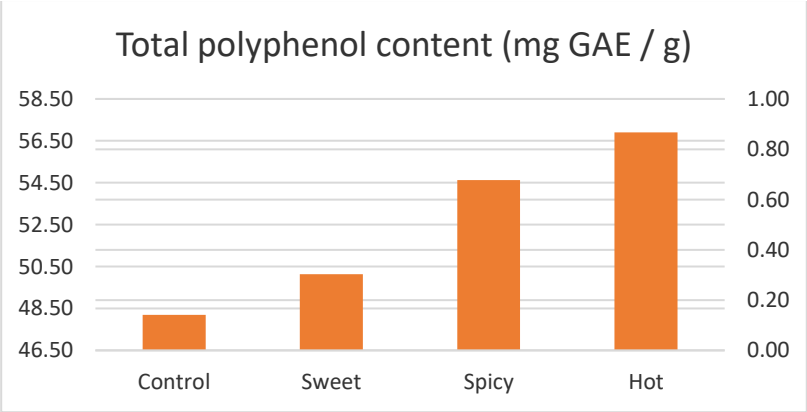



**Figure 2. – Total polyphenols content of rosehip chutney products**

Similar results were obtained by Begic-Akagic *et al.*, (2013)[1] in rosehip pekmez (a jam-like) – 31.57-63.15 mg GAE / g pekmez. Our results were lower than Begic-Akagic

results reported, but were higher than other literature date. Depending on the method used to determine the TPC, literature data show that the TPC for rosehip marmalade was between 6.45 -9.12 mg GAE / g FW marmalade (Yildiz *et al.*, 2012)[15], or 21.14-38.74 mg GAE / g DW marmalade (Sagdic *et al.*, 2015)[9]. For rosehip fruits in a fresh state, the researchers detected TPC values of 54.84 mg GAE/g (Ghendov-Mosanu *et al.*, 2020)[5], 31.08–52.94 mg/g GAE FW (Medveckienė *et al.*, 2020)[6]. These values were superior than TPC from tomato (2.93 și 8.35 mg/g, DW - Tan *et al.*, (2021)[11]. The TPC values found in our study are close to those found in fresh rosehip fruits as a result of the contribution of polyphenols brought by the rest of the ingredients used to obtain rosehip chutney.

*Antioxidant activity*

The RSA values of the rosehip chutney are presented in figure 3.

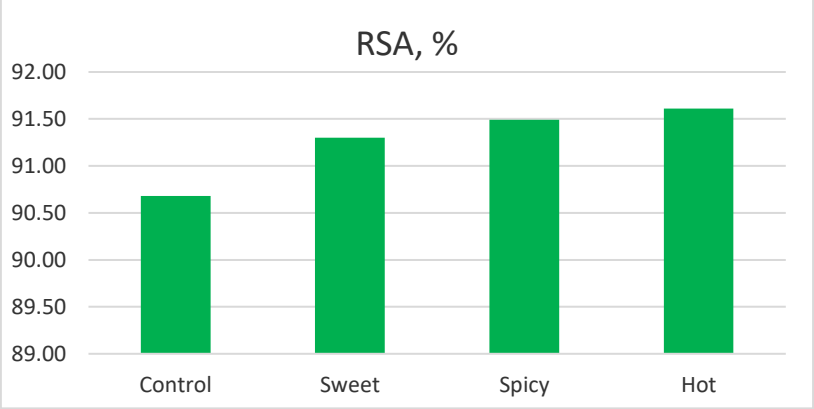



**Figure 2. – Total polyphenols content of rosehip chutney products**

Although thermal treatments were applied to obtain rosehip chutney, their antioxidant activity was still very good, while Sagdic *et al.*, (2015)[9] reported for rosehip marmalade the RSA value of only 49.98%. In our research, both the control sample and the chutney variants had very high RSA values between 90.68±0.34 (control) and 91.61±0.08 (hot chutney variant). For ethanolic extracts (80%) of rosehips fruits, Stoica *et al.*, (2013)[10] found a value of 95.4% for RSA.

The degree of correlation of the three evaluated parameters for the samples analyzed in our study are presented in table 2. It can be observed that there is a direct correlation of TPC with RSA, but the correlation is negative in the case of TPC with vitamin C content, although a negative correlation of vitamin C with RSA was observed. The ingredients added to the rosehip chutney variants caused a slight decrease in vitamin C content, but increases in TPC and RSA in the more complex chutney variants, which is why those negative correlations appear.

**Table 2.** Parameters correlation

| *CORRELATION* | *TPC* | *Vitamin C* | *RSA* |
|---|---|---|---|
| TPC | 1 | | |
| *Vitamin C* | -0.94928 | 1 | |
| RSA | 0.893046 | -0.89244 | 1 |

*Sensory analysis*

All the scores for the evaluated sensory parameters were higher than 4, excepting the taste of the control sample, which accumulated an average of 3.83 points. High scores denote a very good acceptability of the evaluated products. The highest scores regarding appearance, color and smell were assigned by evaluators to the rosehip base (control). For the chutney variants, the appearance and color obtained lower scores than the control (4.22 - 4.61 points). The smell of the chutney variants was appreciated with values of 4.83-4.94, and in the control – 4.89 points. The consistency of the chutney variants far exceeded the score obtained by the control. Regarding to taste, hot chutney variant was less appreciated than the spicy or sweet version. However, the taste of the chutney variants (4.44 - 4.94 points) was clearly superior to that of the control version (3.83 points).

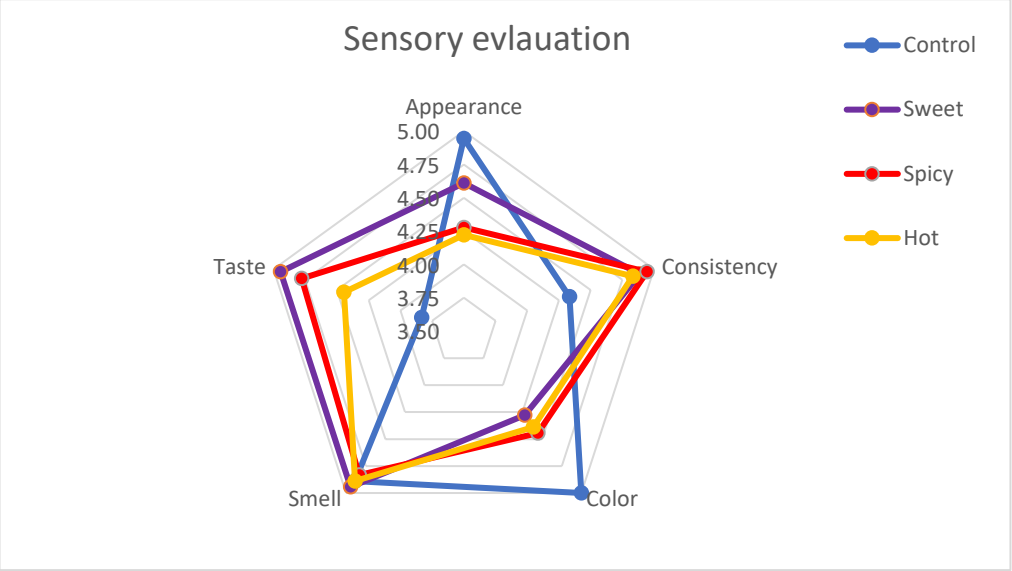



**Figure 4. – Global values of the sensory evaluation of rosehip products**

The degree of acceptability of the chutney variants was higher than the control variant, in the order: sweet, spicy, hot.

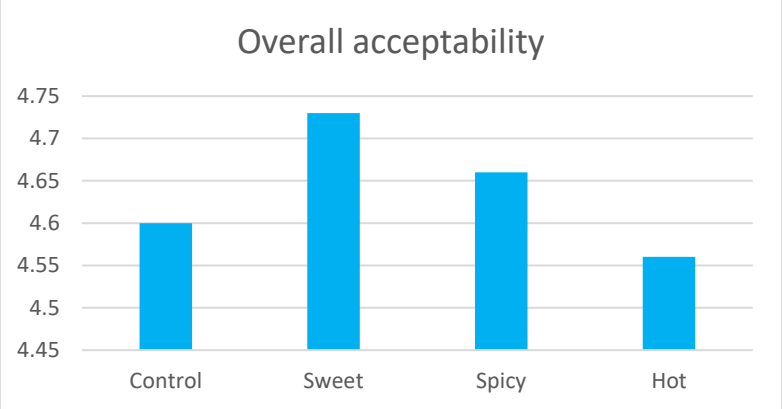



**Figure 5. – Overall acceptability of rosehip products**

## CONCLUSION

Due to the high content of vitamin C and TPC, due to the very good antioxidant activity, rosehip chutney is very valuable from a nutritional point of view. Our results show that the addition of other ingredients besides rosehip enhanced the value of rosehip chutney. The spicy and hot chutney variants showed higher RSA and TPC values than the sweet variant and much higher than the control variant. Regarding the vitamin C content, the control version proved to be the most valuable.

The presence of the other ingredients alongside the rosehip substantially modified the sensory properties of the rosehip chutney. The best degree of acceptability was found in the sweet variant. As the hot taste increased, the degree of acceptability decreased. However, the chutney variants were much better appreciated than the control variant. Due to the sensory characteristics, these products are recommended for consumption as such with cheese, meat products, pasta, pizza, etc.

The advantages of using rosehip in chutney form as an alternative to tomato ketchup are that they are pesticide-free and a cheap source of vitamin C and polyphenols.

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# SIX-MEMBERED HETEROCYCLIC SULFONAMIDES: SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL PROPERTIES

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## ABSTRACT

Sulfonamides are appropriate ligands for the preparation of coordination compounds with biological activity, as they possess bactericidal, antiviral, antidiabetic, and diuretic properties and some sulfonamide compounds are under clinical evaluation for cancer treatment. Metal complexes provide highly versatile platforms for drug design. Considering the biological potential of the quinoline nucleus, but also of the sulfonamide scaffold (especially antimicrobial), as well as that of the quinoline-sulfonamide combination we have decided to combine the two pharmacophore fragments. And at last, the complexation with divalent metals - M2+ (M2+: Zn2+, Cu2+, Co2+ and Cd2+) will bring complementary biological activity given by the cation with the final goal of achieving improved biological activity and better pharmacokinetic properties. The synthesized molecules were characterized by HRMS and NMR spectroscopy. The compounds were preliminary screened for their antibacterial and antifungal activity and the obtained results are very promising.

**Keywords:** hybrid compounds, quinoline, sulfonamide.

## INTRODUCTION

The class of nitrogen heterocycles, especially five and six member ring, represents the most abundant class in nature as a structural unit, these heterocycles also constituting the basic skeleton of many synthetic products, such as: medicines, vitamins, pesticides, dyes, pigments, plastics, cosmetics, etc. [1-6]

Sulfamides (also called sulfanilamides or sulfonamides) are organic compounds that contain a sulfonamide group in their molecule. This class of medicinal substances has the greatest therapeutic success due to its high antimicrobial activity and broad spectrum of action on gram-positive and gram-negative bacteria. Sulfamides were the "wonder drugs" before penicillin and are still used today due to their ability to inhibit the growth of bacteria (bacteriostatic action). [1-3, 10, 11,14]

A special class of quinoline derivatives, which have received special attention from the scientific community, are the quinoline-sulfonamide complexes, studied in particular for their photoluminescent (mostly fluorescent) properties. [7,8] Regarding

the activity biologically, these compounds have been evaluated mainly for their antiprotozoal properties [9] and very little data have been found regarding their antibacterial and antifungal activity. [10]

Complexation of sulfonamide derivatives with various metals can bring about significant changes in the toxicology and pharmacology of the compounds. Metal complexes of sulfonamide derivatives showed better carbonic anhydrase (CA) inhibition properties than the starting ligands. [10] Molecules containing in their structure the sulfonamide unit that inhibits the carbonic anhydrase enzyme have applications in the treatment of epilepsy, glaucoma and other disorders.

Considering the biological potential of the quinoline nucleus, but also of the sulfonamide fragment (especially antimicrobial), [11-14] as well as that of the quinoline-sulfonamide combination (anti-HIV), [11,12] we decided to combine the two pharmacophore units, with complementary biological activity given by the M2+ cation (M2+: Zn2+, Cu2+, Co2+, Cd2+), with the ultimate goal of obtaining improved biological activity and better pharmacokinetic properties.

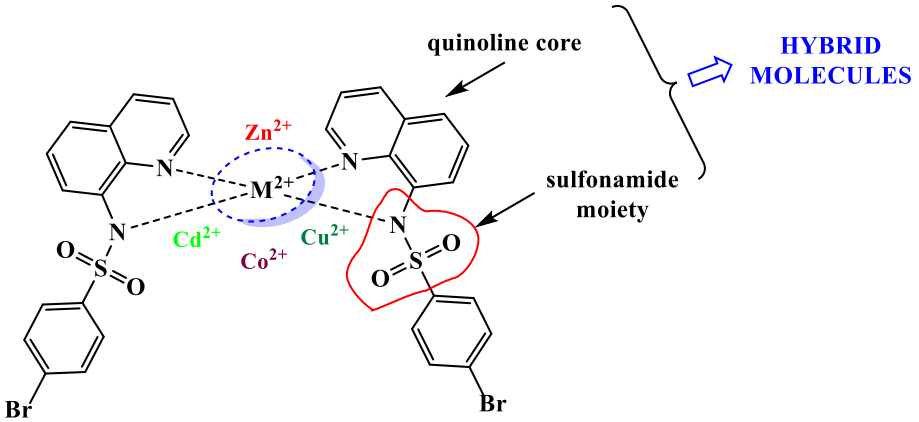



**Figure 1**. Design of the complexes.

## MATERIALS AND METHODS

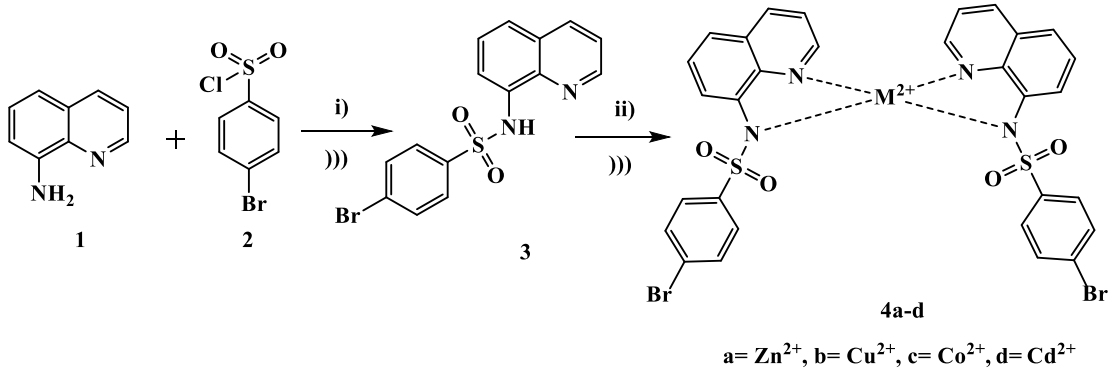
8-aminoquinoline, 4-bromosulfonyl chloride, pyridine and metal salts were purchased from TCI and Sigma Aldrich. All NMR spectra were acquired with a Bruker Avance III 500 MHz spectrometer operating at 500 MHz for $^{1}$H and 125 MHz for $^{13}$C. The program used for acquisition and processing data is TopSpin 3.2 PL5. The mass spectrometry analysis was carried out on a Thermo Scientific Orbitrap Exploris 120 mass spectrometer equipped with a Thermo Scientific OptaMax NG ion source (H-ESI, positive mode).

Ultrasound assisted reactions were accomplished using Sonics (Sonics VCX-130, USA), with a nominal power of 130W and a frequency of 20 kHz. For this ultrasonic reactor the titanium horn (diameter: 6 mm; length: 116 mm) is fixed to the ultrasonic converter. The titanium probe tip was directly immersed in the reaction mixture. Merck silica gel 60 F254 plates were used for thin layer chromatography (TLC) and a UV lamp (λmax = 254 or 365 nm) was used in order to visualize the plates. **Antimicrobial evaluation** *Disk-diffusion method*: The *in vitro* antibacterial and antifungal activity of the ligand (**3**) and its complexes with Zn2+, Cu2+, Co2+ and Cd2+ (**4a-d**) were determined by the Kirby-Bauer disk diffusion method. The method is using Müeller Hinton agar medium for antibacterial assay and Sabouraud nutrient agar

medium for antifungal assay. The *in vitro* antibacterial activity was evaluated against Gram-positive bacteria *Staphylococcus aureus ATCC 25923* and Gram-negative *Escherichia coli ATCC 25922* and the antifungal activity against fungus *Candida albicans ATCC 1023123*. Gentamicin was used as positive control (C+) for *S. aureus* and *E. coli* and Nystatin for *C. albicans*. The negative control (C-) consist of sterile filter paper disks (with no antimicrobial compounds) inoculated with DMSO 3%.

## RESULTS

As in the cases reported in the literature [8,9,11,14] the general way to obtain the compounds involves a simple and efficient two-step procedure. The first step consists in the *N*-acylation reaction of 8-aminoquinoline with *p*-bromobenzenesulfonyl chloride, when the corresponding quinoline-sulfonamide ligand (**3**) is obtained. The second step consists in the complexation of the ligands with various metal ions from acetate (Cu2+, Co2+, Cd2+) or chloride (Zn2+) salts.




**Scheme 1**. Reaction scheme for obtaining hybrid metal quinoline – 4-bromo-benzenesulfonamide complexes. Reaction conditions: i) Py/$CH_2Cl_2$, ii) Metal salt/$CH_3OH$

*a. Synthesis of ligand*

The solution that contains 8-aminoquinoline (3 mmol) and corresponding 4-bromobenzenesulfonyl chloride **2** (3.3 mmol) was placed in a reaction vessel and was exposed under US irradiation. The best results were obtained applying a pulse irradiation (5 s pulse / 5 s pause, 50% from the full power of the generator). After each minute of irradiation TLC was performed. Once the irradiation cycle was completed, the reaction vessel was removed and processed as indicated for CV. The crude product was washed with HCl (1 M), then with a solution of saturated $NaHCO_3$. The organic extract was dried on $Na_2SO_4$ and the solvent was removed in vacuum. The solid obtained was purified using column chromatography ($CH_2Cl_2$/AcOEt=70/30) giving the corresponding sulfonamide ligand **3**.

*b. Synthesis of complexes*

In a tube reaction vessel was placed a methanolic solution (25 mL) of metal salts $ZnCl_2$, $Cu(CH_3COO)_2$ x $H_2O$, or $Co(CH_3COO)_2$ x $6H_2O$ or $Cd(CH_3COO)_2$ x $H_2O$ (0.2 mmol) (and 2 mL $NH_4OH$ for chlorides) and sulfonamide ligand (**3**) (0.4 mmol). The solution mixture was exposed under US irradiation. The best results were obtained by applying a pulse irradiation (5 s pulse / 5 s pause, 100% from the full power of the reactor), with a exposure of 6 to 9 minutes. Once the irradiation cycle was completed, the reaction vessel was removed and the obtained precipitate was separated by filtration.

## DISCUSSION

In order to prove the structure of the obtained compounds we performed spectral analysis. The NMR spectroscopy and HRMS spectrometry confirm the structure of the new molecules. In the $^1$H-NMR spectra the most noteworthy signal is those one who is corresponding to the hydrogen atom from sulfonamide group **H9**, which appear in ligand at a chemical shift of 10.21 ppm while in complexes spectra is absent; this is a solid proof of coordination with the metal ion. We may also noticed in the $^1$H-NMR spectra the slightly shift towards down field values of the signals that are closely to the metal ion, **H2**, **H3**, **H4** (from pyridine ring), as a result of electron density transfer from the ligand to the metal ion. [11, 14]

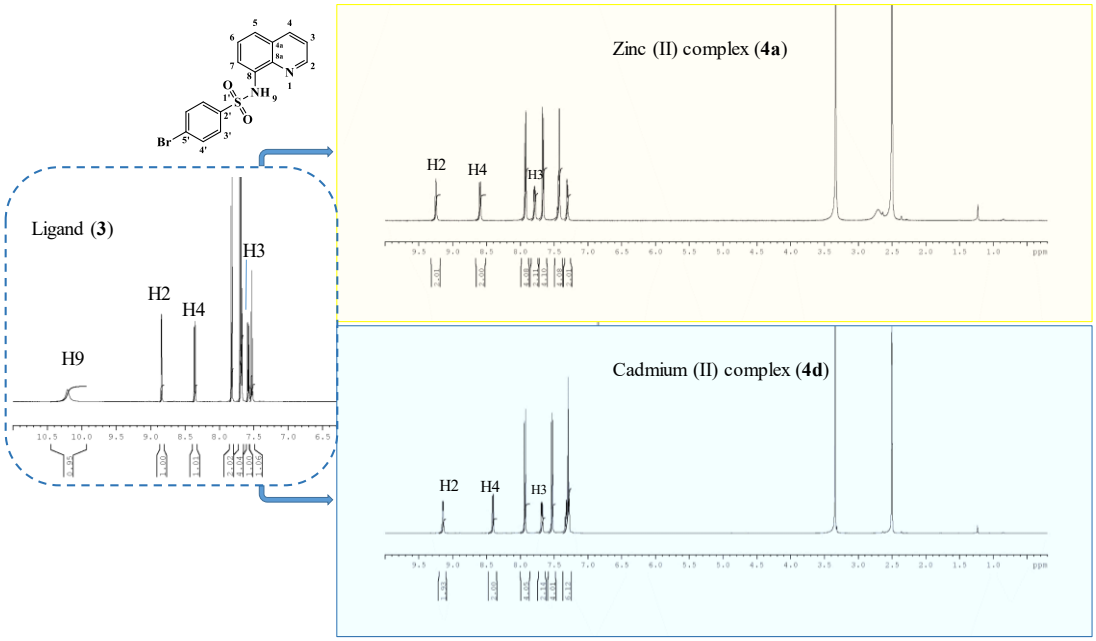



**Figure 2**. $^1$H-NMR spectra of sulfonamide ligand (**3**) and its complexes with Zn2+ (**4a**) and Cd2+(**4d**) in DMSO-*d6*.

**Tabel 1**. Chemical shifts of sulfonamide ligand (**3**) and Zn2+ and Cd2+ complexes (**4a**, respectively **4d**).

| Compound/ Proton | H9 | H2 | H3 | H4 |
|---|---|---|---|---|
| **3** | 10.21 | 8.84 | 7.57 | 8.35 |
| **4a** | - | 9.24 | 7.78 | 8.59 |
| **4d** | - | 9.14 | 7.68 | 8.40 |

On the other hand, the HRMS spectrum for the Zn(II) complex (**4a**) demonstrate the complexation process (*Figure 3*). The $[ZnL_2+Na]^+$ peak evidence the complexation precess. The HRMS spectra of the zinc(II) complex showed a molecular ion peak at an m/z= 808.8484 $\{ZnL_2+Na+]^+\}$ (calculated m/z= 804.8481) that corresponds to the 2:1 complex between sulfonamidic ligand and Zn2+. The isotopic peak pattern observed (*Figure 3-A*) with this peak resembles that of the calculated one (*Figure 3-B*) supporting the presence of Zn2+ in this species, and thus confirms the formation of the complex.

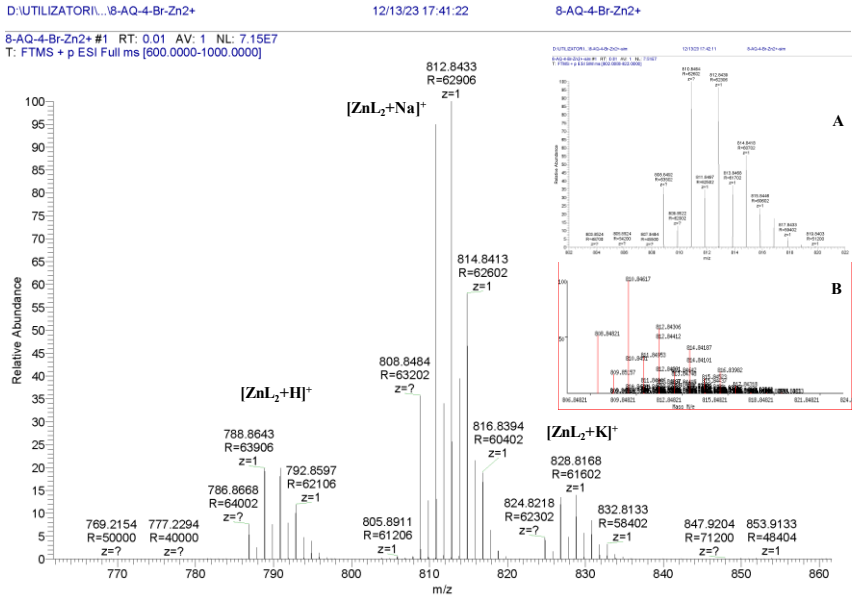
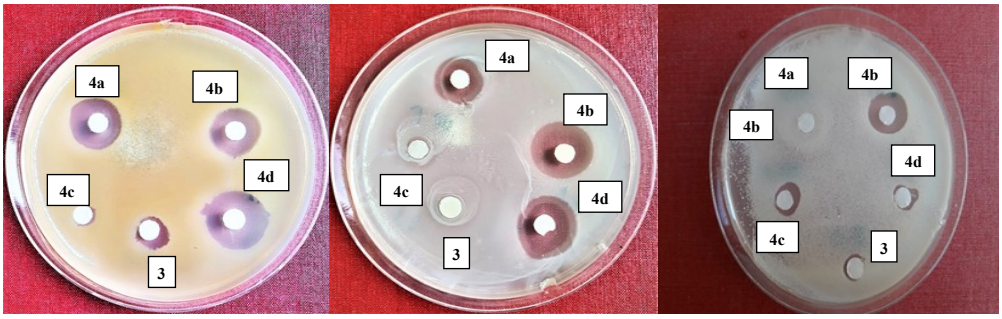



**Figure 3**. The obtained HRMS spectra: molecular ion peak observed in (H)ESI-MS with its isotopic peak pattern for the [Zn(**L**)$_2$] complex (**4a**): A-obtained, B-calculated.




*Staphyloccous aureus* *Escherichia coli* *Candida albicans*

**Figure 4.** The antibacterial and antifungal activity of ligand (**3**) and its complexes with Zn2+ (**4a**), Cu2+ (**4b**), Co2+ (**4c**) and Cd2+ (**4d**).

In *Figure 4* it can be observed that the preliminary screening reveal that copper complex (**4b**) is active against all strains tested (gram-positive bacteria *S.aureus*, garm-negative bacteria *E.coli* and fungus *C. albicans*), zinc(II) complex and cadmium(II) complex (**4d**) are selective and exhibit only antibacterial activity. Sulfonamidic ligand (**3**) and cobalt(II) complex (**4c**) did not show any activity on the three strains evaluated.

## CONCLUSION

In this paper we reported a sulfonamide ligand and four new complexes which were characterized by NMR and HRMS spectroscopy and were evaluated against three strains (gram-positive bacteria *Staphyloccocus aureus*, gram-negative bacteria *Escherichia coli* and fungus *Candida albicans*). The synthesis of the complexes is simple end efficient which involves two steps: *N*-acylation of aminoquinoline and complexation with metal salts (acetates or chloride). The complexes were characterised by NMR and HRMS spectroscopy. Also, the new molecules were screened regarding antibacterial and antifungal activity which reveals that copper(II) complex (**4b**) is active against *S. Aureus*, *E.coli* and fungus *C. Albicans*; zinc(II) complex (**4a**) and

cadmium(**4d**) complex are selective and exhibit only antibacterial activity against *S.aureus* and *E.coli.* The obtained results are very good, highlighting in this way that the complexation with various metal cations brings improvements to the biological activity of the molecules.

## ACKNOWLEDGEMENTS

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# STUDIES REGARDING BIOACTIVE FINGERPRINT OF CAULIFLOWER VERSUS BROCCOLI

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## ABSTRACT

This study aims to determine the specific fingerprints of cauliflower and broccoli available on the Romanian market based on mathematical models and chemical analysis (moisture content, minerals content, total antioxidant capacity and total polyphenols content). These vegetables (cauliflower and broccoli) are considered essential for the human diet due to their bioactive content and nutritional value. The content of minerals and antioxidant compounds varies depending on their varieties, environmental conditions, ripening stage, collecting and storage methods. The studied vegetables are high in essential micronutrients copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), nutrients which are associated with enzymatic activities. Research shows that eating cruciferous vegetables brings many health benefits due to their active sulfur and nitrogen-containing compounds. The obtained results were compared to literature and database data and the graphical fingerprint were created. The generalized linear model was applied for analyzed vegetables, cauliflower and broccoli samples and the correspondence equation were obtained. The Mantel correlogram was used to test the hypothesis that innovative food products and or diets can be created based on *the* nutritional content of the vegetables and the nutritional needs of the consumer.

**Keywords: mathematical model, minerals, antioxidant activity, polyphenols**

## INTRODUCTION

The role of food fingerprints is to offer information about the identity of food products. This type of analysis has gained a lot of importance in recent years due to the importance of obtained information regarding the identity of food products. These applications are usually constructed based on spectroscopic as well as spectrometric data and provide the capability for a all-inclusive description of the considered matrices [1]. The exploration for these fingerprints is not only associated to the quality of food products, but also to the safety of food for human health [2]. Vegetables are considered essential ingredients for the human diet due to their high nutritive value and their bioactive content [3]. Cauliflower and broccoli, together with other cruciferous species are important components of a healthy diet, providing a multitude of health-related micronutrients and phytochemicals [4].

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Broccoli and cauliflower are different plants, having the same species name, *Brassica oleracea* L., are recommended by their high mineral and phytochemical content [5]. The content of cruciferous vegetables minerals and antioxidant compounds varies primarily depending on their growing conditions, the stage of maturity of the vegetables, the stress due to environmental factors, the method of storage after harvesting, as well as variety [4]. Studies have shown that the enrichment of high-quality proteins, minerals, vitamins and antioxidant compounds, especially organic-sulfur phytochemicals and other phytochemicals, significantly improves public health [4]. Crucifers contain a multitude of biologically active molecules as well as nutritional components and are an outstanding source of biologically active sulfur and nitrogen-containing compounds. Research shows that eating cruciferous vegetables brings many health benefits [6]. This study aims to determine the specific fingerprint of cauliflower and broccoli available on the Romanian market based on moisture content, minerals content as well as antioxidant capacity and polyphenols content.

## MATERIAL AND METHODS

All studied samples are of Romanian origin and were available on the Romanian market.

*Preparation of samples for analysis*

The cauliflower and broccoli samples were washed to remove any external contaminants, then cut and homogenized to ensure a representative and uniform sample.

*Analysis of samples*

The moisture content was determinate using thermogravimetric method, the mineral content by atomic absorption spectrometry using ContrAA-300, Analytic-Jena device., total antioxidant capacity using DPPH method and total polyphenols using Folin Ciocalteu method as described by Bordean, 2016. All analyses were made in triplicate and the mean values were reported [7]. The experimental data were compared to literature data and statistically evaluated. The fingerprint of the experimental data was also constructed using mathematical models.

*Statistical Analysis*
The experimental results were compared with data presented by different scientific articles and databases (USDA, FoodB) and statistically evaluated using PAST Version 2.17c [8] and MVSP.

## RESULTS AND DISCUSSIONS

The results are presented in table 1 and figures 1 and 3.

**Table 1.** Experimental and literature data comparation for Cauliflower and Broccoli - Mean ± standard error (SE),

| Evaluated characteristics | M.U. | Exp-C | Exp-B | Lit-C | Lit-B |
|---|---|---|---|---|---|
| MC | % | 90.76 | 91.59 | 92.6 [9] | 91.5 [10] |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 90.3 [11] | 90.7 [11] |
| K | mg·kg−1 DW | 3754.12 ± 14.15 | 5221.15±22.87 | 4764.44 ± 24.48 [4] | 7271.56±3.318 [5] |
| Ca | mg·kg−1 DW | 2391.62 ± 15.19 | 1121.53±18.53 | 1891.52 ± 13.02 [4] | 1013.1±0.054 [5] |
| Mg | mg·kg−1 DW | 2104.25 ± 31.76 | 279.55 | 2454.25 ± 11.91 [4] | 80.93±0.041 [5] |
| Fe | mg·kg−1 DW | 22.55 ± 1.16 | 12.69 ± 2.57 | 31.98 ± 0.19 [4] | 1.22±0.01 [3] |
| Zn | mg·kg−1 DW | 24.62 ± 0.55 | 31.98 ± 0.25 | 21.62 ± 0.32 [4] | 58.4 ± 0.8 [12] |
| Cu | mg·kg−1 DW | 3.81 ± 0.12 | 3.15 ± 0.32 | 4.25 ± 0.01 [4] | 3.6 ± 0.5 [12] |
| Mn | mg·kg−1 DW | 7.76 ± 0.15 | 11.54 ± 0.31 | 5.42 ± 0.11 [4] | 20.00 ±0.2 [3] |
| DPPH | µmol Trolox/ 100 g DW | 2867.29 ± 31.99 | 2543.10 ± 115.67 | 3455.04 ± 71.43 [13] | 3420.18±94.15 [13] |
| TPC | mg GAE/ 100 g DW | 326.20 ± 7.33 | 298 ± 9.23 | 381.49 ± 12.50 [13] | 356.06±9.71 [13] |

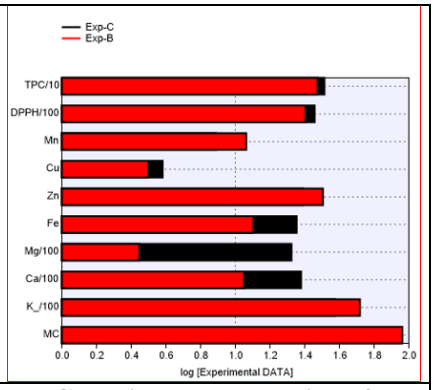
Values are presented as means ± SD of triplicate experiments

Legend: M.U.= measurement unit; Exp-C = experimental results obtained for cauliflower, Exp-B = experimental results obtained for broccoli, Lit-C= literature data for cauliflower, Lit-B= literature data for broccoli

In order to be able to compare the data efficiently and to obtain correct graphical fingerprints of data, some of the obtained values were simultaneously parted to 100 or 10. To evaluate the correlation between the obtained experimental data with the literature data, we applied Pearson's correlation. The evaluation of the correlation coefficient between cauliflower and broccoli is presented in figure 1.

| 0 | Exp-C | Exp-B | Lit-C | Lit-B |
|---|---|---|---|---|
| Exp-C | 0 | 6.72E-05 | 1.18E-06 | 0.00695 |
| Exp-B | 0.93616 | 0 | 0.000159 | 5.33E-05 |
| Lit-C | 0.97704 | 0.92044 | 0 | 0.009687 |
| Lit-B | 0.7866 | 0.93982 | 0.76661 | 0 |

**Pearson's correlation "r" between cauliflower and broccoli**




**Graphical representation of experimental data**

Figure 1 Evaluation of cauliflower vs broccoli based on experimental and literature data using Pearson correlation and graphical fingerprint of logarithmic data

Legend: Exp-C = experimental results obtained for cauliflower, Exp-B = experimental results obtained for broccoli, Lit-C= literature data for cauliflower, Lit-B= literature data for broccoli, TPC= total polyphenols content; DPPH= total antioxidant capacity based on DPPH method, Ca, K, Mg, Mn, Cu, Zn, Fe = evaluated macro and microelements, MC= moisture content

Pearson's correlation coefficient is a measure of the linear relationship between two continuous variables, X and Y and it can range from -1 to +1. The value ( -1) indicates a perfect negative linear relationship, while the value (+1) indicates a perfect positive linear relationship, while 0 indicates no linear relationship [14].

The generalized linear model shows distribution of parameters in analyzed vegetables, cauliflower and broccoli samples. To obtain the correspondence equation was used the normal distribution and the identity link function.

The equation $(\mathbf{y = 1.0685 \cdot x - 4.0966})$ able to identify the correspondence between cauliflower and broccoli ingredients is presented in figure 2.

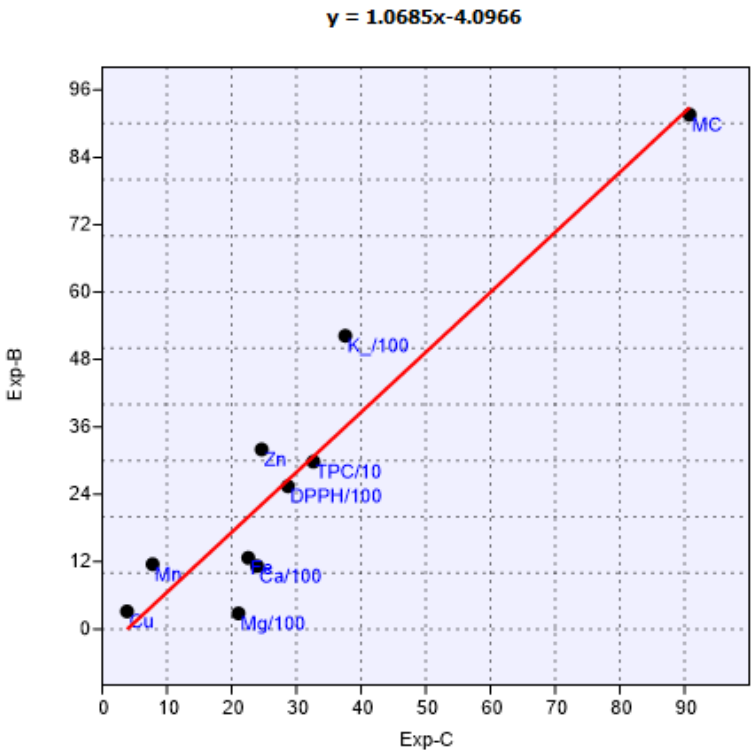



Figure 2 Identification of potential correspondence between cauliflower and broccoli based on experimental data

Legend: Exp-C = experimental results obtained for cauliflower, Exp-B = experimental results obtained for broccoli, Lit-C= literature data for cauliflower, Lit-B= literature data for broccoli, TPC= total polyphenols content; DPPH= total antioxidant capacity based on DPPH method, Ca, K, Mg, Mn, Cu, Zn, Fe = evaluated macro and microelements, MC= moisture content

The graphical fingerprint of chemical characteristics of the evaluated assortments of cauliflower and broccoli samples available on the Romanian market are presented in figure 3.

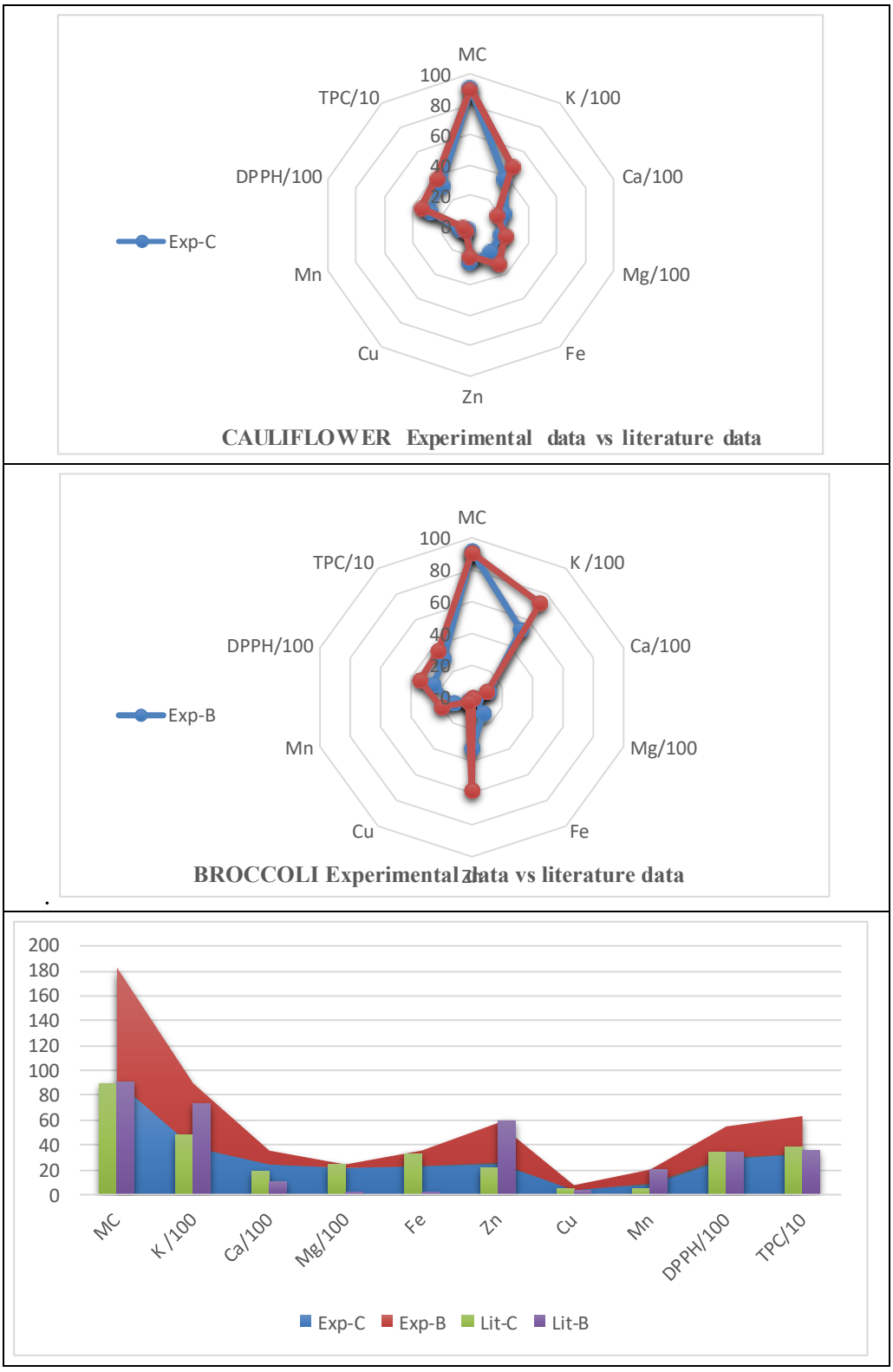
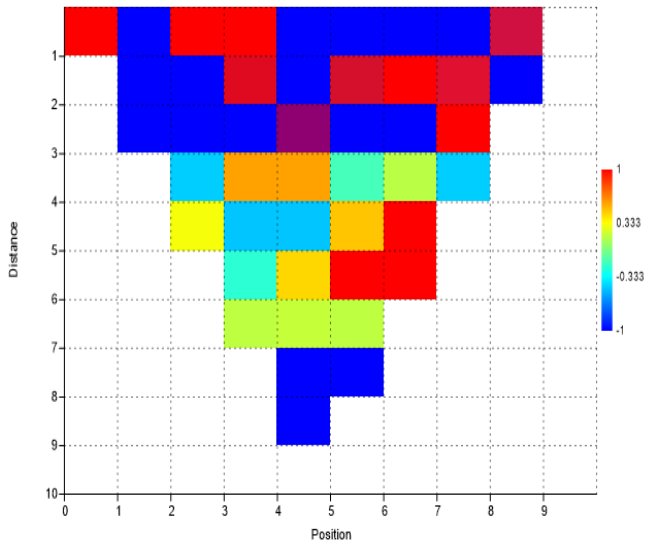



Figure 3 Fingerprint of cauliflower and broccoli, both experimental and literature based data

Legend: MC = moisture content, K = Potassium, Ca = Calcium, Mg = Magnesium, Fe = Iron, Zn = Zinc, Cu = Copper, Mn = Manganese, DPPH = total antioxidant capacity, TPC = total polyphenol content

If we apply Mantel correlogram (figure 4) we obtain the correlation between C-Exp and B-Exp. The Mantel correlogram is obtained using correlation as distance measure and the scalogram plot style. The highest positive correlation corresponds to the red colored plots, while the negative correlations correspond to the blue colored plots. The similarity of each Lag points its attached to the figure.




| Lag | Similarity |
|---|---|
| 0 | 0.88995 |
| 1 | -0.2212 |
| 2 | -0.1101 |
| 3 | -0.5386 |
| 4 | -0.1101 |
| 5 | 0.08995 |

Figure 4 Mantel correlogram of data

The Mantel correlogram was used to interpret all data (Mean ± SE) and to test the hypothesis that knowing the cauliflower nutritional content we can create innovative food products and or diets based on the nutritional profile of the consumer and his/her nutritional needs. In this testing approach, one matrix contains response data, while the other contains a representation of an a priori model to test. The 'model matrix' thus represents the alternative hypothesis of the test.

## CONCLUSIONS

Both, cauliflower and broccoli are high in potassium, calcium as well as different micronutrients (Fe, Cu, Zn, Mn). All the studied micronutrients are important for the plant metabolism as well as for the nutritional value of food products. Studies have shown that many new foods products lack in several mineral elements, and are supplemented artificially with minerals and or antioxidants.

Due to the fact that vegetables and fruits are considered the highest source of micronutrients such as minerals, antioxidants and polyphenols, it is important to be able to create a database of food fingerprints, based on these characteristics, which will help the producers and consumers to easily analyze the created food products. The present study offers the fingerprints for 2 vegetables (cauliflower and broccoli) both of Romanian origins and helps to identify the presence of one / both in different foods.

The most frequently detected food frauds are adulteration (e.g., the addition of water, minerals or antioxidants), sophistication and counterfeiting for economic advantage. When a food is suspected of adulteration, fingerprints databases and rapid methods are of high importance to support the investigation, withdrawal of these products and act against the detected frauds.

The mathematical models and graphical representations are highly appreciated rapid methods for food safety, food frauds and or environmental studies and investigations. The presented method can be used for various vegetables and fruits to improve databases and to create correlated equations which can help improve analyses.

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# STUDY OF ANTIBACTERIAL ACTIVITY OF DIOECIOUS *CANNABIS SATIVA* L. EXTRACTS

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## ABSTRACT

Dioecious *Cannabis sativa* L. is grown for derived products that are of industrial, food and therapeutic importance. The research aims to highlight the therapeutic potential. The study's main objective is to examine the antibacterial activity of dioecious hemp extracts on two bacterial strains. The plant material comes from 4 varieties of dioecious hemp CS (*Cannabis sativa* L.): CS1-Armanca, CS2- Lovrin 110, CS 3- Silvana and CS 4- Teodora. The antibacterial property of the plant biological material was tested on two bacterial strains: *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The evaluation of the antibacterial activity of plant extracts was performed by determining the bacterial growth rate (BGR%), bacterial inhibition rate (BIR%) and MIC (minimum inhibitory concentration). The antibacterial activity of the hydroalcoholic extracts of dioecious *Cannabis sativa* L was positively correlated with the increase of the concentration tested and inhibitory values. The results recommend them as possible candidates as compounds in products with antibacterial efficacy.

**Keywords:** dioecious hemp extracts, antibacterial activity, bacterial growth rate, bacterial inhibition rate

## INTRODUCTION

Industrial hemp (IH) is an annual herbal plant of the cannabis species (*Cannabaceae*) which is cultivated for fiber, oil, and dietary supplements [1]. IH is widely cultivated worldwide and has a long planting history in China for its applications in textile and consumption. In recent years, various kinds of nutraceuticals and functional foods made from IH leaves (IHL) and seeds have been presented [2].

A recent review depicted the applications of *C. sativa* from ancient times to the present, including the ethnological, botanical, chemical, and pharmacological features, with a focus on pharmaceutical research, to show the plant's potential therapeutic activities [3]. Hemp (*Cannabis sativa* L.) is an herbaceous plant belonging to the family *Cannabaceae* that humans have used for 5000 years [4]. The genus *Cannabis* is cultivated for different purposes, including fiber, food, and medicine [5]. It is promising that the whole above-ground part, from stem to flower, and industrial hemp seeds could be processed for further use [6,7].

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Especially the morphological parts, such as the inflorescence, have significant importance due to the presence of potentially biologically active compounds [8,9].
*Escherichia coli* (*E. coli*) is one of the bacteria that seriously endanger the healthy development of humans and animals [10]. Under certain conditions, it would cause diarrhoea in humans and animals [11]. It also could cause the spoilage of food, which is the main topic in food preservation [12].
*Staphylococcus aureus* is a gram-positive and aerobic bacterium and opportunistic pathogen [13]. The main sites of colonisation for *S. aureus* are the skin and mucous membranes. It could cause a wide range of diseases, including common problems such as skin infections and some severe problems such as endocarditis, bloodstream infections, and osteomyelitis [14]
Many studies suggest that cannabidiol (CBD) has potential antimicrobial effects, especially against drug-resistant strains of *Staphylococcus aureus* [15,16,17]
Cannabis extracts and purified cannabinoids have proven to be more active against Gram-positive bacteria, including multidrug-resistant microorganisms.[18]

## MATERIALS AND METHODS

The plant material (1 g) is extracted with 10 ml 70% ethanol for 30 minutes under vigorous shaking. The extracts obtained are filtered using high-porosity filter paper. After extraction, samples were diluted 1:100 with 70% ethanol. The concentrations used were: 25%, 50% and 75%.

Microbiological method

A $10^{-3}$ dilution of the fresh culture in the case of bacteria and a $10^{-2}$ dilution of the fresh culture in the case of fungi were used to perform the test, an inoculum equivalent to a 0.5 McFarland standard. Bacterial strains were revived by growing overnight in Brain Heart Infusion (BHI) broth (Oxoid, CM1135) for 24 hours at 37 °C. Culturile au fost apoi diluate la o densitate optică (OD) de 0,5 McFarland standard ($1,5 \times 10^8$UFC×mL) folosind bulion BHI și evaluate cu un densitometru McFarland (Grand-Bio, Anglia). Dilutions were pipetted 100 µL into each well of the 96-well microdilution plate using a Calibra 852 digital multichannel pipette.

Test extracts were added in amounts of 25 µL, 50 µL, 75 µL and 100 µL. Plates were covered and left for 24 hours at 37 °C. After 24 h, OD (optical density) was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Triplicate assays were performed for all samples. Strain suspensions from BHI were used as a positive control.
The broth microdilution method is one of the most basic antimicrobial susceptibility testing methods [Clinical and Laboratory Standards Institute., 2017, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically: Approved Standard-8th Edition, CLSI Document M07-A8, Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2009. Medicines, 4, 58]. The technique involves testing double dilutions of the antimicrobial agent tested in a liquid culture medium distributed in 96-well microtiter plates. MIC is the lowest concentration of antimicrobial agent that inhibits the organism's growth. CLSI has standardised the broth microdilution method to test aerobically growing bacteria, yeasts and filamentous fungi.

The EUCAST microdilution method is similar to CLSI [Clinical and Laboratory Standards Institute., 2017, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically: Approved Standard-8th Edition, CLSI Document M07-A8, Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2009. Medicines, 4, 58], with modifications typically addressing certain test parameters such as inoculum preparation, size, and MIC reading.

## RESULTS AND DISCUSSION

Results are presented as bacterial growth rate (BGR%)/mycelial growth rate (MGR%) and bacterial inhibition rate (BIR%)/mycelial inhibition rate (MIR%), rates calculated using formulae (1), (2):

$$\text{BGR/MGR\%} = \frac{OD_{PROBA}}{OD_{CONTROL\ NEGATIV}} \times 100(\%) \quad (1)$$

$$\text{BIR/MIR\%} = 100 - \text{CC}\ (\%) \quad (2)$$

Bacterial growth coefficients were negatively correlated with increasing tested concentration in all tested extracts.

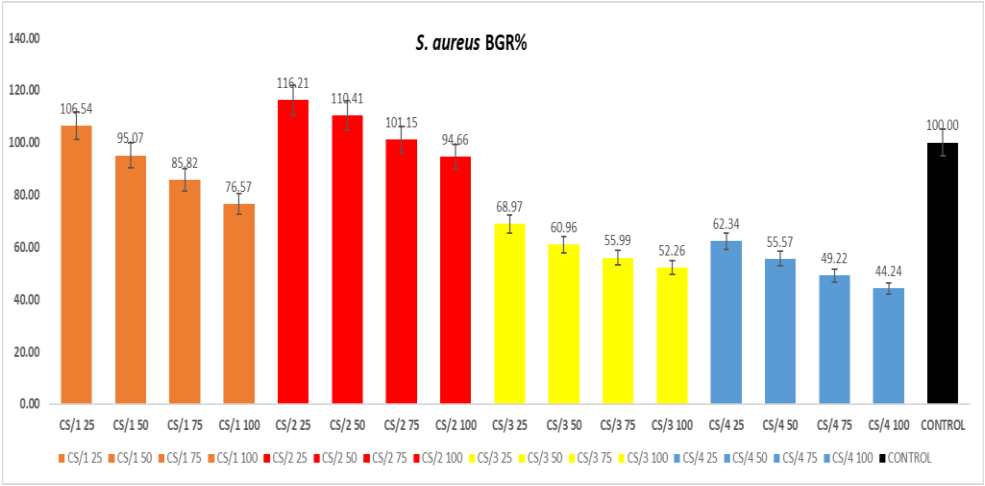



**Figure 2**. Graphical representation of the activity of CS extracts against *Staphylococcus aureus* expressed as BGR%.

Thus, as the amount of extract increases, the BGR% coefficient decreases, as shown in Figure 2.

Figure 3 shows a graphical representation of the antibacterial activity of hemp dioecious extracts, expressed as a percentage inhibition coefficient. The first two extracts showed low efficacy.

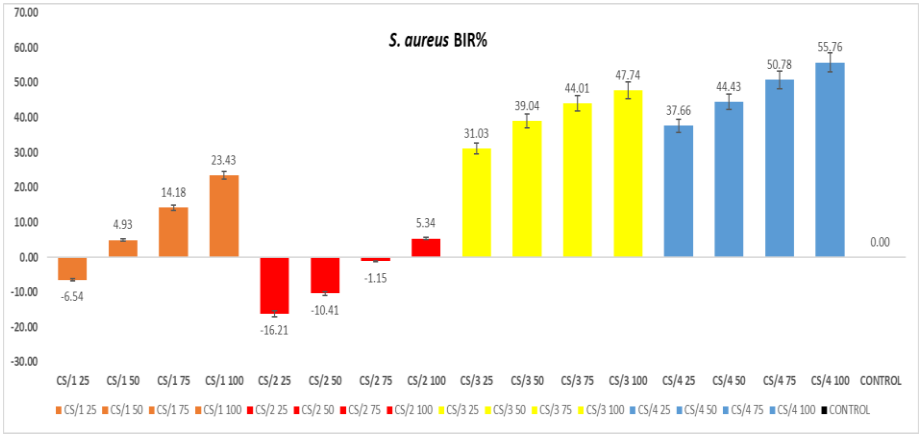



**Figure 3.** Graphical representation of the activity of CS extracts against *Staphylococcus aureus* expressed as BIR%.

Thus, in the case of the first extract, the first value obtained was negative, with a BIR% value of -6.54%, while the other concentrations tested showed positive values, with a maximum value of 23.43%. CS2 showed a roughly similar trend. CS3 and CS4 had the best BIR% values obtained, and the results were also supported by the values obtained in the analysis of total PF content. Thus, both had a high efficacy, with maximum BIR% values of 47.74% for CS3 and 55.76% for CS4. The antibacterial activity of CS extracts is shown in Figure 4 in graphical form, expressed as a percentage, as bacterial growth coefficient.

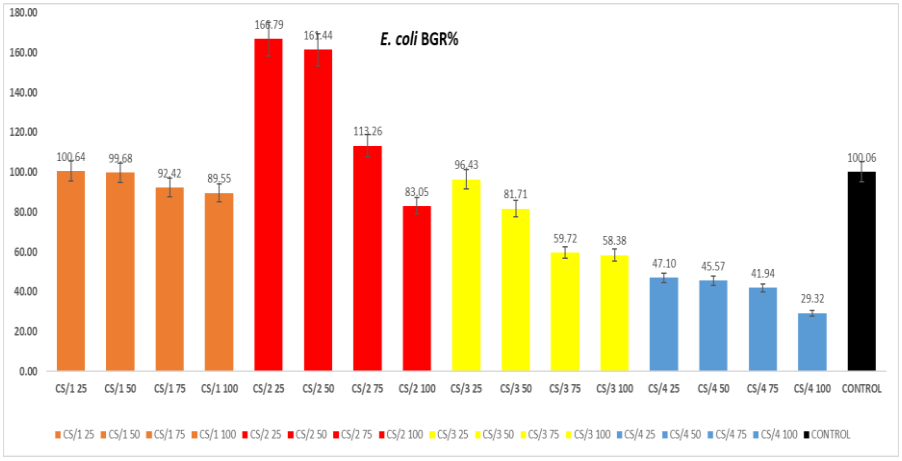



**Figure 4**. Graphical representation of the activity of CS extracts against *Escherichia coli* expressed as BGR%.

Thus, in terms of BGR%, the extracts have a negative trend, the increase being influenced by the increase in concentration tested. The highest increase was recorded for extract CS2, with maximum values of 166.79% at the first concentration tested, and the lowest value was recorded for extract CS4.

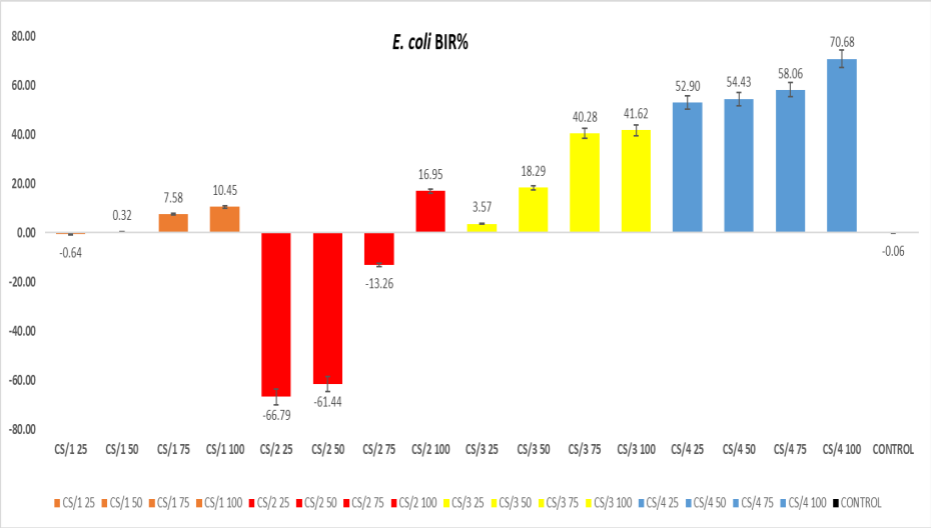



**Figure 5.** Graphical representation of the activity of CS extracts against *Escherichia coli* expressed as BIR%.

Figure 5 shows the inhibition values obtained for CS extracts against *Escherichia coli* strain. CS2 extract, in correlation with the value obtained from the chemical analysis, had the lowest efficacy, the only value obtained being at the highest concentration tested, with a BIR% value of 16.95%. CS2 also has low efficacy, although it has obtained 3 positive values.

## CONCLUSION

From the analysis of the results obtained in the laboratory following the determination of the antibacterial properties of hemp extracts, we can formulate the following aspects: The plant extracts of hemp dioecious are negatively correlated with the level of increase of the tested concentration and, simultaneously, the bacterial growth cofactors. The first value that was obtained was negative, and the other values of the tested concentrations showed positive values CS2 showed a similar trend, with CS3 and CS4 having the highest values of BIR% obtained, results determined also by the values achieved in the case of the determination of the total PF content. Thus, the two had a higher efficacy, recording maximum BIR% values of 47.74% in CS3 and 55.76% in CS4. The extracts CS3 and CS4, in correlation with the values obtained in the chemical analysis, showed good efficacy, which in the case of CS3 had BIR% values ranging from 3.57% - 41.62%, in the case of CS4 ranged between 52.90%-70.68% BIR%, values that recommend them as products with antibacterial efficacy.

## ACKNOWLEDGEMENTS

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# THE CRAFT BEER – AN APPRECIATED SUSTAINABLE BEVERAGE

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## ABSTRACT

This study aims to provide an overview of the craft beer industry and consumption in order to identify consumer preferences. More specifically, it aims to identify a target group of consumers and their willingness to pay (WTP) for craft beer and to enrich knowledge about consumer preferences and the product attributes that influence these preferences. Data were collected through a questionnaire completed by 231 participants. To evaluate the WTP for a 0.33 cl craft beer, they were asked to choose from five price levels starting from "> 3 and < 4 euro" with an increase of 1 euro for each interval. To assess the influence of intrinsic and extrinsic characteristics of beer quality on consumer choice, a Likert scale was proposed on: color, transparency, alcohol content, consistency, aroma, bitterness, raw materials, provenance, brand, price, label, market availability. The results of the study reveal that the majority choose to consume craft beer because they believe it is of higher quality and tastes better, which is why they are willing to spend more than on industrial beer. Consumers' preferences regarding craft beer attributes highlighted as most significant: body, aroma, brand and price. No recent studies have examined a model comparing these aspects, so this study could contribute to the development of new knowledges. Moreover, craft beer is more sustainable than the industrial one, so consumers attitude to buy and drink it should be encouraged.

**Keywords:** craft beer, quality perception, willingness to pay, sustainability

## INTRODUCTION

Beer is popular all over the world and is considered the oldest and most consumed alcoholic beverage.
This study aims to provide an overview of the craft beer industry and consumption in order to identify consumer preferences.
In Italy, the growth of the beer sector has only recently started, mainly due to the growing interest in craft beer [1]. The motivation for this growth trend lies in the consumer preferences to which beer is addressed [2]. Nowadays, the attention of consumers has changed considerably, in fact they are now increasingly looking for quality, healthy and sustainable products that are linked to their local area [3]. These aspects are perfectly fulfilled by craft beer.
The benefits of moderate consumption of craft rather than industrial beer are indeed quite popular [4]. Craft beer does not undergo heat treatment to which many bioactive

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compounds in it are sensitive [5]. The substances it provides the body with are nutrients, vitamins and minerals, dietary fibers, as well as a whole range of biologically active compounds that have antioxidant activity [6], such as polyphenolic and other antioxidant compounds that help capture free radicals and reduce the damage they cause [7].
Moreover, craft beer possesses both a strong capacity for differentiation due to the different combinations of grains used, which attracts consumers who appreciate aromatic complexity, high quality and authenticity of flavours [8]. It also possesses a sustainability that only craft breweries can guarantee, as they have an inherent environmental sustainability in production and transport through the use of local ingredients and sales in local markets [9]. This type of beer thus responds to the growing consumer focus on the consumption of genuine, quality, unique and sustainable products, but also with a lower environmental impact and more committed to sustainability [10].
Scientific studies about the consumption behaviour of craft beer are growing accordingly. In recent years, in fact, several studies have investigated consumer preferences regarding craft beer attributes, motivations for consuming craft beer and factors influencing the choice of craft beer [11]. However, some gaps can still be identified. One of these is a recent association between all these aspects mentioned above and consumers' WTP.
Based on the identification of this gap, a direct survey of consumers was carried out.
The aim of the study is manifold: firstly, it aims to estimates willingness to pay (WTP) for a target group of consumers; secondly, it aims to enrich knowledge about consumer preferences and the product attributes that influence these preferences.
As these aspects are completely unknown at regional level, the results could help direct companies towards the targeted fulfilment of consumer demands, not only regionally but potentially in many other contexts.

## EUROPEAN BEER MARKET OVERVIEW

When it concerns beer production on a European level, as shown in Table 1, the undisputed leaders are Germany and UK, a position they have maintained over at least the last ten years. Primacy is also held at the level of beer consumption, which on the European continent is higher than any other alcoholic beverage; in this respect, Germany also leads the world [12].

**Table 1**. Evolution of the European beer market over the last 10 years (beer production in Europe in 1,000 hl).

| Country | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Germany | 95,545 | 94,618 | 94,365 | 95,600 | 95,623 | 94,957 | 93,013 | 93,652 | 91,610 | 87,027 | 85,443 |
| United Kingdom | 45,701 | 42,962 | 42,420 | 41,204 | 44,054 | 44,000 | 40,480 | 41,530 | 39,247 | 32,217 | 38,399 |
| Poland | 37,850 | 39,290 | 39,560 | 39,870 | 40,900 | 40,731 | 40,382 | 40,930 | 39,740 | 39,066 | 38,200 |
| Spain | 33,600 | 33,031 | 32,700 | 33,535 | 34,775 | 36,200 | 37,621 | 38,370 | 39,513 | 34,738 | 38,000 |
| Belgium | 18,150 | 18,751 | 18,069 | 18,000 | 19,811 | 20,000 | 21,200 | 21,000 | 25,205 | 23,573 | 25,000 |
| France | 17,100 | 19,732 | 18,500 | 18,750 | 24,020 | 24,680 | 21,727 | 22,375 | 22,300 | 21,600 | 22,200 |
| Netherlands | 23,600 | 24,272 | 24,000 | 23,696 | 24,012 | 24,559 | 24,271 | 24,530 | 24,058 | 22,140 | 22,086 |
| Czech Republic | 18,191 | 18,703 | 18,605 | 19,648 | 20,091 | 20,476 | 20,322 | 21,272 | 21,608 | 20,122 | 19,600 |
| Ukraine | 30,510 | 30,050 | 27,600 | 24,200 | 19,460 | 17,980 | 17,800 | 18,070 | 18,020 | 17,970 | 17,071 |
| Romania | 17,000 | 18,200 | 16,500 | 14,900 | 15,950 | 16,000 | 16,110 | 17,600 | 16,690 | 16,750 | 16,600 |
| Italy | 12,510 | 12,791 | 12,688 | 12,968 | 14,671 | 16,218 | 15,552 | 15,700 | 17,271 | 15,829 | 16,600 |

Our elaborations on data from: *Barth Report Hops*, 2021 [13].

The Italian beer industry has developed considerably over the last decade, both in terms of beer consumption and production. In fact, as can be seen from Figure 1, these have clearly increased, except for a slight decrease in 2020 due to the Covid-19 pandemic [13].

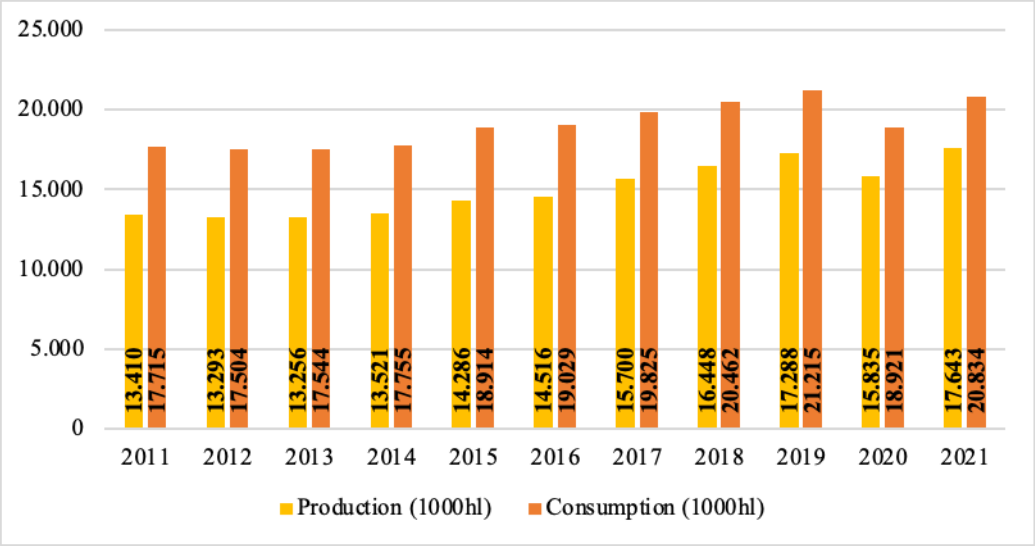



**Figure 1.** Evolution of beer consumption and production in Italy over the last 10 years. Our elaborations on data from: AssoBirra, 2021 [13].

However, despite the considerable increase that has taken place, within the European panorama, Italy reaches only about one fifth of the quantities reached by nations such as Germany, France or Poland [13], where the beer cult is well established. This gap is mainly dictated by the limited Italian demand. Similarly, within this panorama, in light of the growing interest of consumers at European level in beers produced on a small scale, in particular craft beers [12], microbreweries have also entered the scene in Italy and have undergone considerable development, to the point of doubling in number over the last ten years [14], as shown in Figure 2.

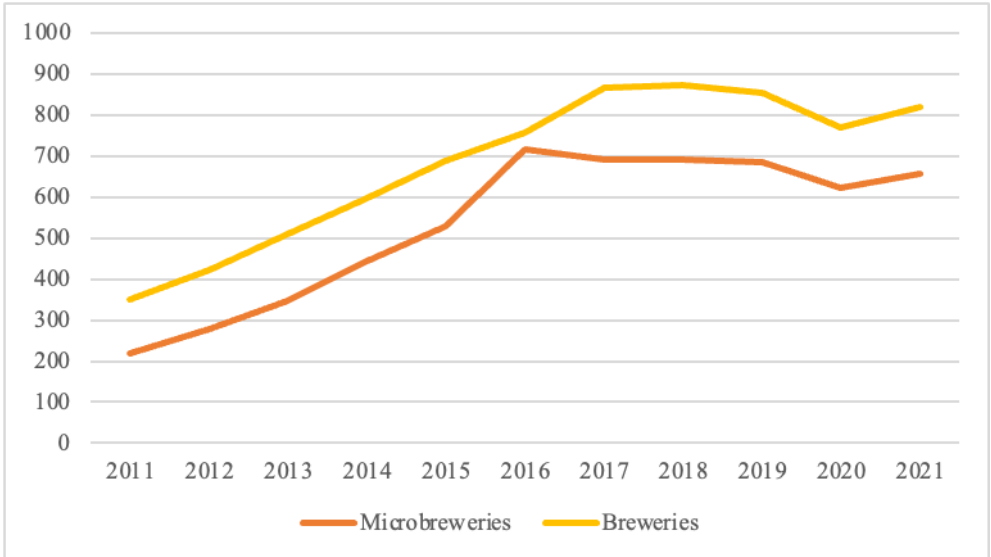



**Figure 2.** Evolution of the number of breweries and microbreweries in Italy over the last 10 years.

Our elaborations on data from: AssoBirra, 2021 [14].
In Italy, despite having never been one of the main beer producing or consuming countries, thanks to the small production capacity of microbreweries, has become increasingly central to the beer market, especially microbreweries.
The motivation for this intense propagation of microbreweries is to be found in the preferences of the consumer targeted by beer production, and that is what this study set out to do.

## MATERIALS AND METHODS

Based on the above, the study aims to enrich knowledge on the consumer's perception of product quality attributes, which is an aspect of fundamental importance for the development of the sector. This research in fact delves into an aspect lacking in the literature, namely the behavioural intentions and preferences of consumers towards craft beer and the socio-demographic and commercial factors that influence them.
In order to acquire the aforementioned knowledge, data collection was carried out from December 2022 to March 2023 through the administration of an online questionnaire on the 'Google Forms' platform, which was distributed on various social and messaging platforms. This study was conducted on a sample of consumers selected on the basis of age and beer consumption. In particular, they had to be beer consumers over the age of 18.
The structure of the questionnaire was designed with the precise aim of achieving the following objectives, to know:

- consumer preferences on industrial and craft beer,
- how often industrial and craft beer is purchased,
- how often and on what occasions industrial and craft beer is consumed,
- the WTP on the purchase of industrial and craft beer,
- the consumers' interest in craft beer,
- which of the extrinsic characteristics that can be assessed for a craft beer may influence the choice of one craft beer over another,
- the purchasing choices for a craft beer.

The purpose of the study was made clear to all participants before they took the questionnaire, and in order to not influence their answers, the anonymity of the data processing was reiterated to all participants, as they were processed in aggregate form.
The questionnaire consisted of 26 dichotomous and multiple-choice questions in the form of a Likert scale. The questions were divided into 3 sections, concerning: industrial beer consumption, craft beer consumption preferences and socio-economic characteristics of the participants.
Prior to the first section, the definition of industrial beer was prefixed as the objective was to investigate the consumption habits of industrial beer and the participants' WTP for it. After, the following questions were asked: (I) frequency of purchase, (II) frequency of consumption, (III) main places of consumption, (IV) time of main consumption, (V) WTP for an anonymous 33 cl industrial beer.
The second section, anticipated by the definition of craft beer, was instead aimed at acquiring data on the consumption and qualitative perception of craft beer. In detail, 10 questions were asked: (I) frequency of purchase, (II) frequency of consumption, (III) main places of consumption, (IV) time of main consumption, (V) level of interest in craft beer on a scale of 1 to 7, (VI) the reasons why drinks or would like to drink craft

beer, (VII) the perceived quality level of craft beer compared to industrial beer on a scale of 1 to 7, (VIII) the WTP for an anonymous 33 cl craft beer, (IX) how much the intrinsic characteristics of the beer influence the quality of a craft beer on a scale of 1 to 7, (X) how much the characteristics of the beer influence the choice to buy a craft beer on a scale of 1 to 7.

The frequency of consumption (II) was assessed on a scale from 1 (low frequency) to 7 (high frequency) and the number of times on a scale from "at least once a week" to "once in a while". Four places were considered as places of consumption: at home, in a bar/pub, at a live event, on holiday. Furthermore, participants were asked to indicate the occasion on which they consume beer, whether during meals or outside meals. About the reasons why they drink or would like to start drinking craft beer, five possibilities were offered: it is something different, it has/could taste better than industrial beer, I like the culture of craft beer, it is trendy, it is of higher quality than industrial beer.

Respondents were asked how much they were willing to pay for a 0.33 cl industrial beer between five price levels starting with "< 1 euro" and ending with "> 5 euro" with an increase of 1 euro for each interval. Furthermore, they were asked how much they were willing to pay for a 0.33 cl craft beer among five price levels starting with "> 3 and < 4 euro" and ending with "> 7 euro" with an increase of 1 euro for each interval.

With regard to their own judgement on the influence of intrinsic and extrinsic characteristics on the quality of a craft beer, the following were proposed as intrinsic characteristics to be evaluated on a scale of 1 to 7: color, transparency (absence of residues), alcohol content, consistency (body), aroma, bitterness, raw materials used for production; as extrinsic characteristics, the origin of the product, brand, price, labelling, market availability.

The third section concerned the socio-economic characteristics of consumers: gender, age, province of residence, household unit, number of adults, occupation, educational qualification, income bracket.

## RESULTS

Data analysis was performed with STATA SE-Standard Edition 17.0. The survey of the study involved 231 participants. The analysis revealed a very heterogeneous consumers profile about gender (46% female, 54% male), age group (25% between 18 and 24, 41% between 25 and 34, 19% between 35 and 44), on average the respondents live in households with three or four members (29% three, 35% four), the majority of whom are employed or students (61% employed, 31% students), while a minority are not employed or are retired. As far as annual income is concerned, respondents fall into two main income bracket groups: between €10,000 and €30,000 (44%) and between €30,000 and €50,000 (30%), while a minority, around 13% each, below €10,000 and above €50,000. Finally, educational qualifications are somewhat less heterogeneous as the majority of subjects are highly educated and hold a university degree (80%), while only a minority hold a high school diploma (20%).

The results of the habit analysis show that the respondents are already consumers of craft beer. In fact, more than 80% of the respondents stated that they consume craft beer, of which about 40% consume it weekly and the remaining 40% monthly. The majority of consumers choose to consume craft beer because they believe it is of higher quality and tastes better than industrial beer. In fact, almost all of the respondents considered craft beer to be of higher quality on average than industrial beer.

In addition, more than half of the respondents stated that they are willing to pay the price levels “>4 and <5 euro” and “>5 and <6 euros” for a 33 cl bottle of craft beer and they consume it mainly in a bar/pub or at home.
Finally, consumer preferences regarding the intrinsic and extrinsic attributes of beer were obtained using the ANOVA method. Table 2 shows the results obtained, which reveal the statistical differences of each attribute for craft and industrial beer respectively.

**Table 2**. ANOVA results for intrinsic and extrinsic attributes.

| **Attributes** | **Beer** | |
|---|---|---|
| | **Craft** | **Industrial** |
| Color | 2.52** | 3.93*** |
| Transparency (absence of residues) | 1.78* | 2.42** |
| Alcohol content | 1.37* | 2.37** |
| Consistency (body) | 3.33*** | 5.10*** |
| Aroma | 2.35** | 1.74* |
| Bitterness | 1.79* | 1.85* |
| Raw materials used for production | 2.15** | 2.87*** |
| Product origin | 2.06* | 3.26*** |
| Brand | 2.48** | 1.50* |
| Price | 2.27** | 0.47* |
| Label indications | 1.78* | 3.24*** |
| Market availability | 1.28* | 0.81* |

*, **, ***, indicate significance at 0.1, 0.05 and 0.01 level, respectively.

Our investigation into consumer preferences has shed light on the intrinsic and extrinsic attributes that significantly influence the valuation of craft beer. Notably, consumers accorded paramount importance to the intrinsic attributes of craft beer, such as body and aroma, which are distinctive characteristics of the craft beer. In terms of extrinsic attributes, instead, the brand and the price, which underscore the product's territorial identity and reflect consumers' WTP a premium for this type of beer, also played an important role in shaping preferences.
The discernible trend is that consumers, despite the higher cost associated with craft beer, are inclined to prioritize sensory experience and the distinctive regional connection offered by these products. This underscores a shift in consumer consciousness towards more quality-driven choices, transcending economic considerations.
In light of these findings, it becomes evident that sustainability, both in terms of sensory enjoyment and the preservation of regional identity, is a key driver in the consumer choice of craft beer over industrial. So, as producers seek to enter in this competitive market, to retain consumers should emphasise on sustainable practices, both in terms of sourcing quality ingredients and supporting local communities, can significantly increase the attraction of craft beer. This insight underscores the market potential for sustainable craft products.

## CONCLUSION

No recent studies have examined a model comparing the aspects of WTP analysis, knowledge analysis of consumer preferences, and identification of the intrinsic and extrinsic attributes of the product that are most desired by consumers and that consequently most influence their purchasing choices.
The results of this study identified that motivational factors influence the consumption behaviour of craft beer consumers. As such, they offer a number of useful implications for meeting the multiple expectations of beer consumers and for providing a cue for further corporate differentiation strategies in the beer market.
Consistent with what is reported in the literature, the consumer segment has been identified among millennials [15], particularly those with a high level of education;, the interest in the intrinsic attributes of beer, the predilection towards consumption in bars/pubs and the WTP of a sample with a heterogeneous income was identified in the two price levels “>4 and <5 euro” and “>5 and <6 euros” . In order to attract consumers towards the consumption of a craft rather than an industrial product, producers should take into consideration these elements. To this end, it could prove useful to emphasise the local origin of the raw materials used for the product, which guarantees the uniqueness of its characteristics and quality, the place of distribution and the selling price.
Despite the recruitment of participants based on their real interest in participating in the survey and in the subject matter of the survey, the limitations of the study can mainly be found in its hypothetical nature. The method of data collection, in fact, having taken place at a theoretical-exploratory level remotely and not in an actual purchasing condition, could be less significant, especially for the purposes of assessing WTP and understanding purchasing decisions. Furthermore, through a sensory analysis of the product to be carried out in the survey phase, a wider range of intrinsic product attributes, which have proven to be fundamental, could be evaluated.

## ACKNOWLEDGEMENTS

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# THE DIRECTION OF CHANGES IN THE GRASSLANDS SURFACES IN THE MOUNTAIN AREA OF BANAT

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## ABSTRACT

Grasslands, some of the most important resources of mountain areas, have been and are subject to changes, both in time and in space, under the action of natural and/or anthropogenic factors. In this context, the research aims, in the first stage, to identify and quantify the areas of grasslands subject to changes, both through surface losses and gains, and in the second stage, to establish the general direction of changes, at regional level. As a case study, the area of the Banat Mountains, from southwest Romania, was selected, a very complex area from a physical-geographical and socio-economic point of view. The analysis of grassland areas and implicitly the changes produced, was made in the GIS environment, based on Corine Land Cover data, over a period of approximately thirty years. As a result of the research, it was found that the areas of grasslands were "mobilized" by adding areas from other categories of use (orchards, lands with natural vegetation and so on), but also by switching to other categories of use, especially in high mountain areas, through afforestation. Also, the study showed important differences in territorial profile, of great importance for local communities and the economy of the mountain area. Monitoring changes in grassland areas is also particularly important in terms of management strategies or sustainable development of mountain areas.

**Keywords:** grassland areas, Corine Land Cover, change analysis

## INTRODUCTION

Grasslands are subject to important changes in time and space, both under the influence of ecological factors and under the influence of human action. The influence of the human factor is reflected by the way grazing is organized or by the way some improvement works are applied ([11], [9]) and/or by the abandonment of grasslands ([3], [8]).

Monitoring the changes produced in the case of grassland surfaces, regardless of the factors that produce them, can be done, with remarkable results, based on the Corine Land Cover datasets ([13], [2], [15]). In this way, changes in space, as a result of surface changes, but also changes in time, given the existence of successive data sets from several time periods, can be followed.

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In designing this study, we started from the following working hypothesis: in general, mountain areas are depopulated [12] and therefore "fragile" in terms of the stability of the land base; grasslands are essential resources for mountain areas, both economically and ecologically [14] and information related to the dynamics of grassland surfaces is vital in development and management strategies [4].

In this context, the research aims, in the first stage, to identify and quantify the areas of grasslands subject to changes, both through surface losses and gains, and in the second stage, to establish the general direction of changes, at regional level.

## MATERIALS AND METHODS

### Study area

For the delimitation of the study area, the cartographic and textual documentation contained in the Mountain Law no. 197/2018 [10], from Romania, the Banat Mountains being included in the mountain zone - Group 8. The area is located in the southwest of Romania, on the territory of Caraş-Severin and Mehedinţi counties (Figure 1).

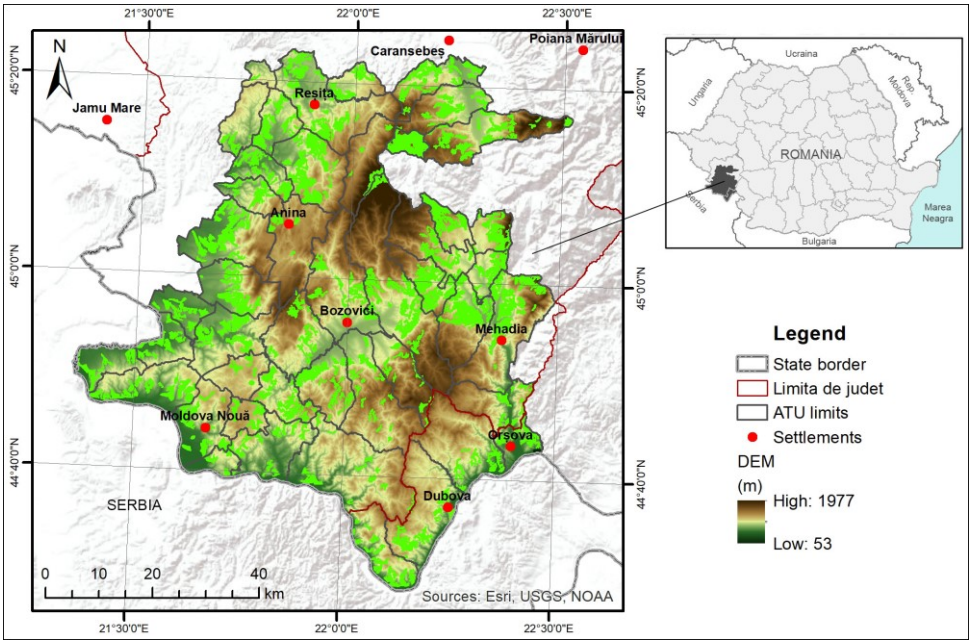



Figure 1 Location of the study area and grassland areas (processing after [5], [6], [7])

From the point of view of the relief, it is grafted on an altitudinal range between 54 - 1977 m (Figure 1). The lowest altitudes are found in the southern part of the area, in the Danube Gorge, and the highest altitudes are reached in the Ţarcu Mountains (1977 m).

### Work methodology

***1. The identification and zoning of grassland surfaces*** was carried out starting from pre-existing geospatial data sets, respectively the Corine Land Cover database, 2018 edition [6]. To update and validate this dataset, orthophotoplans, maps and cadastral plans [1], specialized platforms (Google Earth Pro), but also satellite images, processed by assisted spectral classification, were used;

***2. The analysis of the spatio-temporal changes of the grassland surfaces,*** assumed the processing, in a GIS environment, of the Corine Land Cover databases, the editions of 1990, 2006 and 2018 [6] (updated to the level of 2023, in the previous stage). For these datasets, verification and validation was done with Google Earth Pro, satellite images and high spatial resolution images (orthophotoplans). The data were processed in the GIS environment, through overlay analysis (spatial intersections) and through the Land Change Modeler tool). The analysis was carried out over two time intervals, 1990 – 2006 and 2006 – 2023, but also over the entire period, respectively 1990 – 2023.

ArcGIS and TerrSet software were used for geospatial data processing. When carrying out the localizations and spatial analyses, in addition to the previously mentioned data sets, auxiliary data sets were used, such as: state border, county limits, localities.

## RESULTS

### 1. Changes in land use in the Banat Mountains

The area related to the Banat Mountains, in Romania, delimited according to the Mountain Law, is 503821 ha (Table 1). At the level of this area, forest areas cover approximately 66% of the total area, and grassland areas occupy approximately 14%. The surface difference belongs to other categories of land use, with much lower weights, a situation also found in other mountain areas ([8], [11], [12], [13]).

Table 1 The area and weight of land use classes in the Banat Mountains (1990 – 2023)

| CLC code | Land use | Area in 1990 | | Area in 2006 | | Area in 2023 | |
|---|---|---|---|---|---|---|---|
| | | ha | % | ha | % | ha | % |
| 112 | Built space (urban) | 6930 | 1.38 | 6581 | 1.31 | 7150 | 1.42 |
| 121 | Areas with human activities | 2564 | 0.51 | 1638 | 0.33 | 2170 | 0.43 |
| 211 | Arable land | 26760 | 5.31 | 32699 | 6.49 | 34528 | 6.85 |
| 221 | Vineyards | 1601 | 0.32 | 841 | 0.17 | 550 | 0.11 |
| 222 | Orchards | 17762 | 3.53 | 12532 | 2.49 | 9838 | 1.95 |
| **231** | **Grasslands** | **65860** | **13.07** | **70291** | **13.95** | **70366** | **13.97** |
| 242 | Complex cultivation patterns | 5827 | 1.16 | 13319 | 2.64 | 13429 | 2.67 |
| 243 | Agricultural area with natural vegetation | 23835 | 4.73 | 15250 | 3.03 | 13764 | 2.73 |
| 311 | Forest areas | 313714 | 62.27 | 334705 | 66.43 | 336446 | 66.78 |
| 322 | Transitional woodland-shrub | 29005 | 5.76 | 5952 | 1.18 | 5927 | 1.18 |
| 332 | Areas without vegetation | 0 | 0.00 | 208 | 0.04 | 208 | 0.04 |
| 511 | Water surfaces | 9963 | 1.98 | 9805 | 1.95 | 9446 | 1.87 |
| | Total area | 503821 | 100 | 503821 | 100 | 503821 | 100 |

### 2. Analysis of changes in grassland areas in the period 1990 – 2023

***2.1. Between 1990 and 2006,*** an area of 50580 ha of grasslands did not undergo changes. There was a net increase of the surfaces by 4431 ha. In territorial profile, the area of grasslands increased by 19711 ha, in some areas, but decreased in other areas by 15280 ha, which means a net increase of 4431 ha (Table 2).

Areas from other land use classes contributed to the increase and/or reduction of grassland areas (Figure 2).

Significant changes have occurred through:

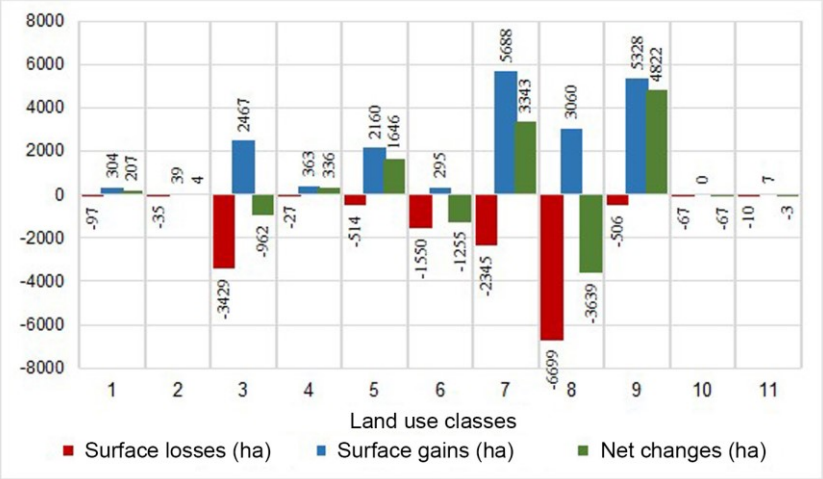
- afforestation, on an area of 6699 ha, a situation encountered at the contact of the grasslands with the forest field, in all mountain units; in the opposite direction, 3060 ha of forestry were deforested in the marginal areas, the respective areas being included in the category of grasslands;

Table 2 Transitions matrix in land use, in the Banat Mountains, between 1990 - 2006

| 2006 / 1990 | 112 | 121 | 211 | 221 | 222 | 231 | 242 | 243 | 311 | 322 | 332 | 511 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 112 | 5448 | 71 | 100 | 6 | 40 | 304 | 472 | 300 | 176 | 0 | 0 | 14 | 6930 |
| 121 | 411 | 1382 | 10 | 5 | 15 | 39 | 178 | 136 | 382 | 0 | 0 | 6 | 2564 |
| 211 | 95 | 59 | 22577 | 183 | 213 | 2467 | 632 | 439 | 89 | 0 | 0 | 5 | 26760 |
| 221 | 16 | 0 | 203 | 482 | 219 | 363 | 146 | 90 | 82 | 0 | 0 | 0 | 1601 |
| 222 | 39 | 0 | 367 | 0 | 10115 | 2160 | 2894 | 832 | 1097 | 258 | 0 | 0 | 17762 |
| 231 | 97 | 35 | 3429 | 27 | 514 | 50580 | 1550 | 2345 | 6699 | 506 | 67 | 10 | 65860 |
| 242 | 155 | 0 | 1365 | 0 | 389 | 295 | 2932 | 536 | 156 | 0 | 0 | 0 | 5827 |
| 243 | 124 | 24 | 3896 | 119 | 764 | 5688 | 3296 | 7306 | 2248 | 127 | 3 | 241 | 23835 |
| 311 | 149 | 61 | 147 | 3 | 41 | 3060 | 235 | 589 | 307629 | 1686 | 51 | 61 | 313714 |
| 322 | 29 | 5 | 602 | 17 | 221 | 5328 | 984 | 2296 | 16075 | 3341 | 83 | 24 | 29005 |
| 511 | 18 | 0 | 3 | 0 | 0 | 7 | 0 | 381 | 73 | 34 | 3 | 9444 | 9963 |
| Total (ha) | 6581 | 1638 | 32699 | 841 | 12532 | 70291 | 13319 | 15250 | 334705 | 5952 | 208 | 9805 | **503821** |

Legend: 112- Built space (urban); 121-Areas with human activities; 211-Arable land; 221-Vineyards; 222-Orchards; 231-Grasslands; 242-Complex cultivation patterns; 243-Agricultural areas with natural vegetation; 311-Forest surfaces; 322-Transitional woodland-shrub; 332-Areas without vegetation; 511-Water surfaces

- passing the grasslands into the arable land class, on an area of 3429 ha, especially in the Danube Gorge, but also isolated, in other depressed areas; on the contrary, the abandoned arable land was transferred to the category of grasslands on an area of 2467 ha, thus resulting in a net reduction of 962 ha of grasslands;




Legend: 1-Built space (urban); 2-Areas with human activity; 3-Arable land; 4-Vineyards; 5-Orchards; 6-Complex cultivation patterns; 7-Agricultural areas with natural vegetation; 8-Forest surfaces; 9-Transitional woodland-shrub; 10-Areas without vegetation; 11-Water surfaces

Figure 2 The contribution of land use classes to changes in grasslands

- the transition from grasslands to areas with shrubby vegetation, on a surface of 506 ha and in the opposite direction, by the transition from shrubby vegetation to grasslands, on the surface of 5328 ha, brings a net increase of grasslands by 4822 ha.

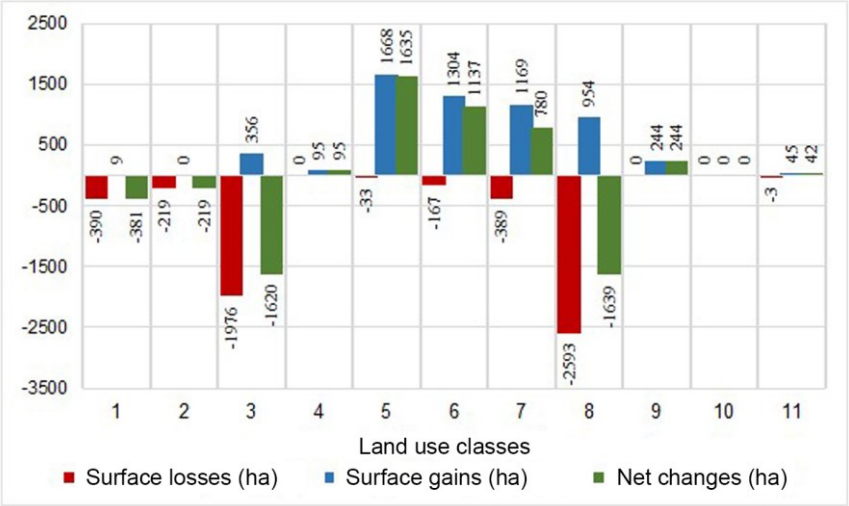
***2.2. Between 2006 and 2023,*** grasslands did not undergo changes on an area of 64521 ha. The area of grasslands increased by 5845 ha, in some areas and decreased by 5770 ha, resulting in a net increase of 75 ha (Table 3).

The reduction of grassland areas was caused by their transition, mainly, to the following land use categories: forest areas (1639 ha) and arable land (1620 ha) (Figure 3).

Table 3 Transitions matrix in land use, in the Banat Mountains, between 2006 - 2023

| 2023 / 2006 | 112 | 121 | 211 | 221 | 222 | 231 | 242 | 243 | 311 | 322 | 332 | 511 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 112 | 6459 | 0 | 9 | 0 | 4 | 9 | 1 | 26 | 73 | 0 | 0 | 0 | 6581 |
| 121 | 16 | 1611 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 1638 |
| 211 | 22 | 6 | 31105 | 36 | 7 | 356 | 806 | 290 | 55 | 17 | 0 | 0 | 32699 |
| 221 | 0 | 0 | 229 | 515 | 0 | 95 | 0 | 1 | 1 | 0 | 0 | 0 | 841 |
| 222 | 0 | 0 | 325 | 0 | 9745 | 1668 | 708 | 47 | 29 | 8 | 0 | 0 | 12532 |
| 231 | 390 | 219 | 1976 | 0 | 33 | 64521 | 167 | 389 | 2593 | 0 | 0 | 3 | 70291 |
| 242 | 188 | 32 | 72 | 0 | 13 | 1304 | 11504 | 121 | 85 | 0 | 0 | 0 | 13319 |
| 243 | 22 | 55 | 664 | 0 | 23 | 1169 | 158 | 12636 | 479 | 44 | 0 | 0 | 15250 |
| 311 | 51 | 100 | 79 | 0 | 13 | 954 | 80 | 81 | 332550 | 796 | 0 | 0 | 334705 |
| 322 | 0 | 0 | 2 | 0 | 0 | 244 | 2 | 73 | 570 | 5061 | 0 | 0 | 5952 |
| 332 | | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 208 | 0 | 208 |
| 511 | 2 | 147 | 66 | 0 | 0 | 45 | 2 | 100 | 0 | 0 | 0 | 9444 | 9805 |
| Total (ha) | 7150 | 2170 | 34528 | 550 | 9838 | 70366 | 13429 | 13764 | 336446 | 5927 | 208 | 9446 | **503821** |

Legend: 112- Built space (urban); 121-Areas with human activities; 211-Arable land; 221-Vineyards; 222-Orchards; 231-Grasslands; 242-Complex cultivation patterns; 243-Agricultural areas with natural vegetation; 311-Forest surfaces; 322-Transitional woodland-shrub; 332-Areas without vegetation; 511-Water surfaces




Legend: 1-Built space (urban); 2-Areas with human activity; 3-Arable land; 4-Vineyards; 5-Orchards; 6-Complex cultivation patterns; 7-Agricultural areas with natural vegetation; 8-Forest surfaces; 9-Transitional woodland-shrub; 10-Areas without vegetation; 11-Water surfaces

Figure 3 The contribution of land use classes to changes in grasslands

The increase in the areas of grasslands (Figure 3) was caused by the transition to this category of land areas originating, mainly, from: orchards (1635 ha), complex crops (1137 ha) and agricultural areas with natural vegetation (780 ha).

***2.3. In the interval 1990 - 2023,*** changes produced in the class of grassland surfaces had the following components: 48468 ha did not undergo spatial changes; 21898 ha were added from other land use categories; 17391 ha of grasslands passed to other categories of use (Table 4). Under these conditions, the general balance sheet of the changes produced in the grassland class shows a net increase of 4507 ha.

The loss of grassland areas occurred, for the most part, through (Table 4): the transition to the category of forest areas, on 8237 ha; the transition to the category of agricultural land with natural vegetation, on 2056 ha and to the category of land with shrubby

vegetation, on 472 ha; conversion to arable land, on 4169 ha and to complex crops, on an area of 1528 ha. The expansion of grassland areas occurred, for the most part, by the transfer of areas from the following land use categories (Table 4): agricultural lands with natural vegetation, on 6742 ha and with complex crops, on an area of 522 ha; lands with shrubby vegetation, on 5603 ha; orchards, on 3473 ha; forest areas on 3257 ha.

Table 4 Transitions matrix in land use, in the Banat Mountains, between 1990 - 2023

| 2023 / 1990 | 112 | 121 | 211 | 221 | 222 | 231 | 242 | 243 | 311 | 322 | 332 | 511 | Total (ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 112 | 5543 | 67 | 101 | 6 | 38 | 267 | 348 | 274 | 273 | 0 | 0 | 14 | 6930 |
| 121 | 479 | 1483 | 14 | 5 | 15 | 26 | 91 | 69 | 377 | 0 | 0 | 6 | 2564 |
| 211 | 110 | 59 | 23270 | 139 | 24 | 1576 | 988 | 483 | 101 | 6 | 0 | 5 | 26760 |
| 221 | 16 | 0 | 537 | 268 | 153 | 382 | 71 | 82 | 89 | 4 | 0 | 0 | 1601 |
| 222 | 45 | 0 | 149 | 0 | 8713 | 3473 | 3197 | 849 | 1099 | 237 | 0 | 0 | 17762 |
| 231 | 393 | 222 | 4169 | 27 | 206 | 48468 | 1528 | 2056 | 8237 | 472 | 67 | 13 | 65860 |
| 242 | 162 | 0 | 1356 | 0 | 117 | 522 | 3028 | 473 | 170 | 0 | 0 | 0 | 5827 |
| 243 | 143 | 30 | 4061 | 89 | 337 | 6742 | 2944 | 6762 | 2428 | 178 | 3 | 117 | 23835 |
| 311 | 200 | 98 | 206 | 0 | 55 | 3257 | 284 | 571 | 306679 | 2253 | 51 | 60 | 313714 |
| 322 | 40 | 64 | 661 | 17 | 180 | 5603 | 948 | 1943 | 16666 | 2776 | 83 | 24 | 29005 |
| 511 | 20 | 147 | 3 | 0 | 0 | 51 | 1 | 203 | 328 | 0 | 3 | 9207 | 9963 |
| Total (ha) | 7150 | 2170 | 34528 | 550 | 9838 | 70366 | 13429 | 13764 | 336446 | 5927 | 208 | 9446 | **503821** |

Legend: 112- Built space (urban); 121-Areas with human activities; 211-Arable land; 221-Vineyards; 222-Orchards; 231-Grasslands; 242-Complex cultivation patterns; 243-Agricultural areas with natural vegetation; 311-Forest surfaces; 322-Transitional woodland-shrub; 332-Areas without vegetation; 511-Water surfaces

From the point of view of net changes, significant increases in the class of grasslands occurred through areas originating from: agricultural areas with natural vegetation (4686 ha), areas with shrubby vegetation (5131 ha) and orchards (3267 ha). Significant losses were caused by the transition of grasslands to the following use classes: forest areas (4980 ha) and arable land (2593 ha).

## DISCUSSIONS

Through the action of different natural and/or anthropogenic factors, in the way of land use in the area related to the Banat Mountains, quantitative changes occur, through the transfer of land surfaces to different classes. The detailed analysis of changes through the LCM tool allows establishing the contribution of other land use classes to the dynamics of grassland surfaces, depending on the temporal thresholds.

The loss of grassland areas, in general, occurred mostly through:

a. the transition to the category of forest areas, a phenomenon encountered at the contact of forestry and pastoral domains, in the entire analyzed area, but especially in the Semenic Mountains, Locvei Mountains, Almăjului Mountains or at the contact of the Timiş-Cerna Corridor with the mountain area;

b. moving to the category of agricultural land with natural vegetation, in areas with lower altitudes and to the category of land with shrubby vegetation, in areas with higher altitudes; these phenomena represent the early stages of grassland afforestation and are also due to their underexploitation or abandonment ([4], [11]);

c. the conversion to arable land occurred in depression areas, with higher population densities and therefore more intense agricultural activity, namely the Danube Gorge, at the contact with the low area of the Almajului Mountains and the Locvei Mountains; in

these areas it is intended to be exploited as arable land in order to obtain basic products (cereals, vegetables, fruits); the situation is similar in the case of the transformation of grasslands into complex crops.

The expansion of grassland areas occurred, for the most part, by the transition of some areas from the following categories of use:

a. agricultural lands with natural vegetation - the transformation occurred in the depressed area and the low hills (Bozovici Depression, Tîrnovei Hills, Oraviţei Hills, Timiş-Cerna Corridor, Danube Gorge), as a result of the "cleaning" of those lands in order to use them for grazing and mow; the situation is similar in the case of lands with complex crops;

b. lands with shrubby vegetation - the transformation took place, for the most part, in the low mountain area (Semenic Mountains, Locvei Mountains), where it was desired to expand the areas of grasslands by removing non-specific shrubby vegetation;

c. orchards, the phenomenon occurred in depression and hill areas (Timiş-Cerna Corridor, Oraviţei Hills, Bozovici Depression) and highlights the abandonment of fruit processing and commercialization activities, one of the traditional occupations for the study area [9]; the "reconversion" towards the exploitation of agricultural lands and animal breeding is observed;

d. forest areas - the transformation occurred dispersedly, over the entire area, through deforestation.

## CONCLUSION

Major changes were noticed between 1990 and 2023, on the one hand, through the afforestation of some lands used as grasslands, and on the other hand, through the "recovery" of some grassland areas from lands with shrubb vegetation, arable land, fruit plantations, lands with complex crops and agricultural lands with natural vegetation.

The study showed important differences in territorial profile, regarding the "mobility" of grasslands, particularly relevant aspects in the economy of mountain areas.

The data obtained by monitoring the changes produced in the grassland surfaces, based on geospatial data, can be used in projects regarding management strategies or sustainable development of mountain areas.

## ACKNOWLEDGEMENTS

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# THE EFFECT OF BLUEBERRIES ON PHYSICAL, CHEMICAL AND SENSORIAL PROPERTIES OF TURKISH DELIGHT

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## ABSTRACT

Turkish delight (lokum) is a sweet product originating in Turkey made from sugar, food flavors and starch. The delights dates back more than 300 years and is one of the oldest sweets in the world. Technologies have evolved and with them the demands of consumers have also diversified. People are in search for everything new and healthy in terms of nutrition, this being the reason for the development of a new kind of Turkish delight. The aim of this study is to analyze the physico-chemical and sensorial properties of two types of blueberries Turkish delight. First type is made from the culture blueberry and the second one is made from the forest blueberry. The physico-chemical properties analyzed for both varieties of blueberries and as well on the finished products are: pH, total acidity, viscosity, dry substance, moisture content, protein, ash, total polyphenol content and the antioxidant activity. It was determined that Turkish delight sample containing forest blueberry it has a greater amount of active biological compounds. According to the results, for the raw material, the pH values indicate that cultivated blueberries are less acidic than wild blueberries. And as for the finished product, compared to the control sample, it is found that due to the thermal process and the concentration of sugar present in the blueberries, the pH value decreases for the two varieties made. Regarding the acidity, it was found that cultivated blueberries have a medium acidity, and forest blueberries have a low acidity. And for the finished product, the Turkish delight obtained with wild blueberries has a higher acidity. From the point of view of sensory analysis, the product obtained from cultivated blueberry puree has higher sensory properties than the one obtained from forest blueberry puree.

**Keywords:** total polyphenol content, antioxidant activity, acidity, pH, viscosity.

## INTRODUCTION

Turkish delight, or Lokum, is a traditional sweet from Turkey that has been made for centuries (Yilmaz, 2008)[5]. It's a family of confections based on a gel of starch and sugar. Premium varieties consist largely of chopped dates, pistachios,

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hazelnuts, or walnuts bound by the gel; traditional varieties are often flavoured with rosewater, mastic gum, bergamot orange, or lemon (Batu, 2009)[1].

It holds cultural, historical, and economic importance that contributes to its significance. Regarding the cultural significance, Turkish delight is deeply rooted in Turkish culture and is considered a traditional dessert. It is often consumed during special occasions, such as weddings, religious festivals, and holidays like Ramadan and Eid. The sweet treat has become an integral part of Turkish cuisine, representing the rich culinary heritage of the country.

Historical Significance: Turkish delight has a long history dating back several centuries. It was initially created in the Ottoman Empire and gained prominence during that time. It was even used as a delicacy in the royal courts, becoming a symbol of wealth and luxury. Throughout history, Turkish delight has been enjoyed by people of various backgrounds and is known internationally as a prominent Turkish confectionery.

Export and Trade: Turkish delight has not only gained popularity within Turkey but also across the world. It has become a significant part of the country's economy through its export and trade. Turkey is one of the leading producers and exporters of Turkish delight globally. This sweet treat is widely imported and enjoyed by people from diverse cultures, contributing to the economy of the country.

Culinary Tourism: Turkish delight plays a crucial role in promoting culinary tourism in Turkey. Many tourists and visitors are attracted to the country because of its diverse and delicious food, including Turkish delight. Locals often gift Turkish delight to visitors as a gesture of hospitality, showcasing the importance and pride attached to this confectionery.

Symbol of Turkish Cuisine: Turkish delight has become an iconic representation of Turkish gastronomy. It perfectly reflects the essence of Turkish flavours with its unique combination of textures, aromas, and tastes. The confectionery comprises ingredients like sugar, cornstarch, natural flavourings such as rosewater or lemon, and is often adorned with nuts or dusted with powdered sugar. Its presence on menus and in culinary discussions helps to solidify its importance as a symbol of Turkish cuisine.

Technologies have evolved and with them the demands of consumers have also diversified. People are looking for new and healthy foods, which is the reason for the development of a new range of Turkish delight, with wild blueberries and culture blueberries.

Blueberries are often referred to as a “superfood” due to their numerous health benefits. They are packed with nutrients and have a variety of benefits for your body. Blueberries are believed to have one of the highest antioxidant levels of all common fruits and vegetables [Gomez, 2017][6]. The main antioxidant compounds in blueberries belong to a family of polyphenol antioxidants called flavonoids1. One group of flavonoids in particular — anthocyanins — is thought to be responsible for much of these berries’ beneficial health effects.

Blueberry delight is a combination of the traditional Turkish delight dessert, which has a soft texture and special sweetness. Through the combination of the blueberry, with ascorbic acid in the processing technology, results a functional product with multiple benefits for those who consume it.

The main objective of the current study is to evaluate the sensorial properties present in the Turkish delight on the taste, texture, color and stability of, and also, was monitored the pH parameters, acidity, dry matter, polyphenols (TPC) in blueberries and

compared to their values on the two types of Turkish delight obtained. The blueberry used in our study were harvested in September 2022 from the Făgăraș area, Romania.

## MATERIALS AND METHODS

The technological process for obtaining the Turkish delight is presented in Figure 1. The process and the recipe are made after the research of Batu et al, 2009[1]. The raw material is starch, citric acid, blueberries fruits, glucose and sugar. The addition of acid is used to prevent crystallization, and the use of ascorbic acid makes the structure of the turkish delight soft and the shelf life longer.




Figure 1. The technological process for obtaining the Turkish delight

The use of fruits in different forms in the processing of turkish delight makes the thermal processing time after the addition of fruits shorter resulting a high quality product. Long processing results in undesirable color, taste deterioration and an extremely hard texture.

Blueberry juice, water, sugar, citric acid and glucose syrup are brought to a boil temperature and boiled 15 minutes. Water was used only in the control sample (P3). Than was add the starch milk in a thin layer so as not to interrupt the cooking. It is important to slowly add the starch suspension so that gelatinization of the starch occurs gradually. Depending on the recipe and the properties of the materials used, starch gelatinization takes about 1-1,5 hours after the addition of glucose syrup. The completion of the gelation process is completed when the dry substance reaches 82%. After at least 24 hours, the mass cools and becomes consistent so that it can be cut and packaged.

*pH measurement*

For the pH analysis, 10 g of the sample was homogenized with 100 ml of distilled water by using a homogenizer (IKA, Germany) The pH was measured using the electrochemical method (Webster, 2003) with a potentiometer (Consort C1010, Consort, Turnhout, Belgium).

*Total Acidity*

The total acidity values of the samples were determined with the method describe by Hanoglu, 2023[4]. The total acidity value of each sample mixture was determined by

monitoring the pH meter (Hanna, HI 2211, Romania) during titration with 0.1 N NaOH until the pH value reached 8.1. The total acidity value of the samples was calculated using the amount of base consumed in the titration as % aqueous citric acid.

*Dry substance*

To determine the dry substance content of the Turkish delight samples for products with a harder consistency or containing sugar crystals, for example because the viscosity is too high and the boundaries between the regions are not clear, the sample is diluted by one volume equal to water. To do this, weigh 5 or 10 grams of sample into a vial with a cap (or in a Berzelius beaker covered with a watch glass) and with a rod accurate to 0.01 gram and add the volume (in cubic centimeters) with a pipette of quality distilled water, mix until dissolve. To speed up dissolution, heat in a water bath to 50-60°C, then bring at a temperature of 20°C. An accuracy of 0.01 g is used to determine the dry substance. Place 2 drops of sample on the lower prism of the refractometer. The prism approaches and se move the eyepiece until the marker overlaps the line separating the 2 fields of view. Then one can read the dry substance content as a percentage directly on the scale at a temperature of 20 °C.

*Viscosity*

The viscosity (η [cP]) was determined using the Brookfield Rheometer (Braive Instruments, Liege, Belgium).

*Moisture content*

Moisture content was determined in hot air oven. Firstly weigh 10 g sample accurately and subject to oven drying at 110℃ for 4-5 hour. Oven dried samples were cooled in desiccators and weighed. The drying was repeated until the constant weights were obtained or until the difference between two successive weighing was not more than 0.002g. The resultant loss in weight was calculated as percent moisture content (Okey F. O., Sunday L.E. and Chidinma O. E., 2016).

$$\%Moisture = \frac{Initial\ weight - Final\ weight}{Initial\ weight} x100$$

*Determination of protein content of Turkish delight*

Protein content was determined by Kjeldhal method. Approximately 1 g of sample was hydrolysed with 15 ml concentrated sulphuric acid ($H_2SO_4$) containing two copper catalyst tablets ($CuSO_4$ / $K_2SO_4$) in a heat block at 420℃ for 2 hours. After cooling, H2O was added to the hydro lysates before neutralization and titration. After digestion in conc. H2SO4, the total organic nitrogen is converted to ammonium sulphate. Ammonia is formed and distilled into boric acid solution under alkaline conditions. The borate anions formed are titrated with standardized (HCl) hydrochloric acid, by which is calculated the content of nitrogen representing the amount of crude protein in the sample. Most proteins contain 16% of nitrogen, thus the conversion factor is 6.25 (D.A. Goulding. 2020).

$$\%\ Nitrogen = \frac{(ml\ of\ standard\ acid - ml\ of\ blank) \cdot N\ of\ acid \cdot 1{,}4007}{Wheight\ of\ sample\ in\ gram}$$

Calculate % Protein as:

$$\%\,Protein = \%\,Nitrogen \times 6.25$$

*Determination of ash content of Turkish delight*

Ash content was determined in muffle furnace. Firstly, note the empty weight of the crucible and the weigh 10 g sample of Turkish delight in the crucible. Keep the crucible in muffle furnace and set its temperature and time at 550℃ at 4 hours. Then cool the dishes in desiccator and weigh the content. Then note the difference of content and calculate the ash content in % (Hanoglu, 2023)[4].

$$\%Ash = \frac{Final\ weight}{Initial\ weight} x100$$

*Total polyphenolic content*

Using gallic acid as a reference, the total polyphenolic content (TPC) was calculated using the Folin-Ciocalteu spectrophotometric method and represented as micrograms of gallic acid equivalents per millilitre (g GAE/mL). Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), 1.25 mL, was applied to a 0.5 mL sample after being diluted 1:10 (v/v) with distilled water. 1 mL of Na2CO3 60 g/L was added after the mixture had been incubated for 5 minutes at room temperature. The sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany) after 30 min of incubation at 50 °C. Gallic acid was used as the standard, with concentrations ranging from 5 to 250 g GAE/mL, to create the calibration curve[2,7].

*Sensory analysis*

The sensory analysis of tea samples was made by 15 people (untrained; 9 females and 6 males; aged 20 to 41 years), who were selected from among students and teachers from the Food and Tourism Faculty, Transilvania University from Brasov. All the panels were informed to conduct sensory evaluations before the test.

The evaluation of Turkish delight samples was made using the hedonic test, a five-point scale using a single-blind approach. Were evaluated considering the criterion of color, brightness, clarity, astringency, aroma and bitterness. A scoring scale in the range of 0–5 was used for all parameters. Scoring was as follows: 0 means absent; 1 - very weak; 2 - rather weak; 3 - neither weak nor strong; 4 - considerably strong; and 5 - very strong. (Polat et al., 2022; Dasdemir et al., 2023)[8,3].

## RESULTS AND DISCUSSION

The results of physico-chemical properties are presented in the Table 1.

Analysing the results from Table 1 it can be seen that for the raw material, the pH values indicate that blueberries of culture are less acidic than wild blueberries. And as for the finished product, compared to the control sample, it is found that due to the thermal process and the concentration of sugar present in the blueberries, the PH value increase for the two varieties.

Regarding the acidity, it was found that cultivated blueberries have a medium acidity, and the wild blueberries has a low acidity. And for the finished product, it is found that the delight obtained with wild blueberries has a higher acidity.

The dry substance content is present in a higher amount in cultivated blueberries compared to wild ones, due to the pulp content. As for the finished product blueberries influenced the dry substance of the cultivated blueberry assortment.

Table 1. The results of physico-chemical properties are presented in the

| Properties | P1 | P2 | P3 | P4 | P5 |
|---|---|---|---|---|---|
| pH | 3.5±0.07 | 3.1±0.11 | 2.8±0.13 | 4.1±0.02 | 3.4±0.09 |
| Acidity [%] | 0.65±0.09 | 0.47±0.03 | 0.28±0.12 | 0.82±0.023 | 0.59±0.015 |
| Viscosity [cP] | 0.989±0.032 | 0.948±0.056 | 0.969±0.017 | 0.998±0.04 | 0.971±0.031 |
| Dry substance [°Brix] | 15±0.92 | 10±0.76 | 75±1.12 | 75±1.87 | 73±1.65 |
| Moisture [%] | 88.2±1.9 | 87.02±2.09 | 38.25±1.34 | 48.24±1.19 | 42.38±1.11 |
| Protein [g/100g] | 0.74±0.03 | 1.1 ±0.08 | 0.0±0.00 | 0.16±0.06 | 0.54±0.05 |
| Ash [g/100g] | 1.8±0.09 | 2.3±0.12 | 0.04±0.012 | 0.13±0.04 | 0.76±0.05 |
| TPC [mg GAE/L] | 367.03±3.42 | 602.5±5.54 | - | 289.2±12.02 | 500.6±6.71 |

P1-culture blueberries; P2-wild blueberry; P3-control sample; P4-Turkish delight with cultured blueberries; P5-Turkish delight with wild blueberries

The results are expressed as the mean value of the three replicates ± the standard deviation (SD).

The rheological behavior of the two types of blueberries that had a different solids content. Depending on the temperature and the total content of substances dried shear thinning behavior emerged, the food product having characteristics non-Newtonian pseudoplastics.

Regarding the moisture content, it was found that the control sample has the lower value (38,25%). The higher moisture content it was obtained at the culture blueberry (88.2%) due to the high pulp content.

The Ash content and the protein content it doesn't very much, they register higher values in the case of Turkish delight obtained from wild blueberries.

The dosage of polyphenols was carried out by means of the Folin-Ciocâlteu method. As a result of the measurements, it emerged that the value of polyphenol concentrations is affected by the thermal process to which the raw materials consisting of blueberries, even if the blueberries are added at the end of the thermal process when the temperature is decreasing.

The results of the sensorial properties are presented in the Figure 1.

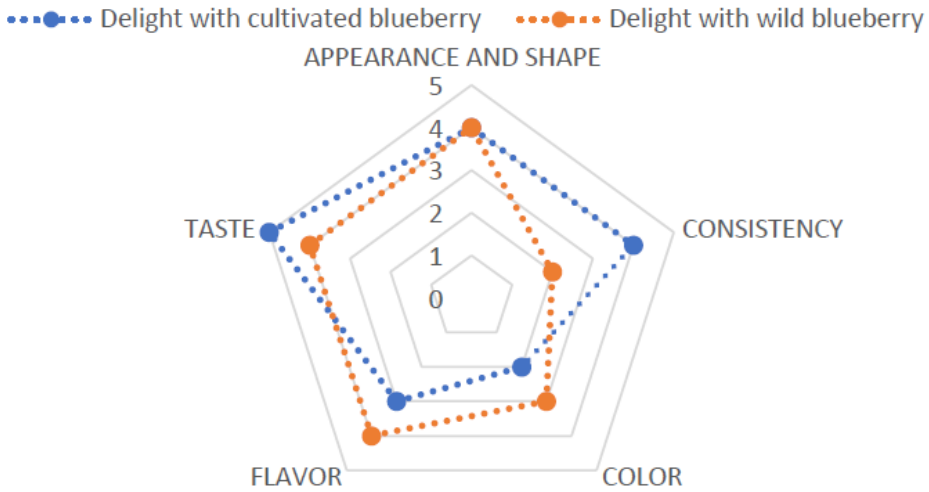



Figure 1. Sensory analysis of Turkish delight with culture and wild blueberry

Research results have shown that due to the increased juiciness of the puree obtained from wild blueberries, the consistency of the finished product was more defective soft and sticky, and in terms of color, the presence of anthocyanins in a larger amount in wild blueberries they have greatly intensified this property. Analyzing the figure of the sensory analysis (Figure 1), it can be seen that the Turkish delight obtained from culture blueberries has a higher score. This is due to the maximum taste score, having a sweeter and more pleasant taste compared to the wild Turkish delight, as well as due to the firmer consistency.

## CONCLUSION

In order to analyze the physico-chemical properties of the obtained product, standardized methods were used, and the obtained results demonstrated the fact that the Turkish blueberry delight products can be classified as a functional product.

Turkish delight holds importance not only for its delectable taste but also for its cultural, historical, economic, and culinary significance. It is a cherished dessert that represents Turkish tradition and heritage while enticing people worldwide with its unique flavors and textures.

The technological process has a very important role in determining the quality of the finished product; a high temperature and an increased heat processing time affect viscosity as well the number of polyphenols of the finished product; the acidity of the finished product is influenced by acidity fruits, as well as the PH.

The dry substance content is present in a higher amount in cultivated blueberries compared to wild ones, due to the pulp content.

Depending on the temperature and the total content of substances dried shear thinning behavior emerged, the Turkish delight products is having characteristics non-Newtonian pseudoplastics.

The Ash content and the protein content it doesn't very much, they register higher values in the case of Turkish delight obtained from wild blueberries.

Regarding the sensorial properties, the Turkish delight obtained from culture blueberries has a higher score in comparation with the Turkish delight obtained from wild blueberries.

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# THE ENHANCING IMPACT OF FOLIAR FERTILISERS ON QUALITY INDICES OF WHEAT

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## ABSTRACT

Achieving profitable crop production milestones hinges on the establishment of a robust soil fertility program. Such programs demand foresight and meticulous planning. To ensure the profitability and efficiency of wheat cultivation, it is imperative to provide the right amount of essential plant nutrients precisely when and where the crop requires them.

The application rates of fertilizers hold little significance if these vital nutrients are not distributed at the appropriate locations and times. Wheat, in particular, exhibits a heightened sensitivity to fertilization, responding favorably to the application of fertilizers. This is due to the fact that grains consume relatively modest quantities of nutrients, primarily because their roots explore a smaller volume of soil. Additionally, they possess a limited capacity for solubilizing and absorbing nutrients in highly soluble forms when compared to other crops. The influence of foliar fertilizers on various plant species is diverse and contingent upon the specific plant type and even its variety. Foliar application can offer the advantage of improved nutrient accessibility for crops, ultimately leading to higher yields.

The judicious fertilization of wheat crops, contingent upon soil fertility, significantly contributes to the quality of grain production. The presence and balance of both macro and microelements play a pivotal role in crop nutrition, ultimately leading to higher yields. The primary objective of this study was to assess variations in several wheat quality indicators under conditions of varying nitrogen (N) levels.

**Keywords:** fertilization, foliar fertilizers, wheat

## INTRODUCTION

Current climate change concerns, as well as biodiversity loss, water scarcity and declining soil fertility, show that agriculture plays an increasingly important role in the sustainable management of natural resources. ([1], [2], [3], [4], [5])

Wheat is considered to be the most cultivated agricultural crop in the world, with over 220 million hectares.

Wheat is particularly pretentious for fertilization. Wheat reacts positively to the application of fertilizers although consumes moderate amounts of nutrients due to the fact that its roots exploit a lower soil volume and has less power to solubilize and absorb

the nutrients in hardly soluble forms compared to other crops. ([6], [7], [8], [9], [10], [11])

The structure and chemical composition of wheat is influenced by certain technological factors, such as crop structure, crop rotation, and fertilization. Also important are environmental factors (temperature, rainfall/soil moisture, air composition). ([12], [13], [14], [15])

The balanced fertilization of wheat crop, depending on soil fertility, contributes to the quality of grain production. [16]

Foliar application can guarantee better nutrient availability for crops and also achieve higher yields.

The role of macro and microelements is critical in crop nutrition and in obtain higher yields. Nitrogen, phosphorus and potassium are of big importance in crop nutrition. Nitrogen is a primary constituent of proteins and therefore of enzymes. Due to the fact that phosphorus is part of compounds such as ATP and ADP, it is involved in most biochemical processes. Several micronutrients are involved in photosynthesis, N-fixation, respiration and other biochemical processes. ([17], [18])

In the case of cereals, 50-70% of nitrogen is accumulated by the plants until flowering, after which the nitrogen is transferred to the grain. Thus, wheat fertilization with N is a current practice to ensure high protein content in grains.

However, with the gradual increase of nitrogen doses, to a certain limit value, the protein content reaches a maximum. This value is maintained constant without increasing the absorption or remobilization of N in the production of grains, thus reducing the efficiency of fertilization with N.

The main purpose of fertilizer use is to obtain high yields with minimal expense. The cost of fertilizers has increased a lot over the last decades as they are obtained with high fossil energy consumption. ([19], [20])

For determining the quantities of fertilizer to be used and the method of application, account shall be taken of:

- specific consumption of nutrients of cultivated plants,
- supply status of the soil with the nutrients essential to plants,
- the farming system and crop rotation,
- the agro-technical measures used,
- remanence of fertilizer applied in previous years,
- time and manner of application in the current year,
- the nature of fertilizers and restrictive economic factors.

## MATERIALS AND METHODS

Experimental conditions

The researches within the mentioned topic were conducted in the experimental field of the Agrochemistry discipline located within the Didactical Resort of the University of Life Sciences King Mihai I and later in the research laboratories from the Soil Sciences Department.

These aim at scientifically and rigorously investigating the problems of increasing production and quality, and reducing environmental pollution, due to controlled use of fertilizers in order to obtain new data and information that will serve to change the current concept of agriculture, towards a sustainable agriculture.

The soil was cambic chernozem with moderate fertility, showed by some indexes such as the humus content of H = 3.1% and pH = 6.90-7.10. The seed was produced by SCDA Lovrin and the biological material was the Sofru wheat cultivar (Caussade).

The main objective is to observe plant health, quality and changes in production, following application of these fertilizers, the basic idea being sustainable agriculture.

The experimental design was subdivided in plots with three replicates and five variants of fertilization (according to the field plot). The size of a plot was 200 $m^2$.

V1- Ferti KEM 10:10:10+ME * dose – 6l/ha
V2- Ferti KEM 8:8:8+ME* dose - 6l/ha
V3- Ferti KEM 15:0:0+2S+1B+ME* dose – 6l/ha
V4- Ferti KEM 24:0:0+3Zn+ME* dose 4 l/ha
V5- Ferti KEM 15:0:0+5Zn+ME* dose 4l/ha
MT-martor

A quantity of 150 kg/ha 15:15:15 and 100 kg/ha of ammonium nitrate was applied to the soil. Two foliar fertilizations with the doses recommended by the producer in the wheat vegetation phases were performed, ranging from 4 to 6 l/ha.
Foliar fertilizers are liquid chemical fertilizers NPK, NP and N in the form of transparent, colourless solutions without any mechanical impurities.

These fertilizers have a higher content of phosphorus in the phase of strengthening the root and flowering, higher nitrogen content in vegetative growth phase and a high potassium content in fruit ripening stage. Foliar fertilizers provide the amounts of essential nutrients needed to develop crops at different stages of development.

Fertilizer application was done by finely spraying the product onto the leaves. The ambient temperature was 27ºC, it was not a rainy and windy day.

The application of foliar fertilizers can be done throughout the vegetation period, in the phases where these elements are highly demanded.

## RESULTS AND DISCUSSION

Grain production is one of the most complex attributes, the achievement of which contributes a lot of elements and attributes that can be more or less influenced by environmental conditions.

The harvesting of the experimental variants was done before the rains started, which is also the result of the quality analyses carried out, it was done in the shortest time and without loss.

**Tabel 1**
**Results on the production of grain for autumn wheat**

| Replicates | Fertilization variant | The type of fertilizer | Production kg/ha |
|---|---|---|---|
| *R1* | **V1-** | Ferti KEM 10:10:10+ME | 5700 |
| | **V2-** | Ferti KEM 8:8:8+ME | 5550 |
| | **V3-** | Ferti KEM 15:0:0+2S+1B+ME | 5850 |
| | **V4-** | Ferti KEM 24:0:0+3Zn+ME | 6000 |
| | **V5-** | Ferti KEM 15:0:0+5Zn+ME | 5600 |
| | **MT-martor** | | 4150 |
| *R2* | **V1-** | Ferti KEM 10:10:10+ME | 5810 |
| | **V2-** | Ferti KEM 8:8:8+ME | 5670 |
| | **V3-** | Ferti KEM 15:0:0+2S+1B+ME | 5980 |
| | **V4-** | Ferti KEM 24:0:0+3Zn+ME | 6210 |
| | **V5-** | Ferti KEM 15:0:0+5Zn+ME | 5780 |
| | **MT-martor** | | 4320 |
| *R3* | **V1-** | Ferti KEM 10:10:10+ME | 5780 |
| | **V2-** | Ferti KEM 8:8:8+ME | 5710 |
| | **V3-** | Ferti KEM 15:0:0+2S+1B+ME | 6010 |
| | **V4-** | Ferti KEM 24:0:0+3Zn+ME | 6190 |
| | **V5-** | Ferti KEM 15:0:0+5Zn+ME | 5720 |
| | **MT-martor** | | 4220 |

In graphical representation, autumn wheat production is shown in Figure 1 and Table 2 represents a synthesis of statistical data in its descriptive form, as evidenced by the average, the median as well as the Standard Deviation.

**Table 2**
**Statistical data on cereal production, highlighted by average, median and Standard Deviation**

| *V1-* | | *V2-* | | *V3-* | | *V4-* | | *V5-* | | *MT-martor* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| Mean | 5763,333 | Mean | 5643,333333 | Mean | 5946,666667 | Mean | 6133,333333 | Mean | 5700 | Mean | 4230 |
| Standard Er | 32,82953 | Standard Er | 48,07401701 | Standard Er | 49,10306621 | Standard E | 66,91619967 | Standard Ei | 52,91502622 | Standard Er | 49,32882862 |
| Median | 5780 | Median | 5670 | Median | 5980 | Median | 6190 | Median | 5720 | Median | 4220 |
| Standard De | 56,86241 | Standard De | 83,26663998 | Standard De | 85,04900548 | Standard D | 115,9022577 | Standard D | 91,6515139 | Standard De | 85,44003745 |
| Sample Var | 3233,333 | Sample Var | 6933,333333 | Sample Var | 7233,333333 | Sample Va | 13433,33333 | Sample Vai | 8400 | Sample Var | 7300 |
| Skewness | -1,20566 | Skewness | -1,293342781 | Skewness | -1,492769076 | Skewness | -1,674209536 | Skewness | -0,93521953 | Skewness | 0,519470297 |
| Range | 110 | Range | 160 | Range | 160 | Range | 210 | Range | 180 | Range | 170 |
| Minimum | 5700 | Minimum | 5550 | Minimum | 5850 | Minimum | 6000 | Minimum | 5600 | Minimum | 4150 |
| Maximum | 5810 | Maximum | 5710 | Maximum | 6010 | Maximum | 6210 | Maximum | 5780 | Maximum | 4320 |
| Sum | 17290 | Sum | 16930 | Sum | 17840 | Sum | 18400 | Sum | 17100 | Sum | 12690 |
| Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 |
| Confidence | 141,254 | Confidence | 206,8458005 | Confidence | 211,2734419 | Confidence | 287,9171692 | Confidence | 227,674982 | Confidence | 212,2448191 |

For the null hypothesis test, we used IBM SPSS Statistics Version 21, the One-sample Kolmogorov-Smirnov test at a significance level of 0.05.

**Hypothesis Test Summary**

| | Null Hypothesis | Test | Sig. | Decision |
|---|---|---|---|---|
| 1 | The distribution of V1 is normal with mean 5.763,333 and standard deviation 56,86. | One-Sample Kolmogorov-Smirnov Test | ,971 | Retain the null hypothesis. |
| 2 | The distribution of V2 is normal with mean 5.643,333 and standard deviation 83,27. | One-Sample Kolmogorov-Smirnov Test | ,960 | Retain the null hypothesis. |
| 3 | The distribution of V3 is normal with mean 5.946,667 and standard deviation 85,05. | One-Sample Kolmogorov-Smirnov Test | ,920 | Retain the null hypothesis. |
| 4 | The distribution of V4 is normal with mean 6.133,333 and standard deviation 115,90. | One-Sample Kolmogorov-Smirnov Test | ,846 | Retain the null hypothesis. |
| 5 | The distribution of V5 is normal with mean 5.700,000 and standard deviation 91,65. | One-Sample Kolmogorov-Smirnov Test | ,991 | Retain the null hypothesis. |
| 6 | The distribution of Martor is normal with mean 4.230,000 and standard deviation 85,44. | One-Sample Kolmogorov-Smirnov Test | ,999 | Retain the null hypothesis. |

Asymptotic significances are displayed. The significance level is ,05.

The influence of the foliar fertilization factor on wheat production is shown in Table 1. Foliar fertilization is an extremely dynamic technological factor that provides a significant increase in production depending on the type of fertilizer used.

The best production results were in V4 fertilization at all 3 rehearsals, and the lowest production was in the control variant.

Production increases reached up to about 2000 kg/ha, reflecting significant production differences.

When using foliar products, there are differences in the production and quality of wheat seeds compared to the control.

The foliar fertilizers used have had a beneficial effect on the plants in all the fertilization variants of the wheat culture, with an increased development.

Foliar fertilization is an effective measure of productivity development, with crop yields being observed.

Similarly, the graphical representations of Wet Gluten% and Humidity% are shown in Tables 5 and 6 and represent the synthesis of statistical data in their descriptive form.

**Table 3**
**The main values of attributes of quality wheat variety Sofru, under the SDE Timisoara**

| Replicates | Fertilization variant | The type of fertilizer | Hectolitric mass MH kg/hl | Wet gluten % | Humidity % |
|---|---|---|---|---|---|
| R1 | V1- | Ferti KEM 10:10:10+ME | 79 | 28,3 | 12 |
| | V2- | Ferti KEM 8:8:8+ME | 77 | 28 | 12 |
| | V3- | Ferti KEM 15:0:0+2S+1B+ME | 81 | 29,5 | 12 |
| | V4- | Ferti KEM 24:0:0+3Zn+ME | 83 | 31 | 12 |
| | V5- | Ferti KEM 15:0:0+5Zn+ME | 80 | 29 | 12 |
| | MT-martor | | 72 | 24 | 12,6 |
| R2 | V1- | Ferti KEM 10:10:10+ME | 80 | 28,5 | 12 |
| | V2- | Ferti KEM 8:8:8+ME | 78 | 28,1 | 12 |
| | V3- | Ferti KEM 15:0:0+2S+1B+ME | 82 | 30 | 12 |

| | | | | | |
|---|---|---|---|---|---|
| | **V4-** | Ferti KEM 24:0:0+3Zn+ME | 83,5 | 32 | 12 |
| | **V5-** | Ferti KEM 15:0:0+5Zn+ME | 82 | 31,2 | 12 |
| | **MT-martor** | | 72 | 24 | 12,6 |
| **R3** | **V1-** | Ferti KEM 10:10:10+ME | 78 | 28,5 | 12 |
| | **V2-** | Ferti KEM 8:8:8+ME | 77 | 27 | 12 |
| | **V3-** | Ferti KEM 15:0:0+2S+1B+ME | 80 | 29 | 12 |
| | **V4-** | Ferti KEM 24:0:0+3Zn+ME | 81 | 30,6 | 12 |
| | **V5-** | Ferti KEM 15:0:0+5Zn+ME | 79 | 29,2 | 12 |
| | **MT-martor** | | 71 | 23 | 12,6 |

**Table 4**
**Statistical data on Hectolitric mass MH kg/hl, highlighted by average, median and Standard Deviation**

| *V1-* | | *V2-* | | *V3-* | | *V4-* | | *V5-* | | *MT-martor* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| Mean | 79 | Mean | 77,33333 | Mean | 81 | Mean | 82,5 | Mean | 80,33333 | Mean | 71,66667 |
| Standard I | 0,57735 | Standard I | 0,333333 | Standard I | 0,577350269 | Standard I | 0,763763 | Standard I | 0,881917 | Standard I | 0,333333 |
| Median | 79 | Median | 77 | Median | 81 | Median | 83 | Median | 80 | Median | 72 |
| Standard I | 1 | Standard I | 0,57735 | Standard I | 1 | Standard I | 1,322876 | Standard I | 1,527525 | Standard I | 0,57735 |
| Sample Va | 1 | Sample Va | 0,333333 | Sample Va | 1 | Sample Va | 1,75 | Sample Va | 2,333333 | Sample Va | 0,333333 |
| Skewness | 0 | Skewness | 1,732051 | Skewness | 0 | Skewness | -1,45786 | Skewness | 0,93522 | Skewness | -1,73205 |
| Range | 2 | Range | 1 | Range | 2 | Range | 2,5 | Range | 3 | Range | 1 |
| Minimum | 78 | Minimum | 77 | Minimum | 80 | Minimum | 81 | Minimum | 79 | Minimum | 71 |
| Maximum | 80 | Maximum | 78 | Maximum | 82 | Maximum | 83,5 | Maximum | 82 | Maximum | 72 |
| Sum | 237 | Sum | 232 | Sum | 243 | Sum | 247,5 | Sum | 241 | Sum | 215 |
| Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 |
| Confidenc | 2,484138 | Confidenc | 1,434218 | Confidenc | 2,484137712 | Confidenc | 3,286205 | Confidenc | 3,794583 | Confidenc | 1,434218 |

The hectolitric mass (MH [kg / hl]) of wheat for baking as required must be above 75 kg / hl and is considered to be very good when the MH is above 78 kg / hl. As can be seen in Table 3 our values are very high, which shows us that foliar fertilization has significantly increased the quality of wheat.

The production increased by 33-45% compared to the control, by application of foliar fertilizers, the hectolitric mass, according to the standard is very good for variants 1, 3, 4 and 5, ie it is higher than 78 kg / hl, and at V2 is good, ie over 75 kg / hl.

Regarding wet gluten content, all variants received foliar fertilizer is classified as very good, that is over 25%. Particularly, V4 containing Zn, which produces the highest production, with higher hectolitric mass and the highest content in wet gluten, is highlighted.

**Table 5 Statistical data on Wet gluten %, highlighted by average, median and Standard Deviation**

| *V1-* | | *V2-* | | *V3-* | | *V4-* | | *V5-* | | *MT-martor* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| Mean | 28,43333 | Mean | 27,7 | Mean | 29,5 | Mean | 31,2 | Mean | 29,8 | Mean | 23,66667 |
| Standard E | 0,066667 | Standard E | 0,351188 | Standard E | 0,288675 | Standard Error | 0,416333 | Standard E | 0,702377 | Standard E | 0,333333 |
| Median | 28,5 | Median | 28 | Median | 29,5 | Median | 31 | Median | 29,2 | Median | 24 |
| Standard D | 0,11547 | Standard D | 0,608276 | Standard D | 0,5 | Standard Deviatior | 0,72111 | Standard D | 1,216553 | Standard D | 0,57735 |
| Sample Va | 0,013333 | Sample Va | 0,37 | Sample Va | 0,25 | Sample Variance | 0,52 | Sample Va | 1,48 | Sample Va | 0,333333 |
| Skewness | -1,73205 | Skewness | -1,67954 | Skewness | 0 | Skewness | 1,15207 | Skewness | 1,679536 | Skewness | -1,73205 |
| Range | 0,2 | Range | 1,1 | Range | 1 | Range | 1,4 | Range | 2,2 | Range | 1 |
| Minimum | 28,3 | Minimum | 27 | Minimum | 29 | Minimum | 30,6 | Minimum | 29 | Minimum | 23 |
| Maximum | 28,5 | Maximum | 28,1 | Maximum | 30 | Maximum | 32 | Maximum | 31,2 | Maximum | 24 |
| Sum | 85,3 | Sum | 83,1 | Sum | 88,5 | Sum | 93,6 | Sum | 89,4 | Sum | 71 |
| Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 |
| Confidenc | 0,286844 | Confidenc | 1,511042 | Confidenc | 1,242069 | Confidence Level(9 | 1,791337 | Confidenc | 3,022084 | Confidenc | 1,434218 |

**Table 6**

**Statistical data on Humidity %, highlighted by average, median and Standard Deviation**

| *V1-* | | *V2-* | | *V3-* | | *V4-* | | *V5-* | | *MT-martor* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| Mean | 12 | Mean | 12 | Mean | 12 | Mean | 12 | Mean | 12 | Mean | 12,6 |
| Standard E | 0 | Standard E | 0 | Standard E | 0 | Standard E | 0 | Standard E | 0 | Standard E | 0 |
| Median | 12 | Median | 12 | Median | 12 | Median | 12 | Median | 12 | Median | 12,6 |
| Standard D | 0 | Standard D | 0 | Standard D | 0 | Standard D | 0 | Standard D | 0 | Standard D | 0 |
| Sample Va | 0 | Sample Va | 0 | Sample Va | 0 | Sample Va | 0 | Sample Va | 0 | Sample Va | 0 |
| Range | 0 | Range | 0 | Range | 0 | Range | 0 | Range | 0 | Range | 0 |
| Minimum | 12 | Minimum | 12 | Minimum | 12 | Minimum | 12 | Minimum | 12 | Minimum | 12,6 |
| Maximum | 12 | Maximum | 12 | Maximum | 12 | Maximum | 12 | Maximum | 12 | Maximum | 12,6 |
| Sum | 36 | Sum | 36 | Sum | 36 | Sum | 36 | Sum | 36 | Sum | 37,8 |
| Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 | Count | 3 |
| Confidenc | 0 | Confidenc | 0 | Confidenc | 0 | Confidenc | 0 | Confidenc | 0 | Confidenc | 0 |

## CONCLUSIONS

The application of granular fertilizers has always been one of the key issues in plant production. High yield modern wheat varieties are increasingly demanding on production technology. High yields and good quality can only be achieved by applying foliar fertilizers that fully satisfy the nutritional requirements of culture.

Foliar fertilization is most effective as it blends harmoniously and under agrochemical analytical control with other agro-phytotechnical measures that potentiate the application of fertilizers.

We can note that besides a very significant influence of the climatic conditions, the most important contribution to the production of the grains during the experimental year was the foliar fertilization application.

No major changes were observed on the wheat culture, the breeding was normal, the foliar surface of the wheat plants was very well developed, the spikes were large and they were not attacked by diseases and pests. Focused foliar fertilization saves fertilizers due to increased absorption and efficiency.

Our research has shown that different fertilization with foliar fertilizers results in a significant increase in wheat.

The implications of our research underscore the importance of tailoring fertilization strategies, specifically through foliar applications, to realize a significant increase in wheat production. As agriculture continues to evolve, the adoption of precision techniques, such as targeted foliar fertilization, becomes integral to meeting the

demands of modern crop varieties and ensuring sustainable and efficient agricultural practices.

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# THE EVOLUTION OF SOME VEGETATION INDICES ON SOME PERMANET GRASSLANDS IN TIMIȘ COUNTY (WESTERN ROMANIA). CASE OF STUDY.

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## ABSTRACT

The areas occupied by permanent grasslands constitute one of the most important ecosystems of the Earth, occupying almost half of it. The use of land covered by grasslands and climate changes are the main stressors of grass communities and implicitly of grassland ecosystems.

Its multifunctionality (food source for ruminants, insect habitat, means of soil and water conservation, as well as outdoor activities, etc.) is gradually affected, thus diminishing the mentioned functions. The purpose of this paper is to analyze the evolution of some indices characterizing permanent grasslands over a three-year period 2020-2023. Observations were made for three years on two permanent grasslands located in the vicinity of Hitiaș and Căpăt (belonging to the same UAT Racovița). The monitored indices were the agronomic ones, namely the pastoral value (PV), the grazing capacity (GC), as well as the ecological ones (the Shannon-Weaver and Simpson indices). For each analyzed permanent grassland, three analysis points were taken and the resulting data represent the average. The analysis methods were the linear one to study the vegetation and the indices, and correlation analysis was used for the statistics.

Thus, it may be concluded that there are differences from one year to the next in the structure of the vegetation and in grassland communities. This aspect is also reflected in the agricultural fodder potential of the studied grasslands. The results of this study enable starting points for urgent measures to improve these permanent grasslands.

**Keywords:** dynamics, vegetation indices, pastoral value, biodiversity

## INTRODUCTION

Meadows represent almost half of the land area excluding the perimeters covered by ice; they intervene in the conservation of water, soil, biodiversity, and, finally, in the regulation of climate change. [1] Biodiversity loss is a challenge scientists must face. [2]

Disturbances generated by human activity, especially because of the improper use of land, lead to disruptions in various circuits (nitrogen, phosphorus, etc.). Globally,

the exclusion of overgrazing has become a large-scale management strategy to restore degraded meadows and sequester carbon. [2]

Grazing is one of the most important forms of land use covered with grassy vegetation, intervening in the maintenance of biodiversity, the microbial activity of the soil, as well as in the cycle of nutrients when it is practiced in an organized manner respecting the load of animals.

Intensive exploitation, under-exploitation (mowing, grazing) to abandonment are the main directions that influence grassy communities both at the level of the plant individual up to a larger scale through the disappearance for a longer period or forever of some species.

In Romania, agriculture is practiced on 56.8% of the territory (including permanent and temporary meadows) [3]. Membership of the European Union has led to a considerable increase in the amount of financial support allocated to agriculture in Romania.

Agricultural policies regarding permanent meadows in Romania have been regulated since 10 years ago. Updating and supplementing the legislation was done permanently in correlation with the situations encountered in the field [4]. However, the condition of the permanent meadows in Romania and in Banat, is still poor-mediocre to mediocre [4].

In this sense, specialists in meadows continuously monitor the main vegetation indices, their dynamics over longer periods of time to outline a general picture of permanent meadows in Banat and Romania.

**MATERIALS AND METHODS,**

In this paper, the grassy cover analysed is in the immediate vicinity of Hitiaș and Căpăt, two localities in the centre of Timiș County, Romania, in the Lugojului Plain, on the left side of the Timiș River, and belonging to the Racovița territorial administrative unit.

The permanent meadows were studied in the fall of 2020, during which the investigation was made regarding their history (form of ownership, management applied in the last 10 years, type of grazing animals). The floristic surveys were carried out three times a year (the first decade of April or the last decade of March, the last decade of June, the last decade of September) in five work points. To assess the analysed permanent meadows, the necessary steps were:

- Investigating the management history applied in the past;
- Determining the floristic composition and analysing the following indices: pastoral value (PV), grazing capacity (GC), and the Shannon-Weaver biodiversity index.

The purpose of this paper was to highlight the current state of the observed meadows, as well as the dynamics of the vegetation indices mentioned above in the current management context of these permanent meadows.

Therefore:

- The Hitiaș permanent meadow (HI) (45°42′59"N - 21°35′28"E) is located at an altitude of 101 m; it was exploited with sheep throughout the year, it is not restricted with an electric fence, and it was not cleaned up after the animals vacated the land. The pastoral investigation showed that it emerged

that there have been no improvement measures, and that it had been abandoned in the last two years.

- The Căpăt permanent meadow (CA) (45°40´50"N - 21°40´08"E) is located at an altitude of 118 m; it was exploited with sheep and, occasionally, with cattle; currently, irrational grazing is practiced, there was no cleaning done after the animals vacated the land (spreading manure, mowing uneaten remains and removing woody vegetation).

The linear method (Daget & Poissonet, 1971) [5] was used as a method to obtain the value of the indices taken into study. Correlation analysis and interpretation were used as a statistical method.

**RESULT DISCSSION**

The grass cover is a mixture of grasses (G), legumes (L) and plants from other botanical families (OBF).

Their coverage (A%) is influenced by the intensity and frequency with which they respond to disturbances related to grazing, mowing, and climatic conditions.The floristic composition of the two points studied is similar, but the distribution and coverage of the participating species is different.

In the Hitiaș permanent meadow (HI), the grasses (G) with the greatest coverage are *Festuca pratensis* L. and *Alopecurus pratensis* L. In 2023, a high presence of the species *Bothriochloa ischaemum* L. was noted.

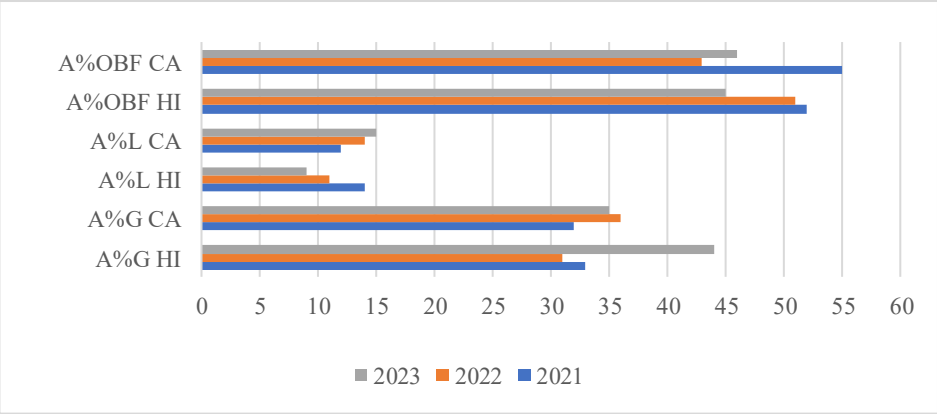



Fig. 1 Dynamics of cover (A%) of the main plant groups in permanent meadows (2021-2023)

The stratification of the vegetation is obvious: the upper layer includes the highest species *Leuchantemum vulgare* L., *Festuca pratensis* L. and *Alopecurus pratensis* L. Species populations such as *Symphitum officinale* L., *Juncus inflexus* L., *Rorripa sylvestris* L., and *Mentha arvensis* L. form the middle layer. The lower layer consists of small species such as *Potentilla reptans* L. and *Ranunculus repens* L.

In the Căpăt permanent meadow (CA), the grasses with the greatest coverage are *Poa pratensis* L. and *Alopecurus pratensis* L., the upper layer being occupied by dominant grasses joined by *Dactylis glomerata* L. and *Vicia grandiflora* L. The middle layer is represented by *Poa pratensis* L., *Trifolium pratense* L., *Hypericum perforatum* L., and *Lotus corniculatus* L. The lower layer is completed by species such as *Ononis*

*spinosa* L., *Trifolium repens* L., *Trifolium incarnatum* L., *Potentila argentea* L. and *Bellis perennis* L.

Analysing the dynamics of the main plant groups, it is seen that grasses in the Hitiaș permanent meadow (HI) increased their coverage to 44% compared to those in the Căpăt permanent meadow (CA) where there are no remarkable differences. Fig.1

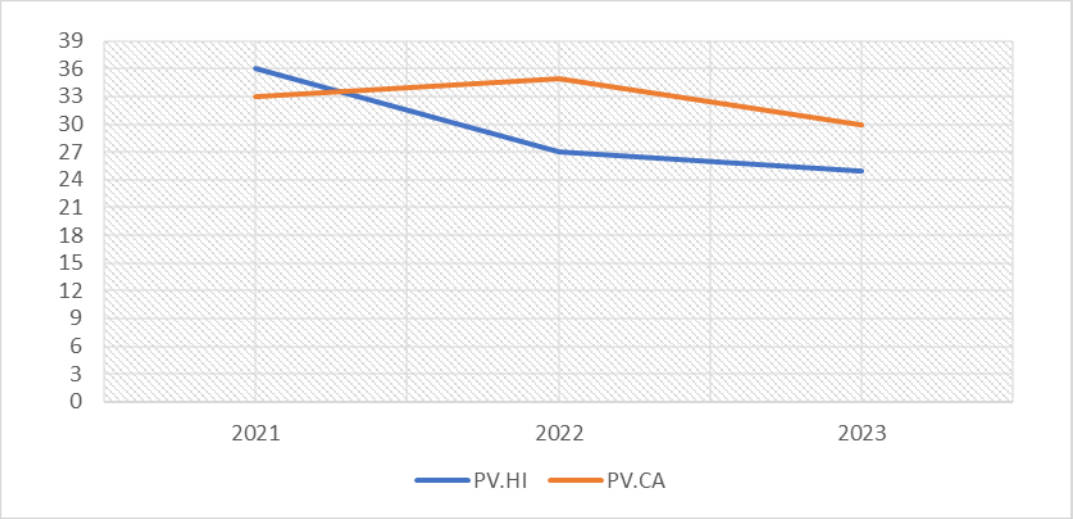



Fig. 2 Evolution of the pastoral value index (PV) on the analysed permanent meadows in the period 2021-2023

Plant species belonging to other botanical families (OBF) during the three years of the study did not differ in cover (A%) at group level, but they did at the within-group species level.

From the floristic surveys, it emerged that species such as *Achillea millefolium* L., *Taraxacum officinale* Weber L. and *Plantago media* L. decreased their coverage considerably in 2023.The modification of the floristic composition, i.e., of the aerial parts of the species, contributed significantly to the agro-economic value of the permanent meadows both from the point of view of green mass production and nutritional properties.

This aspect is eloquent in the Hitiaș permanent meadow (HI) and the dynamics of the pastoral value (PV) is also reflected. This decreases from PV = 36 to PV = 25, compared to that in the Căpăt permanent meadow (CA) where there are no noticeable changes, except, probably, for those related to seasonal fluctuations. Fig. 2

The pastoral value (PV) was associated with the animal load: thus, the critical load that maximizes the pastoral value and forage abilities of the vegetation was established [6]. In our case, the explanation for these differences is that the permanent Hitiaș permanent meadow (HI) was abandoned in the last two years. The evolution of the floristic composition depends particularly on the initial state and on the disturbances that take place over a period. Along with the pastoral value (PV) and the optimal duration of the grazing season, the grazing capacity (GC) is a very important parameter that has been little analysed in literature. In our case, the highest grazing capacity (GC) was in the Hitiaș permanent meadow (HI) – GC = 1.08 UVM (2021) whose dynamics decreased to 0.75 UVM.

Withdrawal of animals or abandonment reduces disturbance related to grazing. In this way, the competition between species is mainly based on the abilities of each of them for survival. This explains the fact that the coverage achieved by the legumes in the permanent Hitiaș permanent meadow (HI) decreased from A% = 14 (2021) to A%= 9 (2023) compared to their dynamics in the Căpăt permanent meadow (CA)

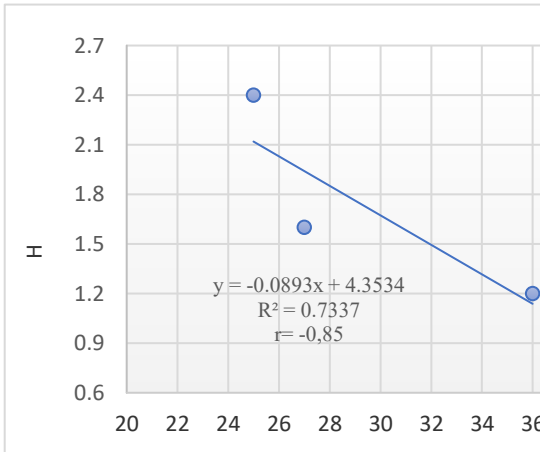



Fig. 3 Correlation between Pv and *(H')* in HI

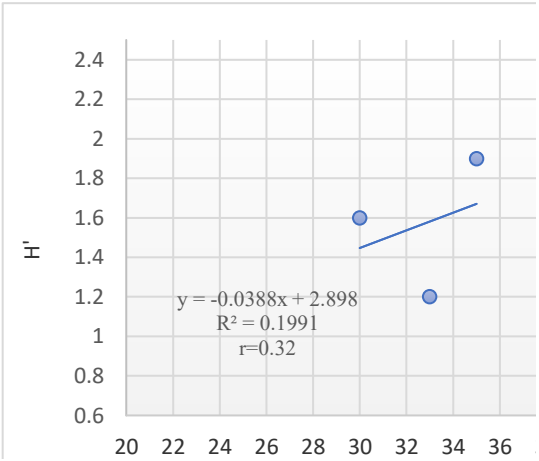



Fig. 4 Correlation between Pv and (*H'*) in CA

Increasing the number of uncut stems and inflorescences will further generate more dead mass at soil level preventing seed germination. This aspect influences, over time, the biodiversity in the analysed permanent meadows, especially in the Căpăt permanent meadow (HI).

The two analysed permanent meadows fall in terms of the Shannon-Weaver index as having a low species richness with evolution towards the average. Interpreting the values of the Shannon-Weaver index, it can be said that it increases from H' = 1.2 to 2.4 in the Hitiaș permanent meadow (HI) compared to the values of the Shannon – Weaver index in the Căpăt permanent meadow (CA) where the differences between the three years are insignificant. The creation of the Shannon-Weaver index value from H' = 1.2 to 2.4 in the Hitiaș permanent meadow (HI) is due to the appearance of annual species such as *Settaria viridis* L., *Anthyllis vulneraria* L., and *Trifolium incarnatum* L.

Thus, analysing the correlation between the values of the PV index and the values of the Shannon-Weaver index in the Hitiaș permanent meadow (HI), it can be said that there is a strong negative correlation between the two variables, the value of the regression coefficient being r = -0.85. Fig.3. In the case of the Căpăt permanent meadow (CA), there was no correlation between the two parameters, the value of the regression coefficient being r = 0.32. Fig.4

## CONCLUSION

In all vegetation samplings, plants from other botanical families were the most abundant compared to grasses and legumes.

The dynamics of the vegetation is in an intermediate stage of succession because of the competition of the participating species in the grass cover to occupy the land area necessary for survival, influencing the forage value (VP) of the studied

permanent meadows. Thus, the dynamics of the pastoral value index VP decreased from VP = 36 (2021) to VP = 25 (2023) in the case of the Hitiaș permanent meadow (HI) compared to the Căpăt permanent meadow (CA) where it did not have a well-defined trajectory.

Regarding the evolution of the Shannon-Weaver index, it increased after the abandonment on the Hitiaș permanent meadow (HI) and fluctuated very little on the Căpăt permanent meadow (CA).

The correlations between the pastoral value indices (PV) and the Shannon-Weaver index showed that the results of the regression coefficients r differ for the two analysed permanent meadows. It can be concluded that the withdrawal of grazing is an important vector in increasing biodiversity but at the expense of the fodder value, the permanent meadow being enriched with species without nutritional value.

Although considerable financial resources have been allocated in recent years for the development of projects whose mission was to implement effective management on permanent meadows in Romania, they hardly exceed the stage of average agro-fodder value.

Each of the two case studies carried out on the permanent meadows of Banat has brought new elements in the current context because studies of this kind were, for a good period, abandoned especially after December 1989; however, in the last 15 years, they were resumed.

Both previous and current research on the impact of management and other disturbance factors requires further investigation of the way they influence the stability of species in the meadow ecosystem and their resistance to fluctuations.

## ACKNOWLEDGEMENTS

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# THE REVOLUTION OF FUNCTIONAL FOOD: A MARKET ANALYSIS

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## ABSTRACT

Consumers are changing their food preferences in favor of healthier foods that have a beneficial effect on health and that embrace sustainable consumption patterns. To meet the demand of modern consumers, the food sector is undergoing a major revolution as more and more new products are appearing on the market. This trend has encouraged the food industry to develop a new category of food products, which is 'functional foods', characterized by the presence of one or more bioactive compounds having beneficial effects on the body. Therefore, functional foods represent a technological progress offering an important strategy to combat nutritional deficiency and diet-related diseases thanks to the presence of one or more bioactive compounds, can provide a benefit to the body. To distinguish such products and highlight their beneficial effect, claims are made on the product packaging about nutritional indication and about potential disease risk reduction. Such claims increase consumers' awareness about the benefit of these products hence can influence the consumer's purchasing decisions.
The aim of this work is to analyse the market size of 'Health and Wellness' foods, and in particular 'Functional Foods', over the last four years (2019-2022), using Euromonitor's database. A worldwide analysis has been carried out to identify the geographical areas with the greatest market activity and the European countries with the greatest economic importance. The analysis of the different food categories and the fortifications of greatest interest for the functional food market has provided an overview of the market for these foods.

**Keywords:** Functional Foods, Health and wellness, Market size, Food product trends.

## INTRODUCTION

Food choices in recent years determined an increase in demand for organic, naturally healthy, 'free from' and functional/fortified foods (FF), which can provide a benefit to the body or prevent the risk of certain diseases [1]. Technological advances in the food industry have improved and increased industrial productivity and enabled the reduction of food waste by facilitating the recovery of bioactive compounds from agro-industrial wastes and by-products. Moreover, since the Covid-19 pandemic, health has taken a leading role and the 'Health and Wellness' (HW) sector in 2020 has experienced significant growth [2].

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For these reasons, food industries have formulated a wide range of products, which fall under the category of 'functional food' (FF) [3]. To ensure the reliability of food products, several countries have started to assess and approve the health effects of certain substances in food through the establishment of specific commissions and/or governmental authorities [4]. For EU countries, the European Commission (EC) and the European Food Safety Authority (EFSA) are responsible for regulating food safety practices. Regulation (EC) No. 1924/2006 regulates nutrition and health claims made on foods in order to ensure the effective functioning of the internal market and a high level of consumer protection. The same regulation aims to ensure that claims on food labels are clear and supported by scientific evidence to protect consumer's health. FF represent one of the most interesting areas of research and innovation in the food industry and, like many food innovations, FF follow a cycle driven by two factors: the 'technology-push' dynamic that implies a strong transfer of technology and know-how from other sectors and the 'demand-pull effects' that depend on consumer acceptance of new products [5]. Therefore, the ability of a company to turn a technological adoption into a true innovation is strongly influenced by the market response. Food companies interested in using innovation as a competitive strategy must analyse changes in consumer choices and preferences [6], [7], [8]. Health claims are an important tool to communicate the health benefits of food to consumers and to guide them towards more informed choices. It is crucial for the food industry to intercept new market trends in order to be able to meet consumer demands, orientate its offer and gain a competitive advantage in the market [9]. Although FFs are becoming increasingly popular, with rosy predictions on their future development and demand, little is known about how these foods are perceived by consumers and how demand is segmented. FFs are still very risky from an investment perspective as new products often fail a few years after their introduction on the market [10]. There are three regions where the FF market is concentrated: Europe, Japan and the United States [11]. This paper shape a picture of the functional food market, by major food categories and enrichments, in quantity, value and per capita spending, in the 5 continents and the main European countries, in order to display the evolution of these foods and the development prospects for the future.

## MATERIALS AND METHODS

The analysis of the FF market was conducted using the Euromonitor database [12], which considers the following macro-areas as follows: Asia Pacific; Australasia; Eastern Europe; Western Europe; Latin America; Middle East and Africa; North America.

In order to be able to compare the continents with each other, the statistics for the individual countries were disaggregated and then re-aggregated homogeneously, by geographical, area to obtain the data for Asia, Europe, America and Oceania. For the African continent, only South Africa was considered because of the lack of further data for this macro-area.

The market segment taken into consideration was defined by Euromonitor as "Health and Wellness" (HW), which includes food and beverages divided into 6 categories: "fortified/functional", "organic", "better for you", "naturally healthy", "Health Benefit" and "Dietary and free from".

In this regard, it results important to underline that the Euromonitor database provides the following definitions for each category:

- *Fortified/functional* includes products to which healthy ingredients and/or nutrients have been added. The fortification must be highlighted on the label or contain a health/nutritional claim.

- *Better for you* refers to products in which the amount of a substance considered less healthy (e.g., fat, sugar, salt, carbohydrates) has been actively reduced during production.

- *Health Benefit* includes products that improve cardiovascular health, bone and joint health, brain and memory, digestive system, skin, eyesight; that boost energy or offer immune support.

- *Food intolerance* (or *'free from'*) comprehends gluten-free, lactose-free, allergen-free, dairy-free and meat-free.

- *Naturally healthy* contains products based on the natural content of a substance that improves health and well-being beyond the pure calorific value of the product.

- *Organic* refers to certified organic products produced, stored, processed, handled and marketed according to precise technical specifications (standards) and certified as 'organic'.

## RESULT

In 2022, the HW segment, globally, registered values for about $2,190 billion and the FF segment alone represented the 25% of the whole sector with values for about $556 billion, ranking first among HWs, followed by 'Better for you' ($552 billion). The FF market in 2022, as shown in Table 1, in terms of quantity, is evenly split among the three main continents leading this category of food: Asia, Europe and America. In absolute terms, however, America surpasses Asia and Europe. Oceania and Africa are negligible.

**Table 1.** Market size in quantity and value of "Health and Wellness" (2022) in the World (*)

| Continent | Functional/ Fortified | Better For You | Dietary/free From | Health Benefit | Natural | Organic |
|---|---|---|---|---|---|---|
| Asia | | | | | | |
| - 000 tonnes | 32,853 | 42,912 | 8,952 | 11,621 | 18,426 | 3,282 |
| - mm $ | 173,321 | 137,939 | 59,547 | 50,468 | 64,648 | 22,577 |
| Europa | | | | | | |
| - 000 tonnes | 31,763 | 35,197 | 21,207 | 6,541 | 21,227 | 4,101 |
| - mm $ | 152,726 | 144,256 | 196,793 | 27,657 | 91,974 | 33,158 |
| America | | | | | | |
| - 000 tonnes | 29,584 | 41,635 | 23,241 | 4,906 | 17,888 | 3,978 |
| - mm $ | 215,495 | 258,091 | 291,476 | 42,502 | 129,260 | 51,913 |
| Oceania | | | | | | |
| - 000 tonnes | 2,174 | 1,904 | 1,049 | 340 | 576 | 88 |
| - mm $ | 12,022 | 9,152 | 9,671 | 2,119 | 4,352 | 892 |
| Africa (**) | | | | | | |
| - 000 tonnes | 511 | 1,234 | 187 | 214 | 258 | 17 |
| - mm $ | 2,048 | 2,802 | 1,269 | 789 | 630 | 76 |

(*) Source: Euromonitor.
(**) Data from the African continent only refer to South Africa, as data from other countries are not available.

In particular, the FF market showed an upward trend in terms of volume over the four years from 2019 to 2022 (Table 2). Asia was the continent recording the highest growth rates, with growth rates of 150 in volume terms, rising from 22 million tonnes in 2019 to 33 million tonnes in 2022, while Europe and the Americas record growth rates of 28% and 44% respectively. The growth trend is also reflected in the market position, with Asia having the largest market size at around 33 million tonnes, followed by Europe at around 32 million tonnes and the Americas at around 30 million tonnes. The fastest growing sector globally in percentage terms was staple foods, followed by hot drinks and snacks. In contrast, in fortification, proteins, probiotics and antioxidants had the highest percentage growth [12].

The global FF market is expected to continue to grow in the coming years, driven by increasing awareness of the health benefits of good nutrition. However, the number of products is expected to decline slightly in the Americas and Europe, while the Asian continent is expected to continue to grow, albeit at a slower pace than in previous years [12].

There is also an overall increased value due to changing product prices over the period considered (2019-2022). America had the highest value in 2022, more than $215 billion; compared to 2019, it showed an increase of 20% in product value due to higher unit prices compared to other continents.

**Table 2.** Trend of market size in quantity and value of "Functional foods" in the World (*)

| Continent | 2019 | 2020 | 2021 | 2022 | 2019 | 2020 | 2021 | 2022 |
|---|---|---|---|---|---|---|---|---|
| | 000 tonnes | | | | mm $ | | | |
| Asia | 21,955 | 23,389 | 27,311 | 32,853 | 139,083 | 147,025 | 162,021 | 173,321 |
| | **100** | **107** | **124** | **150** | **100** | **106** | **116** | **125** |
| Europe | 24,761 | 27,216 | 28,400 | 31,763 | 131,956 | 146,619 | 150,073 | 152,726 |
| | **100** | **110** | **115** | **128** | **100** | **111** | **114** | **116** |
| America | 20,556 | 23,918 | 26,975 | 29,584 | 180,034 | 201,318 | 214,286 | 215,495 |
| | **100** | **116** | **131** | **144** | **100** | **112** | **119** | **120** |
| Oceania | 1,905 | 2,018 | 2,150 | 2,174 | 11,233 | 11,793 | 12,062 | 12,022 |
| | **100** | **106** | **113** | **114** | **100** | **105** | **107** | **107** |
| Africa** | 446 | 525 | 579 | 511 | 1,804 | 2,192 | 2,361 | 2,048 |
| | **100** | **118** | **130** | **115** | **100** | **122** | **131** | **114** |

(*) Source: Euromonitor.
(**) Data from the African continent only refer to South Africa, as data from other countries are not available.

Asia was the continent with the highest growth rate (+25%), rising from $139 billion in 2019 to $173 billion in 2022. Europe saw a steady increase in total value, rising from around $132 billion to around $153 billion, with an indexed value of 116. In the biennium 2019-2020, the European and American continents had the highest growth rates, while in the following biennium (2020-2021) Asia had a higher growth rate. Overall, Asia and the Americas both showed price decreases of around 17%, while Europe showed a decrease of only 10%. Only Oceania and Africa registered a lower fall in unit prices. The analysis of the examined data suggested an evolutionary picture with prices tending increasingly to fall in the near future (Figure 1).

Although the overall FF market appeared to be rapidly growing, the products were not distributed homogeneously across all market segments: the largest market share in volume terms was represented by the 'Dairy and alternatives' sub-category, followed by soft drinks and then 'Staple foods'. However, in terms of food fortification, the most important groups were vitamins and minerals, followed by proteins, antioxidant, fibers and probiotics [12].

The FF market in Europe was diverse and varied significantly from country to country; in fact, culinary culture and different consumer habits impact on the demand's size. In general, there was a higher level of consumer interest in HW and FF products in the Western European countries than in the Eastern European ones. The main European functional food markets, by value and volume, were the UK, Poland, Italy, Turkey, Russia, France, Germany, Spain, Ireland and the Netherlands.

The countries with the highest growth rate between 2019 and 2022 were Poland and Turkey; the latter with an increase rate of 87% went from 1.3 million tonnes to 2.4 million tonnes of product, while Poland saw its market increasing of 51% from 1.9 million tonnes in 2019 to 2.8 million tons in 2022.

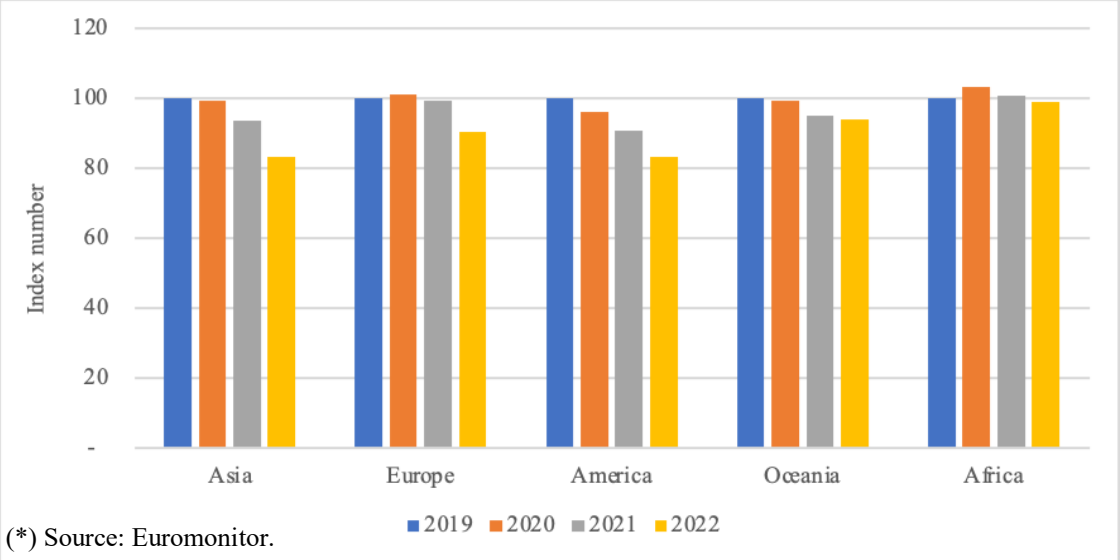



**Figure 1.** Dynamics of the price index for "functional foods" in the world.

For the other European countries, the growth indices over the four-year period showed clear differences (Table 3). In detail, the increase was 36% in Germany, 31% in the Netherlands, 26% in Ireland, 24% in the United Kingdom, 21% in Italy, 7% in France, 3% in Russia and 3% in Spain. However, the growth of FF was not constant, but varied from year to year.

**Table 3.** Trend of market size in quantity and value of "Functional foods" in selected country (*)

| Country | 2019 | 2020 | 2021 | 2022 | 2019 | 2020 | 2021 | 2022 |
|---|---|---|---|---|---|---|---|---|
| | 000 tonnes | | | | mm $ | | | |
| Poland | 1,878 | 2,180 | 2,329 | 2,832 | 6,989 | 8,488 | 8,815 | 9,762 |
| | **100** | **116** | **124** | **151** | **100** | **121** | **126** | **140** |
| Russia | 2,183 | 2,180 | 2,200 | 2,271 | 14,366 | 13,509 | 12,593 | 11,324 |
| | **100** | **100** | **101** | **104** | **100** | **94** | **88** | **79** |
| France | 1,942 | 2,097 | 2,107 | 2,069 | 9,585 | 10,971 | 11,417 | 11,221 |
| | **100** | **108** | **108** | **107** | **100** | **114** | **119** | **117** |
| Germany | 1,508 | 1,741 | 1,738 | 2,045 | 9,446 | 11,464 | 12,849 | 14,197 |
| | **100** | **115** | **115** | **136** | **100** | **121** | **136** | **150** |
| Ireland | 650 | 703 | 759 | 819 | 3,523 | 3,909 | 4,169 | 4,212 |
| | **100** | **108** | **117** | **126** | **100** | **111** | **118** | **120** |
| Italy | 2,324 | 2,467 | 2,677 | 2,818 | 15,798 | 17,367 | 17,851 | 18,332 |
| | **100** | **106** | **115** | **121** | **100** | **110** | **113** | **116** |
| Netherlands | 723 | 904 | 955 | 948 | 4,190 | 5,133 | 5,431 | 5,300 |
| | **100** | **125** | **132** | **131** | **100** | **123** | **130** | **126** |
| Spain | 1,444 | 1,492 | 1,449 | 1,482 | 5,313 | 5,712 | 5,570 | 5,425 |
| | **100** | **103** | **100** | **103** | **100** | **108** | **105** | **102** |
| Turkey | 1,284 | 1,347 | 1,477 | 2,395 | 3,628 | 3,655 | 3,546 | 3,697 |
| | **100** | **105** | **115** | **187** | **100** | **101** | **98** | **102** |
| United Kingdom | 4,840 | 5,307 | 5,591 | 5,998 | 31,580 | 34,033 | 34,476 | 34,882 |
| | **100** | **110** | **116** | **124** | **100** | **108** | **109** | **110** |
| Others Countries | 5,984 | 6,797 | 7,117 | 8,085 | 27,540 | 32,378 | 33,355 | 34,375 |
| | **100** | **114** | **119** | **135** | **100** | **118** | **121** | **125** |
| **World** | **24,760** | **27,215** | **28,399** | **31,762** | **131,956** | **146,619** | **150,073** | **152,726** |
| | **100** | **110** | **115** | **128** | **100** | **111** | **114** | **116** |

(*) Source: Euromonitor.

The two-year period, 2020 to 2021, in the middle of the Covid-19 pandemic, recorded the lowest growth rates, referred mostly to a decrease in the market volume for Germany and Spain, while the other countries kept their market shares broadly stable or even increased them (Poland, Ireland, Italy, the Netherlands, Turkey and the United Kingdom). The country with the highest market share was the United Kingdom with around 6 million tonnes, followed by Russia (2.3 million tonnes), Poland (2.8 million tonnes), Italy (2.8 million tonnes), Turkey (2.4 million tonnes), France and Germany (2.0 million tonnes each), Spain (1.5 million tonnes), the Netherlands (0.9 million tonnes) and Ireland (0.8 million tonnes). In terms of value, there were clear and significant differences among the different countries analysed, due to changes in food prices and the increase in the number of products. Germany and Poland recorded the largest increases of 50% and 40%, respectively, from $9.4 billion to $14.2 billion and from about $7.0 billion to $9.8 billion; Spain and Turkey kept their market share almost stable, despite fluctuations in the two-year period of Covid-19 pandemic (2020-2021), while Russia experienced a 21% decline over the four-year period. In absolute terms, the United Kingdom has the highest value ($33.4 billion), followed by Italy ($17.6 billion) and Germany ($13.6 million). The global trend towards minerals and vitamins as FF ingredients was also confirmed in Europe [12].

HW and also FF tended to be more expensive and for this reason the calculations of the average per capita expenditure confirmed that the countries with the highest per capita income were those in which consumption was highest. Figure 2 showed the per capita consumption of FF population categorized in normal, overweight and obese. In 2022, consumption for the normal population was $832 in Ireland, $519 in the UK and $311 in Italy. The consumption's

level was the lowest in Russia ($78) and Turkey ($43). Overweight and obese tended to consumed more the other categories analysed. Per capita consumption increased by a factor of 3 in the overweight population and by a factor of 4 in the obese population in all selected countries.

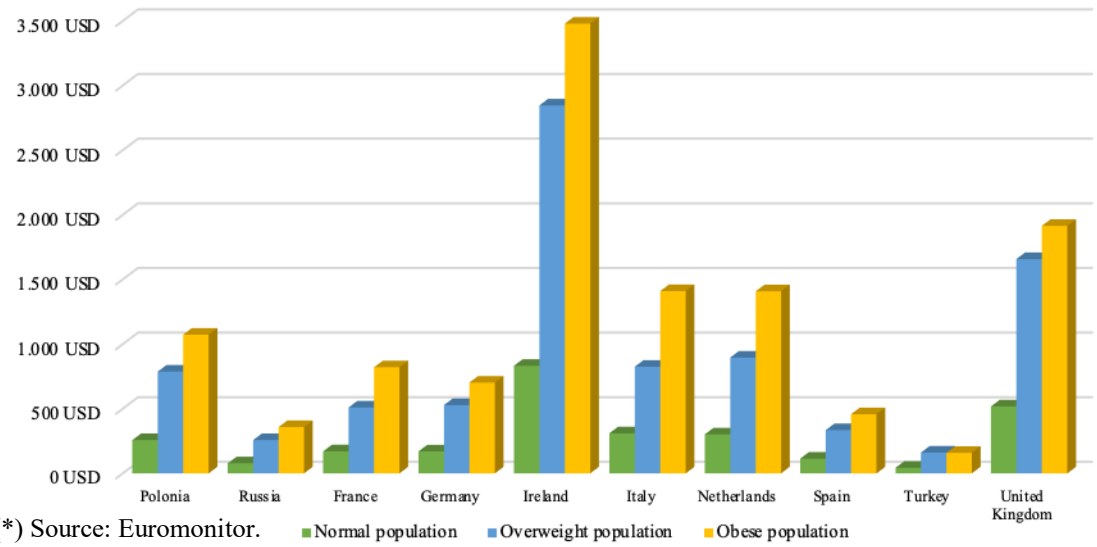



**Figure 2**. Per capita consumption of functional foods in selected European countries (*)

Looking at the population data of the main European countries analysed, the percentage of the overweight population always exceeded 35%, reaching the highest amount in France and Spain (40%), in the Netherlands (41%) and in Italy (44%). The percentage of the obese population was approximately 24% in the Netherlands and reached 33% in the United Kingdom and 36% in Turkey (Figure 3). By analysing the data, it resulted that the health status of the consumers influenced the consumption of HW and FF.

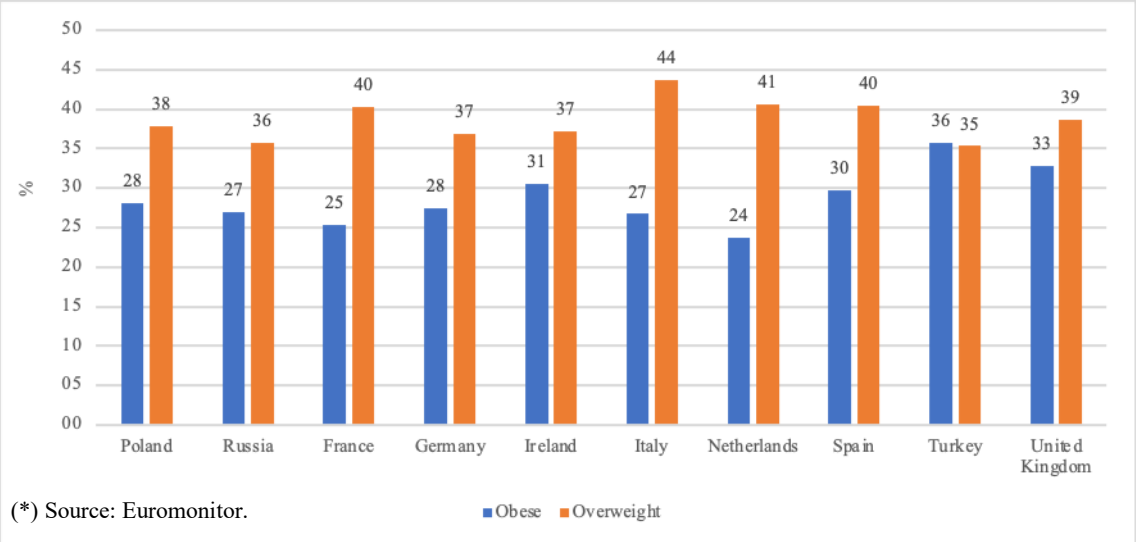



**Figure 3.** Population data in selected EU countries (*).

## DISCUSSION

The food industry is undergoing major change, with an ever-increasing number of new products being launched. The increase in functional food market size between 2019 and 2022 may be linked to the higher level of attention towards health factors stemming from the recent Covid-19 pandemic; in fact, consumers expect to find support in food for their overall health and immune system [13]. The data collected showed that functional food is not equally developed in all categories. The functional ingredients in food and carriers have an impact on consumer choice. In particular, the main added ingredients are minerals and vitamins being well known and perceived as safe by a wider range of consumers [7]. The carrier/ingredient combination was also important: combinations that have been on the market longer and are more familiar to consumers have a larger market size and are preferred by consumers. It resulted clear how food choices are related to family income levels: higher occupational social classes are associated with higher food expenditure and higher consumption of healthy foods. For these reasons, FF consumption is concentrated in countries with higher incomes [14]. The results also showed that consumers' health status, such as overweight or obese, influences their decision to consume FF.

## CONCLUSION

The agri-food sector has undergone significant changes over the last few years. Awareness of the role of diet in maintaining an optimal level of health and in preventing specific pathologies associated with nutritional imbalances (obesity, diabetes, hypertension) is driving the growth of the functional food market. According to the data examined, the functional foods market from 2019 to 2022 has known great growth across the globe. Therefore, these foods play an important role in the global economy, responding to the new habits of consumers, who seek foods that can benefit their health. The functional foods cannot be considered as a single category, but should be studied separately in relation to the different market target. Motivated by the need to gain a competitive advantage and to generate higher returns, several food companies are interested in entering this market. However, the process of new product development is complex, expensive and risky due to high failure rates [3]. In fact, there is no guarantee that a product will be a commercial success, even though the market for functional foods is rapidly expanding. Companies with an interest in this market must be aware of consumer demand, current legislation and the technical production conditions. They also need to have adequate resources for the development of FF and to investigate the perception of the products by consumers in the reference market. To support companies, scientific research on consumer's preferences and perceptions is considered essential to reduce the uncertainty associated with the development of these new products.

## ACKNOWLEDGEMENTS

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# THE USE OF BIOENZYMATIC INDICATORS LIKE PROTEASE AND ASPARAGINASE ENZYMES ON BISCUIT PRODUCTS

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## ABSTRACT

This article presents the rheological study of protease and asparaginase enzymes on dough obtained from white flour used for biscuits. Using alveographic method and falling number method we were able to determine the rheological characteristics of the dough used for biscuits and monitor the effects in the flour and finished products of protease and asparaginase enzymes taking into account different dosages. The technological process of preparing biscuits and crackers using protease and asparaginase becomes more healthy and efficient due to improvements in dough handling, volume and texture of the biscuits. Proteases catalyze the hydrolysis reaction of the peptide bond in the protein between the amino compound of one amino acid and the carboxyl compound of the next amino acid. This leads to the weakening of the gluten structure in the dough. Asparaginase catalyzes the conversion of asparagine and water to aspartic acid and ammonia. This conversion prevents the formation of acrylamide. The falling number and alveograph tests provide results which show that addition of protease and asparaginase to the dough improves the qualities of the finished product, the biscuits being crispier, more porous (they easily melt in the mouth) and more tender. Proteases increase the viscosity of the dough and decrease its stability and tolerance to kneading.

**Keywords**: protease, asparaginase, flour, alveographic method, falling number

## INTRODUCTION

Wheat flour is the most important ingredient in the baking industry. The qualitative characteristics directly and decisively influence the quality of products. Biscuits are obtained from a loose dough subjected to baking, having different forms and flavors due to a rich variety of ingredients used and their proportions, as well as due to different technological processes used. Having a high content of fats and carbohydrates, biscuits present a very important source of energy. Proteases catalyze the hydrolysis reaction of the peptide bond in the protein between the amino compound of one amino acid and the carboxyl compound of the next amino acid. This leads to the weakening of the gluten structure in the dough. Authors Rahil et al. studied wheat flour treated with proteases and

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carbohydrases in different dosages of 30 ppm, 60 ppm and 90 ppm to partially hydrolyze the dough polymers and to initiate molecular rearrangements to ultimately bring improvement in dough rheology and dimensional properties of biscuit.[1]. Study conducted by Hassan et al.showed that proteases significantly reduces the gluten index and reduces the stability, viscosity and softening of the dough [2]. Authors Gazi et al. showed the effectiveness of asparaginase on reducing acrylamide formation in bakery products according to their dough type and properties.[5] Tamba-Berehoiu et al. demonstrated that proteases can be used in baking for flours with strong gluten, or for normal flours with the aim of controlling certain rheological parameters, or in the case of technological processes for obtaining biscuits, wafers.[6]

## MATERIALS AND METHOD

### SAMPLES PREPARATION

The study shows the influence of protease and asparaginase enzymes on the rheological characteristics of wheat flour used in biscuits products. The rheological characteristics were determined using alveographic method and falling number method. The materials used in preparing dough samples include wheat flour, salt, water, yeast, protease and asparaginase enzymes. The preparation involves mixing each sample for 15 minutes in a laboratory mixer and then allowing it to rest for 20 minutes in a temperature-regulated compartment at 25°C. The first sample does not contain any enzyme, the other three samples have different dosages of enzymatic compound based on protease and asparaginase(P&A): 15g, 20g and 30g per 100kg flour. The falling index was determined using Perten Instruments FN 1310 and it indirectly measures the activity of enzymes, rapidly jellifying the aqueous suspension of flour which is introduced in a boiling water bath and it measures the enzymes liquefying action on the starch gel of each flour sample. As long as the falling time is low the enzymatic activity is more intense.

### METHODS OF ANALYSIS

The Falling Number measures the time a stirrer sinks through the gelatinized starch in order to determine the viscosity of a suspension that contains flour and water, which is heated close to the boiling point. The indicator is expressed in seconds and the optimal values should be between 220 - 280sec [3]

The alveographic method measures the strength and deformation characteristics of dough during the mixing and fermentation processes. The alveograph records various parameters during the test, including the maximum resistance the dough can offer (indicating its strength) and the ability of the dough to stretch and expand (indicating its extensibility). The rheological parameters recorded by the alveograme are: P- resistance to deformation (mm $H_2O$); L - extensibility (mm); G - expansion index; P/L - ration of dough strength and extensibility. W - water absorption capacity of the dough (Joule); Ie - elasticity index.[4] The optimal characteristics for flour used in the manufacturing of biscuits are doughs with a low specific resistance (low P values) and extremely extensible (high L values).

## RESULTS AND DISCUSSIONS

- The Falling Number.

An increased enzyme activity results in a reduced viscosity, which is measured by the time a metal rod falls through the starch gel. The method is widely used in commerce to measure the degree of germination of grains. Also, endogenous enzymes are well known for their adverse effects in the case of sprouting before harvesting. Determining the falling number, an empirical test based on the ability of enzymes to reduce the viscosity of heat-treated flour suspensions, is used on a large scale in the milling and bakery industry to estimate and appreciate the quality of bakery products of flour. As long as the falling time is low the enzymatic activity is more intense. The falling number results of the samples are presented in table 1 and figure 1:

Table 1. The falling number results of the samples: Sample A - no enzymes;Sample B - with 15g P&A/100kg flour;Sample C - with 20g P&A/100kg flour; Sample D - with 30g P&A/100kg flour

| **Samples** | **Sample A - no enzymes** | **Sample B - with 15g P&A/100kg flour** | **Sample C - with 20g P&A/100kg flour** | **Sample D - with 30g P&A/100kg flour** |
|---|---|---|---|---|
| **Falling number FN (s)** | **205** | **215** | **233** | **270** |

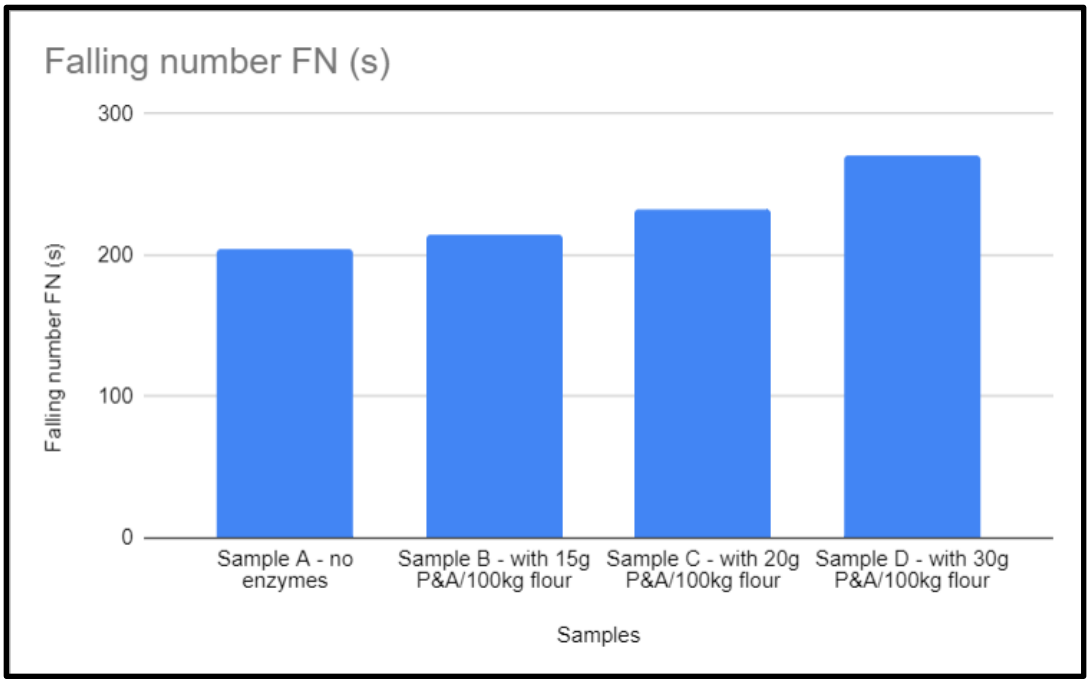



Figure 1. The falling number results of the samples: Sample A - no enzymes;Sample B - with 15g P&A/100 kg flour;Sample C - with 20g P&A/100 kg flour; Sample D - with 30g P&A/100 kg flour

Sample with the most intense enzymatic activity is sample A - no enzymes. Addition of protease and asparaginase enzyme compound increased falling time by 10 seconds for Sample B - with 15g P&A/100 kg flour, by 28 seconds for Sample C - with 20g P&A/100 kg flour and by 65 seconds for Sample D - with 30g P&A/100 kg flour, suggesting that the samples have decreased enzyme activity, making the dough more dense in correlation with the dosage of protease and asparaginase used.

- **Alveographic method**

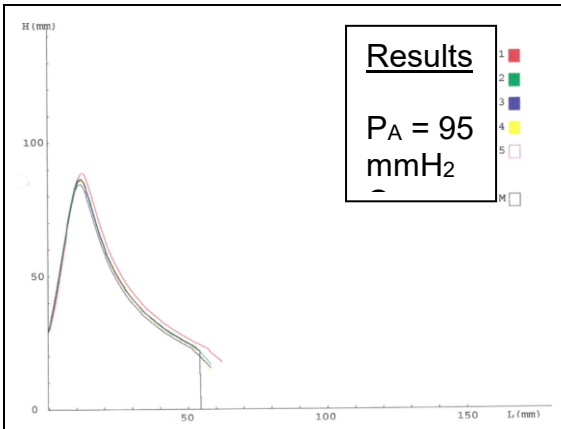



Figure 2. Alveograme of sample A - no enzymes

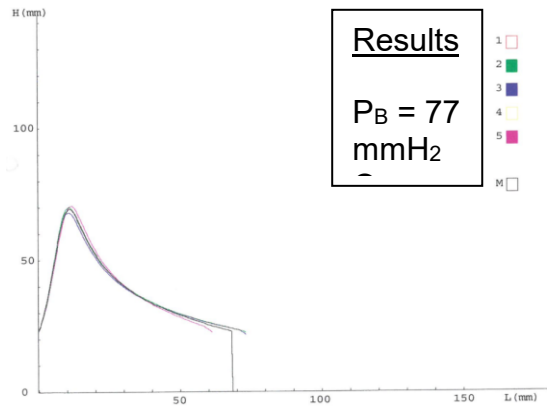



Figure 3. Alveograme of sample B - 15g P&A/100kg flour

In figure 2, the alveograme of sample A - no enzymes represents the dough sample that has no enzyme addition. The parameters present high values for the dough resistance to deformation ($P_A$) is 95 $mmH_2O$; configuration ratio ($P/L_A$) is 1,73 and very low values for the extensibility of the dough ($L_A$)which is 55mm and the extensibility index ($G_A$) which is 16.5. In figure 3 the alveograme of sample B - 15g P&A/100kg flour represents the dough sample that contains 15g of enzymatic compound based on protease and asparaginase per 100kg flour. Compared with sample A there are improvements regarding the dough strength ($P_B$) that decreases with 18 $mmH_2O$. Moreover, the extensibility characteristics have increased. For example dough extensibility ($L_B$) has increased with 13mm and the expansion index ($G_B$) with 1.9. Looking at the absorbed energy during the dough deformation we can see a small increase of $8x10^{-4}$J and regarding the elasticity index there is as well an increase of 11.7%. The $P/L_B$ ratio has decreased with 0.6 because of the dough strength and the dough extensibility. Using 15g of enzymatic compound based on protease and asparaginase per 100kg flour decreases dough tolerance for fermentation and the stability of dough and increases dough viscosity, but it still not enough for the technological processe used for biscuit products.

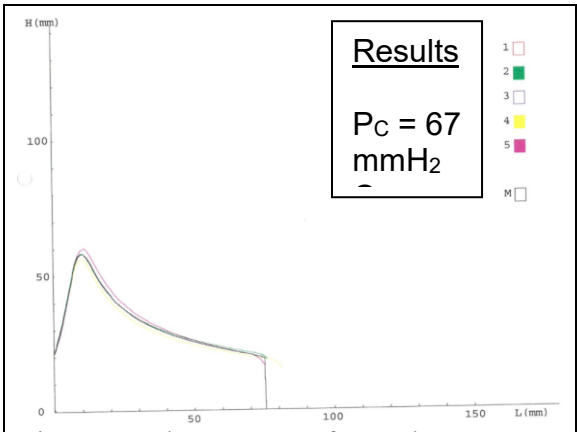



Figure 4. Alveogram of sample C - 20g P&A/100 kg flour

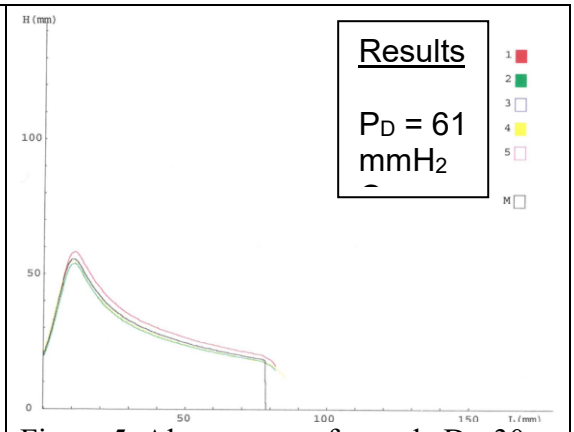



Figure 5. Alveogram of sample D - 30g P&A/100 kg flour

In figure 4 is the alveogram for the dough sample C - 20g P&A/100 kg flour that contains 20g of enzymatic compound based on protease and asparaginase per 100 kg flour. Addition of this dosage of P&A enzymes, decreased the dough strength even more ($P_C$) with 28 $mmH_2O$ compared to sample A - no enzymes. The absorbed energy during the dough deformation ($W_C$) and the $P/L_C$ ratio have been reduced as well and the dough extensibility characteristics ($L_C$ and $G_C$) are much higher than the values of dough sample A - no enzymes. Moreover, the elasticity index ($Ie_C$ ) has increased with 13%. In figure 5 is presented the alveogram of dough sample D - 30g P&A/100 kg flour that has in composition 30g of of enzymatic compound based on protease and asparaginase per 100 kg flour. Compared with sample A - no enzymes there are no significant improvements regarding all parameters. Using 30g P&A/100 kg suggest a better quality of the dough used for biscuit products. The dough resistance to deformation ($P_D$) has decreased and the dough extensibility characteristics ($L_D$ and $G_D$) are much higher than the values of dough sample A - no enzymes. Moreover, the elasticity index ($Ie_D$ ) has increased with 14% and the total quantity of absorbed energy during the dough deformation ($W_D$) is with $9x10^{-4}J$ lower than the value of dough sample A - no enzymes. In table 2 are presented the comparative results of the alveograms for the 4 samples:

Table 2.The alveogram results of the samples: Sample A - no enzymes;Sample B - with 15g P&A/100 kg flour;Sample C - with 20g P&A/100 kg flour; Sample D - with 30g P&A/100 kg flour

| **Samples** | **Sample A - no enzymes** | **Sample B - with 15g P&A/100kg flour** | **Sample C - with 20g P&A/100kg flour** | **Sample D - with 30g P&A/100kg flour** |
|---|---|---|---|---|
| **P($mmH_2O$)** | 95 | 77 | 67 | 61 |
| **L(mm)** | 55 | 68 | 75 | 79 |
| **G** | 16.5 | 18.4 | 19.3 | 19.8 |
| **W($10^{-4}J$)** | 169 | 177 | 160 | 158 |
| **P/L** | 1.73 | 1.13 | 0.85 | 0.77 |
| **Ie(%)** | 37.0 | 48.7 | 50 | 51.0 |

The data in table 2 shows that sample D - with 30g P&A/100 kg flour has the most suitable values for the manufacture of biscuits. The resistance of the dough to deformation ($P_D$) is low, the elasticity index ($Ie_D$) is higher than that of sample A, and the curve configuration ratio ($P/L_D$), as well as the total amount of energy absorbed by the dough when stretching ( $W_D$) are low compared to the control sample. The addition of enzymatic compound based on protease and asparaginase to the dough improves the qualities of the finished product, the biscuits being crispier, more porous (melts easily in the mouth) and more tender. Proteases increase the viscosity of the dough and decrease its stability and tolerance to kneading. Asparaginase minimizes the formation of potentially carcinogenic acrylamide, which is produced, in particular, during the baking of long-life baked goods such as biscuits.

## CONCLUSION

The falling number results present that sample A - no enzymes has the most intense enzymatic activity and that addition of protease and asparaginase enzyme compound increased falling time up to 65 seconds Sample D - with 30g P&A/100 kg flour, suggesting a decrease enzyme activity, making the dough more dense. The alveograph test shows that by using 30g P&A/100 kg flour in the preparation of the dough for biscuits, the resistance of the dough to deformation decreases and the elasticity index increases compared to the other samples. Using 15g P&A/100kg presents small improvements of the dough extensibility characteristics compared to sample A, but it has still lower values compared to samples C and D. Adding protease in the dough improves finished product quality, making the biscuits more crispy, more porous (easily melts in the mouth) and more tender. The proteases increase dough viscosity and decrease its stability and tolerance to kneading also improves dough extensibility and should be added very carefully in controlled amounts. They have irreversible weakening effects on gluten and therefore are used to treat very strong flours. Proteases help gluten softening during long fermentation at low pH of the dough for biscuits. Asparaginase is used in certain foods in order to reduce the amount of the amino acid asparagine that is already present in foods. Under specific cooking conditions, asparagine can react with certain carbohydrates in the food to form acrylamide, a potential human carcinogen. Reducing the amount of asparagine in the food prior to cooking or processing would reduce the amount of acrylamide that can be formed. The choice of the right dosage is made according to the rheological characteristics of the dough, and the correct amount to be added to the dough will be the one that will bring these characteristics to their maximum value.

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# THE VARIOUS STEPS TO PRODUCE BIOPLASTICS AND MEMBRANES FROM INSECTS OR CRUSTACEANS. APPLICATIONS AND GREEN METRICS

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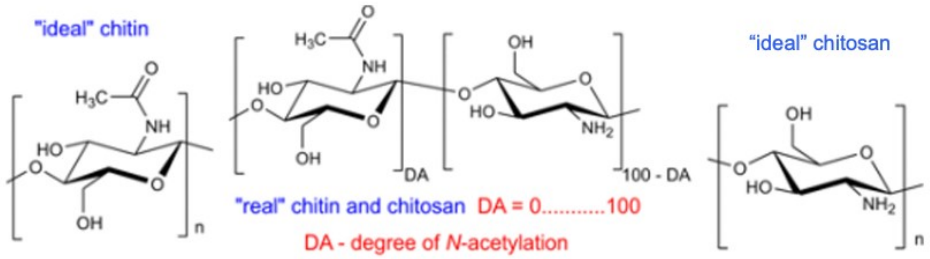
## ABSTRACT

Chitin and chitosan, which due to their wide applicability and abundance are of increasing interest to industries. Chitin is extracted from a wide range of living things by a variety of methods, which may be chemical or biological. Chitosan is obtained from the alkaline deacetylation of chitin by chemical or enzymatic processes.
Due to their different polysaccharide chains, chitin and chitosan have different properties. Chitin is thus an insoluble polymer in most solvents, while chitosan is a soluble polymer in acid solvents because there is protonation of the amide group of this polymer, which makes it soluble in acid solvents.
The source from which the greatest extraction of chitin occurs is from crustaceans such as shrimps, crabs, lobsters, crayfish, and clams. Extraction from crustaceans is an economical process, but seasonal, since most crustaceans are species that only belong to one season of the year. Since crustaceans are living beings that are not in abundance in the environment and in all seasons of the year, insects have been the subject of study for the extraction of chitin, since they exist in abundance in nature, throughout the year and in various parts of the world, being an excellent resource for a large-scale production of chitin and chitosan.
Chitosan is the base for bioplastics and membranes.in this work a review of the existing methods and applications will be surveyed.
Also, some green metrics regarding the synthesis of chitosan will be presented and discussed.

**Keywords**: Chitin; Chitosan; Extraction; Membranes; Bioplastics

## 1. INTRODUCTION

Chitin (poly (b-(1-4)-N-acetyl-D-glucosamine) is extracted from a wide range of food waste by a variety of methods, which may be chemical or biological. Chitosan is obtained from the alkaline deacetylation of chitin by chemical or enzymatic processes. [1] Chitin and chitosan have a similar chemical structure differing only in the percentage and position of the acetamide groups – Figure 1 [2]




100% - DD = DA

Figure 1 Chitin and Chitosan Structure

Chitosan will thus present a lower percentage of acetamide groups, since it comes from an alkaline deacetylation of chitin.[3] Due to their different polysaccharide chains, chitin and chitosan have different properties. Chitin is an insoluble polymer in most solvents [4], while chitosan is a soluble polymer in acidic solvents. This characteristic is due to the protonation of the amide group of this polymer. The protonation of this amide group occurs since this group has a pKb of 6,5.[5] The higher the percentage of deacetylation of chitosan, the greater its solubility in solvents with pH less than or equal to 7. [6] The main sources of chitin are crustaceans, fungi, molluscs, and insects. [7] - Figure 2 [8]

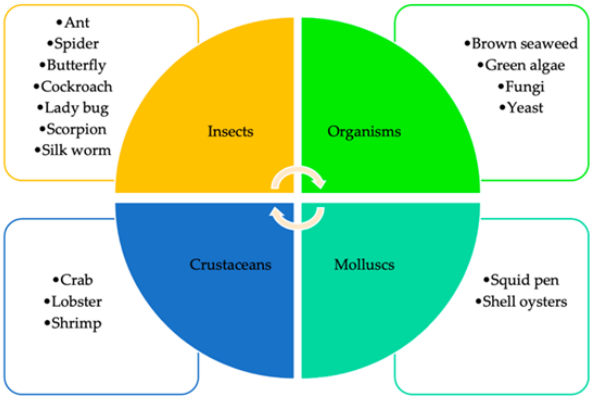



Figure 2 Sources of Chitin

Chitin, is a natural polysaccharide, the second most abundant worldwide, synthesised by various organisms. It has low toxicity and high resistance and flexibility.[9] It has three allomorphic crystalline types, which are found in different organisms. The different crystalline forms differ in the degree of hydration, the size of the unit cell and the

number and orientation of the chitin chains in the unit cells. [10] In the case of α-chitin , acquired from crab and shrimp shells [11], antiparallel chains are found, which are responsible for conferring rigidity to the polymer, due to their compact arrangement granted by hydrogen bonds. In contrast, the β-chitin configuration, found in molluscs, is composed of parallel chains, where the number of hydrogen bonds is reduced, but the number of hydrogen bridges establish with water is higher, as it is a less compact packing) and has a higher solubility. Thus, β-chitin presents a more flexible and softer structure. γ-chitin is a combination of the two types of chains (parallel and antiparallel) with properties referring to the α-type and the β-type. The crystalline form γ-chitin can be found in insect cocoons, fungi, or yeast. - Figure 3

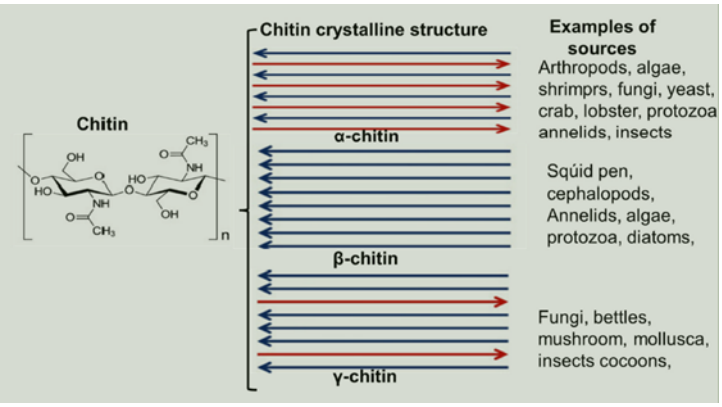



Figure 3 Crystalline allomorphic types of chitins

The greatest source for extraction of chitin currently is from crustacean's waste (from shrimps, crabs, lobsters, crayfish, and clams). [12] Extraction from crustaceans is an economical process, but a seasonal, one, since most crustaceans have a specific life cycle, belonging to one season of the year. Several studies have been conducted on different species of crustaceans, insects, and fungi to understand the amount of chitin these sources possess.[13] From studies carried out on the percentage of chitin in shrimp shells of different species, crabs, mussel shells, lobster and squid, it was found that shrimp and lobster shells have the highest percentage of chitin, which justifies in the case of shrimp its high use as a source of chitin and the vast existence of information on the extraction of chitin from this animal species. Mussel shells have the lowest chitin percentage of the crustaceans studied. In relation to insects, studies of chitin content carried out on different species reveal that pieris butterflies and diptera are a good source of chitin extraction, whereas the crab wasp has very low percentage values of chitin.

To solve the problem of season availability, insects have been the subject of study for the extraction of chitin, since they exist in abundance in nature, throughout the year and in various parts of the world, being an excellent resource for a large-scale production of chitin and chitosan. [14] The amounts of chitin vary with species, however, in general, exoskeletons contain 20-30% chitin, albeit mostly with lipids and pigments, with the remaining exoskeleton composed of proteins and minerals. [15] The fact that the quantities of chitin are identical in crustaceans and insects validates the studies and the interest in insects as an alternative source of chitosan. In the case of fungi, it is not an industrial choice since there is no controlled large-scale production of these species capable of meeting the requirements of reproducibility. The percentage of chitin in in

fungi's is lower when compared to crustaceans and insects. [16] After extracting chitin from a natural source, deacetylation is then carried out so that chitosan can be extracted. As chitosan is biocompatible and biodegradable it is used for various applications [17], such as, pharmaceutical, biomedical, cosmetic, food, textile, agricultural and construction. [18] More specifically, it has currently been used for the manufacture of biological adhesives, biofilm manufacturing and for biomedical applications due to its antibacterial and molecule affinity properties in vivo. [18] It has also been used for the manufacture of food coatings, due to its antimicrobial properties, for water treatment and membrane manufacture. [19] This paper will review chitin extraction methods, chitosan extraction methods and protocols for membrane and bioplastic manufacture. All these steps will be compared in terms of yield and chemical characteristics for each type of living being, crustacean and insect.

## 2. CHITIN EXTRACTION

For chitosan to be used in a wide variety of applications, a chitin extraction method must be applied to different organisms. The isolation of chitin from crustaceans, insects, molluscs, or fungi can be done by the conventional chemical extraction method, by the deep eutectic solvent extraction method or by the biological extraction method. [20]

### *2.1. Conventional chemical extraction*

All these organisms (crustaceans, insects, molluscs, and fungi) have proteins, mineral compounds, and melanin in addition to chitin in their constitution. Thus, chemical extraction consists of 3 stages: demineralization, deproteinization and decoloration, the first and second stages can be performed in the opposite order. - Figure 4 [20]

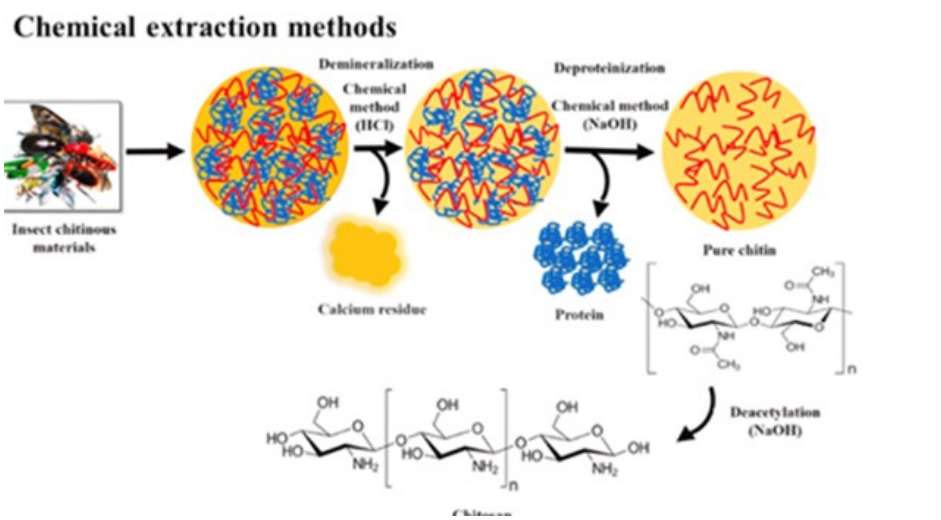



Figure 4 Chemical Extraction Steps

The deproteinization and demineralisation steps are necessary steps in the extraction of chitin from biomass. Although these do not have an order in which to be performed, the demineralization step first may bring benefits in chitin extraction. In addition to the demineralization step being an easier step than the deproteinization step, the deproteinization step being carried out first, increases the contact area between the strong base and the biomass in the deproteinization step which is a more complex step. The decoloration step may be optional.

### *2.1.1. Demineralization*

In demineralization, the minerals present in the organism type are removed. Strong inorganic acids are used for demineralization. The most used acid in this step is hydrochloric acid, which is used either at room temperature, at sub-zero temperatures or at higher temperatures.[21] In addition to hydrochloric acid, sulphuric acid, formic acid and ethanolic acid are also used. [21] As insects have less minerals in their constitution, they only need to have a light demineralization treatment. It is estimated that crustaceans have a percentage of minerals between 20 and 40% and insects a percentage of 10%. Therefore, they can be used in the lower concentration acid insects since they have a lower percentage of minerals in their constitution. [22] In both insects and crustaceans, the demineralisation treatment of chemical extraction has been done with the same acids.

### *2.1.2. Deproteinization*

In deproteinization, the disulphide and peptide bonds of the proteins are broken. A strong base is used for this break. The base used is NaOH in most of the deproteinizations. KOH can also be used. The advantage of using this base compared to NaOH is that NaOH is a base that gives rise to by-products containing sodium ion. This ion is extremely dangerous for the environment. [23]

### *2.1.3. Decoloration*

Decoloration is the last step of the chemical extraction and is an additional step. This step removes pigments from the chitin extracted from the organism. For decoloration NaOCl can be used, H2O2 or KMnO4. It can be seen from the article [24] that the chitin samples that do not undergo decolorization or the samples that are decolored with water are the ones with the highest chitin extraction yields.

Chemical extraction is relatively low cost due to the low cost associated with acidic and basic solutions. It is a method that does not require highly specialised reactors and has low reaction times. Comparing somewhat the chemical extraction method for insects and crustaceans, it is found that using the same extraction method, for example demineralization with 1 M HCl at 100°C for 20 min and deproteinization with 1 M NaOH at 85°C for 24 to 36 hours for the insect species Poriostracum Cicadae a chitin yield of 36.6% is obtained, for the crustacean Pandalus Boreals a yield of 6.89% [25], for the insect species Bombyx mori a yield of 15% is obtained [26] and finally for the crustacean species Penaeus Durarum a yield of 24%. A demineralization with 4 M HCl at 75°C for 2 hours and deproteinization with 4 M NaOH at 150°C for 18 hours in the insect species Melolontha Melolontha produced a yield of 14 % chitin and in the crustacean species Oniscus Asellus a yield of 7 %. [27] In this study, it was also found that chitin extracted from the insect species Melolontha melolontha was more porous and purer than that from the crustacean species Oniscus asellus.

### *2.2. Biological Extraction*

In chemical extraction, deproteinization is done with a strong base (most commonly used is NaOH) and demineralization is done with strong inorganic acids. To avoid all environmental contamination and the costs of organic solvents, chitin extraction by

biological method started to be used. Biological extraction can be done by enzymatic methods where chitin is isolated due to enzymes or by fermentation methods where chitin is isolated due to acids produced by a culture of organisms. In biological extraction, as organisms produce organic acids when they carry out fermentation, demineralization is done from these acids produced by the organisms, whereas deproteinization is done from enzymes produced by them. As well as being environmentally more favourable, this type of extraction produces relatively higher yields of chitin extraction than the conventional chemical extraction method. It was found in the article [28] that for shrimp shells, the deproteinization yield was 99 % and the demineralization yield was 87 % and the final chitin yield was 40 % higher than the yield obtained by chemical extraction. In the extraction of chitin in insects, in this case for the black soldier fly, the same was found. By the biological extraction a chitin yield of 33.9% is obtained and by the conventional method a yield of 9.2% is obtained. A disadvantage of this method is that although the biological extraction method produces higher final yields and are more environmentally friendly, it may not be as effective in removing minerals from biomass when extracting in industrial quantities and therefore sometimes high yields are obtained. Thus, demineralisation by lactic acid can be intensified by microwaves. This intensification leads to lower percentage values of minerals and proteins in the final chitin, so desmineralization is improved with the use of microwaves.[29] In many cases this type of extraction method requires a separate demineralisation step (which can be performed using the reagents used in the conventional method). Another disadvantage associated with this type of extraction process is the long fermentation times. The cost of the enzymes associated with this type of extraction, may also be a disadvantage of the process in question. [29] Regarding the chitin extraction yields presented between crustaceans and insects, in the case of the biological method, information is still rather scarce, especially concerning insects and therefore it is difficult to make comparisons between different organisms using the same method. However, it is still possible, with respect to crustaceans, to make a comparison of chitin extraction yields for the same species with different enzymes/bacteria. In the case of the Litopenaeus Vannamei species, when the acidophilic bacteria Lactobacillus is used, with a reaction time of 3 days, a chitin yield equal to 7.7% is obtained. When the bacteria used are Lactobacillus Ramnoides and Bacillus Amiloliquefaciens, with a reaction time of 7 days, the yield increases to 19.6%. Still with the Litopenaeus Vannamei species, when the protease of Streptomyces Griseus is used in chitin extraction, although its yield is not known, it is known that demineralization and deproteinization have higher yields than previous cases. [29]

### *2.3 Ionic Liquids, Deep Eutetic Solvents and Natural Deep Eutetic Solvents*

For conventional chemical extraction and biological extraction of chitin, demineralization and deproteinization steps are always necessary. Therefore, a new extraction method is used to avoid performing these two individual steps. Ionic liquids are thus used. Ionic liquids avoid performing these two steps and the use of strong solvents such as acids and bases. However, these liquids are hazardous solvents for the environment. As these solvents are environmentally hazardous, the use of deep eutectic solvents and natural deep eutectic solvents has started. To produce the deep eutectic solvents, a hydrogen bonding receptor (HBA) and a hydrogen bonding donor (HBD) are required. Hydrogen bonding receptors are for example choline chloride, betaine, choline bromide and betaine HCl and hydrogen donors for example glycerol, oxalic acid, L-

Lactic Acid, urea among others. After mixing and heating HBA and HBD a eutectic solvent with low melting point is formed. - Figure 5 [30]

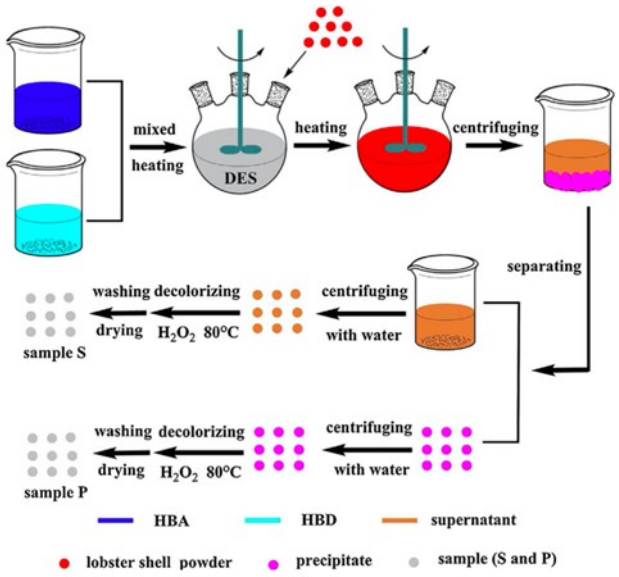



Figure 5 Preparation of Deep Eutectic Solvents

Natural deep eutectic solvents are preferable to deep eutectic solvents because environmentally they are more favourable and are biodegradable, biocompatible and can be recycled. These solvents have low melting points and high solubility. The disadvantage of the use of natural deep eutectic solvents in relation to conventional extraction is that although they produce higher yields of chitin they reduce the purity of chitin when the extraction is done by the conventional method. This can be verified in the referenced papers. These articles compare the yield and purity of chitin when it is extracted by chemical extraction or when it is extracted by deep eutectic solvents. This extraction method (deep eutectic solvents) was compared in insects and several crustacean species. For a deep eutectic solvent with choline chloride as HBA and urea as HBD, at a temperature of 80°C, for the insect Hermetia Illucens a chitin yield of 22.82 % was obtained in 2 hours and for the shrimp species Pandulus Borealis a yield of 18.77% was obtained in 6 hours at 80°C. For an extraction with a deep eutectic solvent of betaine (HBA) and urea (HBD) at a temperature of 50°C, for the insect Hermetia Illucens a yield of 26.71% was obtained, for the shrimp species Agaricus Bisporis a yield of 28.30% was obtained and for a shrimp sheel a yield of 26.30%, however this sample was subjected to microwave radiation. [31]

We can see that the yield of extraction by the same solvent on crustaceans and insects is very similar.

## 3. CHITIN TO CHITOSAN CONVERSION

Chitosan is obtained from the alkaline deacetylation of chitin. The degree of deacetylation is defined by the ratio of glucosamine: N-acetyl glucosamine. Strong bases are used for this deacetylation. Deacetylation of insect or crustacean chitin is done

in the same way. The most commonly used base for deacetylation is NaOH. Besides NaOH, KOH or KOH with ethanol and ethylene glycol can also be used. [21] In addition to using the strong bases NaOH or KOH, the biological method can also be used. In the biological method, enzymes from organisms are used for deacetylation.. Some deacetylation yields of crustacean and insect chitin are summarised below. In all the examples that will be presented, chitin was extracted using HCl demineralization and NaOH deproteinization. The summarised deacetylations were all done with the strong base NaOH between 40 and 55% and at a temperature between 80 and 100°C at different times. It should be noted that it is a little complicated to make comparisons of chitosan yields between crustaceans and insects since the chitosan yield also depends heavily on the method used in the extraction of chitin that often differs in the concentrations of acids and bases and because in some articles the chitosan yield presented is relative to the initial mass of biomass and in other articles relative to the mass of chitin. In some articles it is not mentioned how the yield is calculated. Thus, with regard to insects, for the Potato Beetle species, a chitosan yield of 72% was obtained, for the Mealworm beetle species (larvae) a yield of 80% was obtained (calculated from the mass of chitin extracted in the previous step) and for the Silkworm species a yield between 73% and 97% was obtained depending on the deacetylation time. For crustaceans, a yield of 36% was obtained for one shrimp shells, for prawn shells a yield of 25% (relative to the initial biomass mass) and for one crab a yield of 9.52% (relative to the initial biomass mass). In the article[32], the degree of deacetylation of chitosans from shrimp, beetles, bees and grasshoppers was compared, and it was found that the degree of deacetylation of chitosans from bees and grasshoppers was 96% and from shrimp was around 75%.

## 4. PREPARATION OF MEMBRANES

A membrane is a thin structure that separates 2 phases and controls the transfer of mass between these 2 media. Many membranes are being prepared from chitosan because chitosan is a biodegradable, biocompatible, soluble in acid pH, neutral and highly adsorptive polymer.[33] Chitosan membranes have several applications, namely in medicine and water treatment. In medicine, chitosan membranes can be used in drug delivery systems and for wound healing. For water treatment, chitosan membranes have high applicability because these membranes have a high adsorption character. The methods currently used in water treatment are chemical precipitation, ion exchange resins, electrochemical methods, ultrafiltration, micro and nano filtration, reverse osmosis and electrodialysis. However, the method applied when treating water with chitosan membranes is the adsorption method. The adsorption applied by chitosan is physical adsorption and chemical adsorption. Regarding chemical adsorption, at acidic pH, the amide group and hydroxide groups of chitosan are protonated, giving chitosan a positive charge. This state allows you to establish electrostatic bonds with anions (e.g., dyes), adsorbing them. At basic pH, deprotonation of the OH group of chitosan occurs, which can adsorb positive ions.[34] At a pH close to neutral, adsorption of metals by complexation may occur. Physical adsorption of chitosan may occur due to the establishment of Van der Walls bonds between the pollutant and the OH group of chitosan. Thus, chitosan membranes and composite chitosan membranes have been developed to improve membrane porosity and stability. Most of the articles found do not mention the source of chitosan used and the sources that are mentioned are only

from crustaceans so in this section the information is not enough to be able to make comparisons of membranes made from crustaceans and insects. Thus, the only information that can be analyzed is the characteristics that the membrane has when other compounds are added to it.

### *4.1 Membranes for Water Treatment*

In this section, the articles that will be mentioned do not indicate whether the chitosan used in the membrane comes from an organism, however, they are articles that test the properties of composite chitosan membranes in removing pollutants from water. Chitosan membranes in most articles are membranes that are prepared by dissolving chitosan in acetic acid, which are then dried and washed with an alkaline solution and deionized water. However, pure chitosan membranes are membranes that are low porous, have low mechanical strength and are very unstable. Thus, composite chitosan membranes began to be tested and prepared. The effects of adding crosslinking agents on the adsorption of chromium and mercury. Crosslinking agents are agents that are added to membranes with the aim of stabilizing the membrane, preventing its degradation, and establishing crosslinking of the membrane, improving its resistance. Thus, the adsorption of chromium ions by pure chitosan membranes, chitosan membranes crosslinked with glutaraldehyde and membranes crosslinked with epichlorohydrin were tested. It was found that the crosslinked membranes had greater adsorption power than the pure chitosan membrane and that the maximum adsorption capacity occurred on the chitosan membrane crosslinked with epichlorohydrin at pH 6.0. In article [35] the adsorption of mercury on pure chitosan membranes, membranes crosslinked with glutaraldehyde and membranes crosslinked with epichlorohydrin was also tested. It was also verified that the amount of adsorbed Hg (II) ions increased when the adsorbent was cross-linked, mainly with glutaraldehyde. In addition to the addition of cross-linking agents, the membrane morphology can be changed so that it has a greater adsorption power. In the article [35] the adsorption of ions by membranes with various concentrations of chitosan and various proportions of silica was studied. It was verified that the adsorption of copper ions is increased with the increase of the chitosan concentration (because there are more amide groups) and with the increase of the silica proportion (the surface is more rugosa and amide groups are more available). In addition to crosslinking agents and porogen agents, other polymers and compounds can be added to the membranes such as PVA, PEG or carbon. In article [35] it was found that a chitosan and PVA membrane had twice the adsorption of copper with the presence of MWCNT-NH2 (amino functionalized multi-walled carbon nanotubes). In addition to membranes for adsorbing heavy metals, membranes for adsorbing dyes and salts are also prepared. In article [35] a chitosan membrane is prepared with polyamide nanofilms (NCMs) which has a permeation of 52.8 L/m2·h of NaCl.

### *4.2 Membranes prepared from crustacean chitosan*

In the article [36] chitosan membranes are prepared from shrimp and crab shells and chitosan membranes with polyvinyl alcohol. These membranes were prepared by dissolving chitosan in acetic acid and neutralizing in NaOH solution. Tensile strength and tensile strength have been found to decrease when this NaOH treatment is prolonged. It is also verified that the addition of polyvinyl alcohol reduces the tensile strength and elasticity of the membrane in relation to pure chitosan membranes and that

increasing the degree of chitosan deacetylation decreases the permeability of the membrane. Membranes were also prepared from the dissolution of chitosan from shrimp shells in acetic acid and neutralization with NaOH and water. It was found that membrane permeability to NaCl, glucose and tyrosine is higher for membranes with lower molecular weight chitosan. The mechanical properties of a chitosan membrane from a snow crab with a chitin filament were analyzed. It was found that for a percentage of chitin filament in the membrane of 3%, the tensile strength increases from 38.5 Mpa to 110.3 Mpa and the elongation at break increases from 7.9% to 11.8%. As the percentage of chitin is increased in the membrane these mechanical properties are not improved, on the contrary, the tensile strength and elongation at break decrease. In the article [37] several composite membranes were prepared. It has been proved that the addition of glycerol makes the membrane more flexible, softer, and permeable, however, its mechanical strength is worsened. The addition of dialdehydes increases the mechanical resistance maintaining the Young's modulus, but the permeability is reduced. To improve membrane permeability, artificial pores such as silica and polyethylene glycol can be introduced.

In summary, most chitosan membranes are prepared from the dissolution of chitosan in acetic acid and the membranes may be composed of other components that increase permeability, tensile strength and elongation and porosity depending on the purpose of the membrane.

## 5. PREPARATION OF BIOPLASTICS

### *5.1 Bioplastics*

The wide range of applications of plastics and their low cost, translates into large-scale production of the material. However, their wide use and sometimes disposal and low durability of the material pose a threat to the environment. Some of the plastic waste accumulates in landfills and marine environments becoming a direct threat to living things. In the case of plastics derived from petroleum, they result in an increase of carbon dioxides (CO2) on earth, which in turn increases the greenhouse effect. Thus, the problems associated with the production, degradation, and contamination of plastic, gave rise to the production of materials that could bring advantages at all these levels, the bioplastics, and biodegradable bioplastics. It is important to mention their difference as not all bioplastics are considered biodegradable. Bioplastics are plastics derived from a biological basis, where the associated carbon and produced through renewable resources, partially or totally. Biodegradable plastics are polymers that under certain environmental conditions, the material breaks down into smaller particles transforming them into CO2, water, and biomass through enzymatic actions, they can be of biological origin or not. Adopting the production of bioplastics allows us to reduce our carbon footprint, it is a process that requires less energy and in turn is more environmentally friendly. Their responsiveness to the full range of applications is still under study, given that this is a relatively recent change. However, due to all the possible treatments that can be carried out in order to possess the desired mechanical and chemical characteristics, the applications and industries are equally vast. From packaging to electronic applications, for different sectors such as food, pharmaceutical, construction, textiles, and agriculture. [38]

### *5.2 Chitin and chitosan as a natural resource for bioplastics*

As mentioned, bioplastics are obtained by different natural sources, being chitin and chitosan, the ones discussed in this article. The sources and methods of extraction are those mentioned in sections 2 and 3. In the case of a chitosan-sourced bioplastic, this is a biodegradable bioplastic, with possible waste treatment by composting, high mechanical strength, and good properties for film production. Chitosan makes these bioplastics harder to break, improving their traction and elongation, increases the density of the bioplastic mass and reduces water absorption. (Like other bioplastics, it has a wide range of applications. [38] In the case of the production of bioplastics, it is not yet possible to compare their production with sources in insects and crustaceans, because the published articles only report on crustaceans and most of them do not even reveal the source of chitin and chitosan.

## 6. GREEN METRICS

Green Chemistry faces a constant challenge: evaluating the 'greenness' of chemical processes. It is widely recognized that it is not possible to control processes that cannot be quantified, resulting in the need to solve the measurement of the efficiency of a process. [39] To address this ethical problem, the creation and implementation of metrics becomes fundamental. Currently, several metrics have been developed, based on the Twelve Principles of Green Chemistry, with different levels of sophistication, to enable the estimation of the impacts associated with these processes [39]. Some of them are for specific analytical procedures, while others can be general and applicable to most methods. [40]

Within the various existing green metrics, the following stand out.

1. Atomic economy: focuses on the efficient use of atoms in chemical reactions. The objective is to reduce the production of waste and maximize the conversion of raw materials into desired products. Furthermore, it is desired to reduce the quantity of unwanted by-products produced. [40]

2. Atomic utilization: used to evaluate the efficiency of a chemical process in terms of the use of atoms present in the reactants. This metric compares the quantity of desired products with the total quantity of products, including waste. [40]

3. Carbon efficiency: used to evaluate the environmental performance of a process or product. It considers the amount of carbon dioxide emitted in relation to the resources used. [40]

4. Efficiency factor (E-factor): evaluates the effectiveness of a chemical process in comparison with the amount of waste generated. It is defined as the total mass of all waste generated during a process divided by the mass of the product obtained. [40]

5. Mass Intensity: used in green chemistry to evaluate the efficiency of a chemical process in terms of the amount of raw material used in relation to the mass of the final product. This metric calculates the mass of raw materials required to produce a specific quantity of product. [40]

6. Life cycle assessment: evaluates the effects of a process or product on the environment at all stages of the life cycle, from the extraction of raw materials to manufacturing, use and dismissal. The life cycle takes into account several factors, including use of natural resources, energy consumption, greenhouse gas emissions, impacts on air, water and soil quality, among others. [40]

## CONCLUSION

Throughout this review article, a review was made of the methods of chitin and chitosan extraction and the methods of manufacturing membranes and bioplastics. It was verified that there are more and more studies of chitin and chitosan extraction from insects. It is proven that it is possible to obtain considerable yields from chitin extraction in both insects and crustaceans. Since insects are highly reproducible living beings and are present in all seasons, more studies will have to be carried out in order to start applying chitin and chitosan extracted from insects in the same applications as chitin and chitosan extracted from crustaceans.
The sustainability of chitin extraction and its subsequent conversation in chitosan is a growing concern due to the environmental impacts associated with these processes. These processes not only result in the release of organic waste, but also require the use of chemicals, such as acids, which can have adverse impacts on the environment and human health.
In this work, the objective was to quantify the sustainability of the process, and for this metrics were used for a detailed analysis. The study addressed several aspects, including resource consumption, waste balance and energy use. This is a starting work and more developments will have to be done to improve our knowledge in green metrics.

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# THUJA OCCIDENTALIS AND PLATYCLADUS ORIENTALIS ANTIMICROBIAL ACTIVITY

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## ABSTRACT

The rise of bacterial resistance to currently employed antibiotics is causing growing concerns for public health. The emergence of highly resistant bacterial strains results in the ineffectiveness of antibiotic treatments against many bacterial infections. As a result, there is an ongoing quest for new antimicrobial agents. This pursuit can take two main directions: one involves the design and synthesis of novel agents, while the other involves exploring natural sources to uncover previously undiscovered antimicrobial compounds.
Herbal medications, particularly, have garnered renewed interest due to the perception that they tend to cause fewer adverse reactions when compared to synthetic pharmaceuticals. Moreover, the lower costs of producing plant-based preparations make searching for natural therapeutics appealing.
This study aims to assess the antimicrobial properties of *Thuja occidentalis* (TO) and *Platycladus orientalis* (PO) against Gram-positive and Gram-negative bacteria using specified reference strains: *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19114), *Bacillus cereus* (ATCC 10876), *Clostridium perfringens* (ATCC 13124), *Shigella flexneri (ATCC 12022), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Salmonella typhimurium (ATCC 14028)* and *Haemophilus influenzae type B (ATCC 10211)* Additionally, we determined the minimum inhibitory concentration (MIC). Following ISO 20776-1:2019 guidelines, we assessed the antimicrobial activity by measuring the reduction in microbial mass through spectrophotometry to determine changes in optical density (OD). Our findings indicate that the TO and PO extracts inhibit Gram-positive bacteria, particularly at the initial concentration tested.

**Keywords:** Gram-positive, Gram-negative, MIC

## INTRODUCTION

Before humans possessed knowledge of microbes, it was widely accepted that certain plants presented healing properties and contained what we now describe as active compounds with antimicrobial efficacy. From ancient times onward, people have used plants to treat common infections, and several of these traditional remedies continue to be employed for treating diverse illnesses.

Owing to the negative outcomes and the emergence of resistance among pathogenic microorganisms to antibiotics, research has recently been conducted exploring the extraction and biological activity of compounds derived from plant species, traditional in herbal medicine. Investigating plant-based antimicrobial agents provides a noteworthy and yet-to-be-exploited resource for pharmaceuticals. Thus, further exploration in this field is warranted. Antimicrobial compounds obtained from plants have significant therapeutic potential. They treat infectious diseases effectively and reduce the common side effects associated with synthetic antimicrobial drugs [1].

Thuja occidentalis (TO) and Platycladus orientalis (PO) are evergreen coniferous trees and belong to the *Cupressaceae* family.

TO is native to Eastern North America but is also grown in Europe and Brazil as an ornamental tree and is commonly known as the “tree of life” or “white cedar”. In conventional medicine, TO has addressed liver ailments, bullous bronchitis, psoriasis, enuresis, amenorrhea, cystitis, uterine cancers, diarrhoea, and rheumatism. Additionally, as a tincture, it has been utilised to manage warts, papillomas, and condylomas induced by the human papillomavirus (HPV) [2].

PO is a type of evergreen tree in the *Cupressaceae* family, native to China, known as the “Chinese thuja”. These trees have branchlets arranged in a flat, spreading or ascending manner. Chinese thuja has a long history of being utilised for landscaping purposes in China, with records of its planting in temples, mausoleums, and gardens dating back thousands of years [3]

Traditional Chinese Medicine utilises the branches and leaves of PO for various therapeutic uses, recognising it as one of the 50 essential herbs in China [4]. It is used to treat inflammation-related health problems, such as rheumatism, gout, rheumatoid arthritis, cystitis, cough and bronchial catarrh [5].

## MATERIALS AND METHODS

Plant material and extraction method

The leaves were harvested in March 2023, TO from Liebling, Timis County (45°34′00″N 21°19′54″E) and PO from Alba Iulia, Alba County, Romania (46°04′17″N 23°34′23″E). The leaves were divided into two samples: the first sample of fresh leaves was subjected to analysis, while the second sample was dried at room temperature until a constant weight was achieved.

A plant sample weighing 10 grams of dried leaves of TO and 10 grams of fresh leaves of PO was subjected to extraction using 100 mL of 70% ethanol (obtained from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). This extraction process was carried out with a Holt plate stirrer (manufactured by IDL, Freising, Germany) for 24 hours. Subsequently, the samples were filtered and stored at 4 °C for further microbiological analyses.

## Materials

The reference microbial strains (ATCC) used in this study were obtained from the culture collection of the Microbiology Laboratory within the Interdisciplinary Research Platform of the University of Life Sciences "King Mihai I" From Timisoara.
The sample was tested on the following reference strains: *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* type B (ATCC 10211), *Listeria monocytogenes* (ATCC 19114), *Bacillus cereus* (ATCC 10876) and *Clostridium perfringens* (ATCC 13124).
Minimum Biofilm Inhibitory Concentration (MBIC) is the lowest concentration of the antimicrobial agent to inhibit microorganism growth. Our previous research has described the method as a microbial mass loss by measuring OD by spectrophotometry in accordance with ISO 20776-1:2019.

## Microbiological method

A dilution of $10^{-3}$ of fresh strain was used to perform the test, an inoculum equivalent to a standard of 0.5 McFarland. The bacterial and fungal strains were revived by growing overnight in the Brain Heart Infusion (BHI) broth (Oxoid, CM1135) at 37 °C and subsequently switched to BHI Agar (Oxoid, CM1136) for 24 and 48 hours at 37 °C, respectively. The crops were then diluted to an optical density (OD) of 0.5 McFarland standard ($1.5 \times 10^8$CFU×mL) using BHI broth and evaluated with a McFarland densimeter (Grand-Bio, England). The dilutions were pipetted 100 µL into each well of the 96-well microdilution plate using a Calibra 852 digital multichannel pipette. The tested extracts were added at 1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL, 10 mg/mL, 20 mg/mL, 40 mg/mL and 80 mg/mL. The plates were covered and left for 24 hours at 37 °C. After 24 hours, the DO was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Tripled tests were performed for all samples. Strain suspensions and BHI were used as positive controls.
The MIC was determined by measurement of OD using the spectrophotometric method, according to ISO 20776-1:2019. The MIC is the lowest sample concentration tested at which there is no discernible growth of microorganisms. BGR (bacterial growth rate) and BIR (bacterial inhibition rate) were calculated as indicators for interpreting the results using the following formulas:

$$BGR = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%)$$

$$BIR = 100 - BGR\ (\%)$$

## TABLES AND GRAPHS

The results obtained as OD (optical density) values were used in calculations with the formulas (1) and (2) and the values shown in Figures 1, 2 and 3. All the results obtained and calculated are presented as follows in graphical form. The microbiological analysis of TO effect against Gram-positive bacteria revealed different evolution trends. The effect of TO against Gram-positive bacteria is presented in Figure 1.

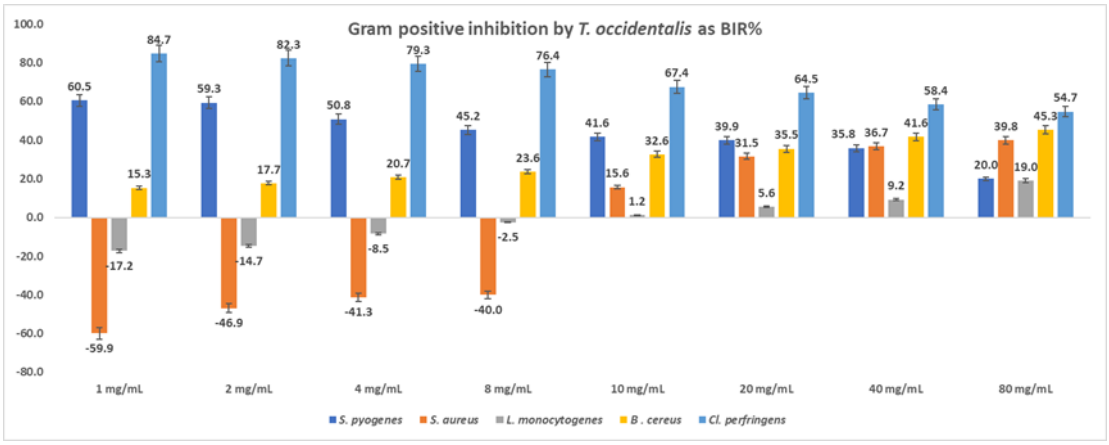



Fig. 1. BIR% of TO against Gram-positive strains tested

Figure 1 presents all the inhibition rates of the Gram-positive strain obtained after applying the 8 concentrations of TO selected (1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL, 10 mg/mL, 20 mg/mL, 40 mg/mL and 80 mg/mL).

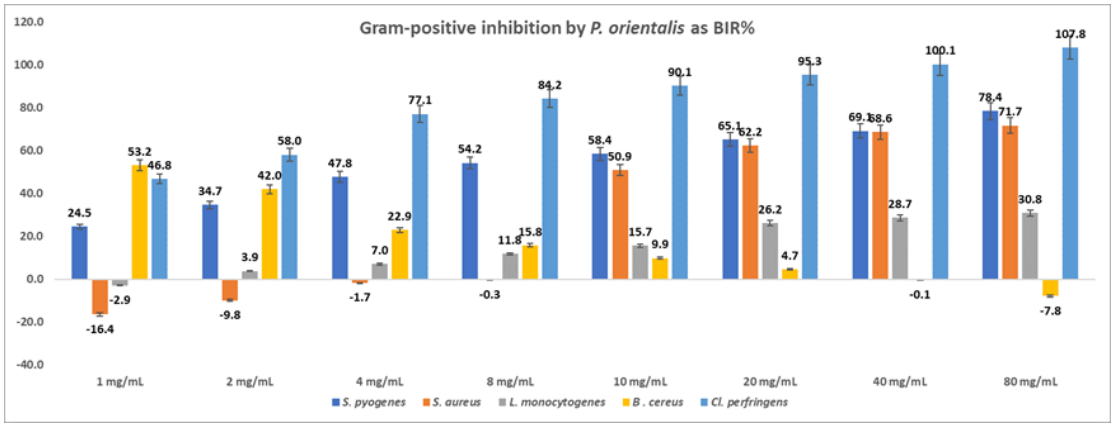



Fig. 2. BIR% of PO against Gram-positive strains tested

Figure 2 represents the BIR% values obtained by PO against the selected Gram-positive strains.

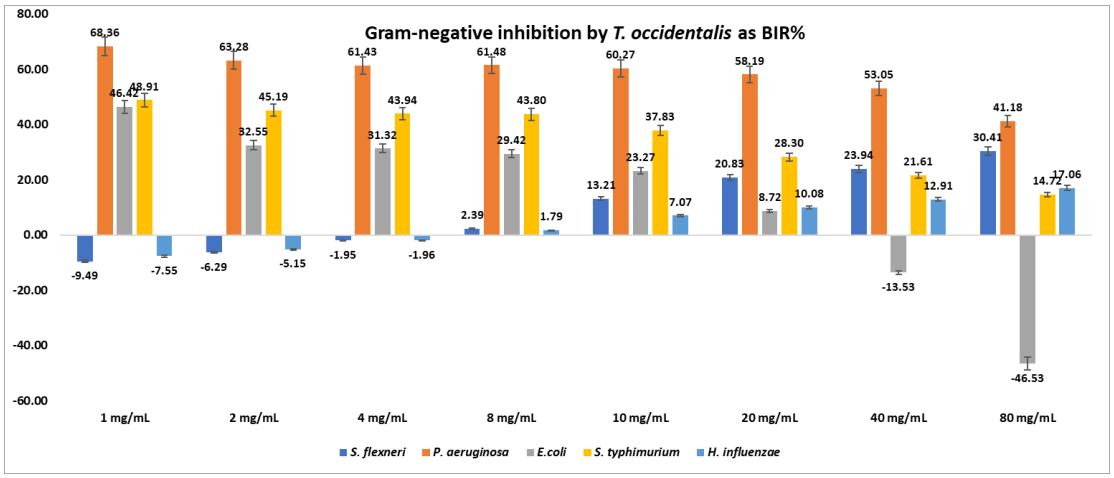



Fig. 3. BIR% of TO against Gram-negative bacteria tested

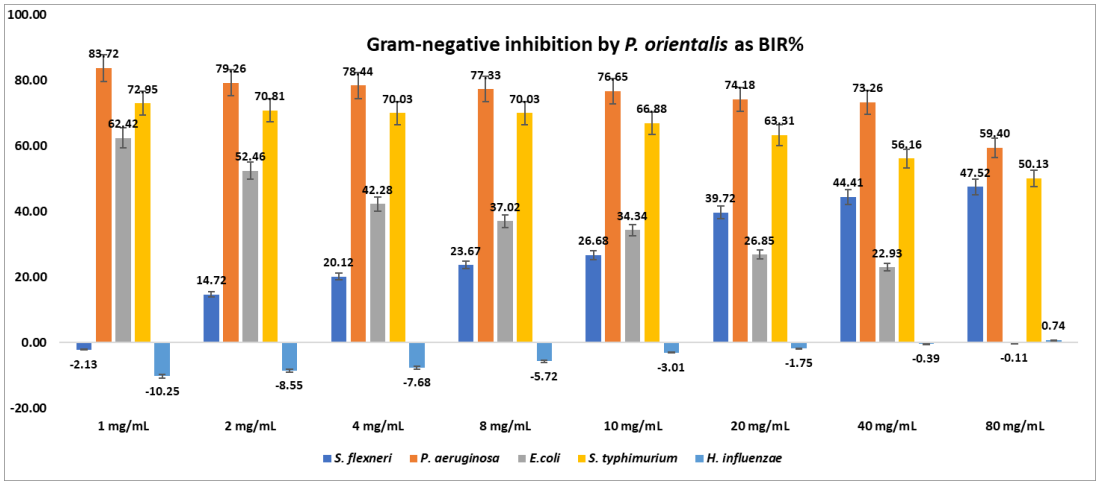



Fig. 4. BIR% of PO against Gram-negative bacteria tested

Figure 3 and 4 presents the BIR% of the selected ATCC Gram-negative strains on which TO and PO were tested at five concentrations (1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL, 10 mg/mL, 20 mg/mL, 40 mg/mL and 80 mg/mL).

Table 1. MIC of TO on the selected ATCC strains

| | *S. pyogenes* | *S. aureus* | *L. monocytogenes* | *B . cereus* | *Cl. perfringens* | *S. flexneri* | *P. aeruginosa* | *E.coli* | *S. typhimurium* | *H. influenzae* |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 mg/mL | | | | | | | | | | |
| 2 mg/mL | | | | | | | | | | |
| 4 mg/mL | | | | | | | | | | |
| 8 mg/mL | | | | | | | | | | |
| 10 mg/mL | | | | | | | | | | |
| 20 mg/mL | | | | | | | | | | |
| 40 mg/mL | | | | | | | | | | |
| 80 mg/mL | | | | | | | | | | |

Table 2. MIC of PO on the selected ATCC strains

| | *S. pyogenes* | *S. aureus* | *L. monocytogenes* | *B . cereus* | *Cl. perfringens* | *S. flexneri* | *P. aeruginosa* | *E.coli* | *S. typhimurium* | *H. influenzae* |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 mg/mL | | | | | | | | | | |
| 2 mg/mL | | | | | | | | | | |
| 4 mg/mL | | | | | | | | | | |
| 8 mg/mL | | | | | | | | | | |
| 10 mg/mL | | | | | | | | | | |
| 20 mg/mL | | | | | | | | | | |
| 40 mg/mL | | | | | | | | | | |
| 80 mg/mL | | | | | | | | | | |

The light grey colour represents the samples in which the MIC was found, but subsequent concentrations showed a potentiating effect, and therefore, the effect decreased together with the concentration. The red colour highlights the samples where the MIC was determined, and the yellow highlights negative values but with a positive correlation, the values increasing alongside the increase in concentration.

## RESULT

*S. pyogenes* proved a high sensitivity to TO starting at the lowest concentrations tested (1 mg/mL), with BIR% values being positive and ranging from 60.5%-20%. Nevertheless, the evolution is negatively correlated with the increase in concentration, with the BIR% values decreasing with the increase of the concentration tested. Concerning the inhibition of *S. aureus*, TO proves a positive trend correlated with the increase in concentration. Therefore, even if the first 4 concentrations tested

demonstrated negative BIR% values ranging from -59.9% to -40%, the evolution is positive, the maximum BIR% obtained being 39.8%. *L. monocytogenes* was the most resistant to the TO antibacterial activity, with small BIR% values that ranged from -17.2% to 19%. *B. cereus* had a positive evolution trend, with BIR% values correlated with the increase in concentration, BIR% reaching 45.3% at the last concentration tested. Regarding *Cl. perfringens*, TO demonstrates the highest BIR% values, even in a negative trend. Still, even if the inhibition decreased with the increase in concentration, the highest concentration tested reached a BIR% of 54.7%.

*P. orientalis* showed better activity against the selected ATTC Gram-positive strains. *S. aureus* was the only strain to present resistance at the first 4 concentrations tested, the positive BIR% values starting at 10 mg/mL tested, reaching a BIR% of 71.7% at 80 mg/mL. *S. pyogenes* and *Cl. perfringens* proved similar trends, in positive correlation with the increase in concentration and with BIR% values reaching 78.4% in the case of *S. pyogenes* and 107.8% for *Cl. perfringens*. *L. monocytogenes* had a higher resistance to the inhibitory activity of PO, reaching only a BIR% of 30.8% at 80 mg/mL. Concerning *B. cereus*, PO extract showed a strain-boosting effect, the BIR% values being negatively correlated with the increase in concentration. Therefore, the BIR% values ranged from 53.2% at 1 mg/mL tested and reached -7.8% at 80mg/mL.

TO exhibited average antibacterial activity against Gram-negative bacteria. *S. flexneri* was the only strain that showed negative BIR% values, the first positive BIR% registering at 8 mg/mL, reaching 30.41% at 80 mg/mL. *P. aeruginosa, S. typhimurium* and *E. coli* had a similar evolution, with high inhibitory values (ranging from 68.36%-41.18% for *P. aeruginosa*, 41.91%-14.72% for *S. typhimurium* and 46.42% to -46.53 for *E. coli*) but with a negative correlation with the increase in concentration.

PO registered good inhibitory results against Gram-negative bacteria. The least sensitive proved *H. influenzae* with BIR% values starting from -10.25% and reaching 0.74%, followed by *S. flexneri* (-2.13% to 47.52%). The common feature was that the evolution trend positively correlated with the increase in concentration. Despite presenting good BIR5 values, all other strains demonstrated negative evolution trends, decreasing efficacy with increased concentration. Nevertheless, P. aeruginosa and S. typhimurium showed high inhibitory values that recommend the usage of PO as an antibacterial agent against the mentioned strains.

Concerning the MIC values regarding TO (Table 1), the smallest concentration tested registered as MIC for most tested strains: *S. pyogenes, B. cereus, Cl. Perfringens, P. aeruginosa, E. coli, S. typhimurium* and *H. influenzae*. The difference was made by *S. aureus, L. monocytogenes* (10 mg/mL) and *S. flexneri* (8 mg/mL).

PO proved that the MIC for most of the strains tested was the 1 mg/mL concentration (*S. pyogenes, B. cereus, Cl. perfringens, P. aeruginosa, E. coli, S. typhimurium* and *H. influenzae*), while *L. monocytogenes* and *S. flexneri* were inhibited starting at 2 mg/mL, and *S. aureus* needed a concentration of 10 mg/mL to become MIC.

## DISCUSSION

Sah and colleagues demonstrated in their study that the extract from the leaves of TO has good antibacterial activity against the selected strains*: E. coli, P. aeruginosa, S. aureus, and Streptococcus spp*. [6].

The methanol-based extract from TO was tested *in vitro* for activity against Gram-positive and Gram-negative bacteria, which included *E. coli, Citrobacter, S. flexneri,*

*Yersinia aldovae, S. aureus,* and *P. aeruginosa.* The results indicated significant efficacy against all types of microorganisms, including those resistant [7].
In a different experiment, Deepika and colleagues examined the antimicrobial properties of a methanolic extract of TO on various microorganisms, including *B. cereus, Pseudomonas spp., Salmonella spp., B. subtilis, A. niger,* and *C. albicans*. They utilised the well diffusion assay method to evaluate the antimicrobial effects of the methanolic extract, which exhibited strong inhibitory activity against the tested microorganisms [8].
Nakuleshwar and colleagues, in their 2020 study, demonstrated that the extract from PO leaves has antimicrobial activity against all the strains selected (*S. aureus, B. subtilis, E. coli, and Agrobacterium tumefaciens*) [9].
Similar results to those in our experiment have been obtained by other researchers who have studied the antimicrobial effect of PO extract [10-13].

## CONCLUSION

Analysing all the data obtained as BIR% and MIC levels, we can state that both TO and PO have a higher efficacy against Gram-negative strains. Comparing the two extracts, PO demonstrated a higher activity as an antibacterial agent against Gram-positive and Gram-negative strains tested than TO. The positive evolution correlated with the increase in concentration suggests the usage of TO and PO against specific bacteria as antagonists to the strain growth. In contrast, the negative evolution trend suggests the existence of synergism between certain active compounds within the extracts and the bacterial cells.

## ACKNOWLEDGEMENTS

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# VOLTAMMETRIC BEHAVIOR AND DETERMINATION OF NICKEL IONS USING BIOPOLYMERS FOR RECEPTOR IMMOBILIZATION

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## ABSTRACT

Voltammetry is an electrochemical technique widely used in quantitative analysis to determine nickel ions in food samples due to its excellent stability. Knowing the exact applied levels of metals such as nickel in different food samples is very important to prevent high nickel consumption, especially in people with allergies and nickel excess. Electrochemical analytical methods appear to be an alternative with many advantages, including high sensitivity, low detection limits on the g/L scale, ease of use, and simple sample preparation. This study describes the voltammetric behavior of nickel biosensors using biopolymers such as agar, chitosan, alginate, and carrageenan, to immobilize the receptors and investigates the analytical performance using three biopolymer concentrations. The analytical performance of screen-printed carbon electrodes (SPEs) immobilized with the biopolymer-biosensor combinations was analyzed by linear sweep voltammograms (LSVs). The voltammetric behavior favored the method using carrageenan in terms of linear sweep voltammetry (LSV) performance characteristics with sensitivities of 6.79 for 0.5%, and 6.87 for 1% ($\mu A\ Mm^{-1}\ cm^{-2}$).

**Keywords:** biopolymers, electrochemical detection, nickel ions

## INTRODUCTION

Nickel is ubiquitous in the environment, present in water, air, soil, and biological materials. However, for humans, the major source of nickel intake is diet [1]. Nickel (Ni), is the metallic component of urease, and it is essential for plants and some livestock. Effects on the human body that have occurred from nickel exposure include damage to the skin, nervous system, lungs, and mucous membranes [2]. Compared with other transition metals, Ni is a moderately toxic element. However, inhalation of nickel and its compounds is known to cause several problems including respiratory cancer [3]. Therefore, knowing the exact applied levels of metals such as nickel in different food samples is very important to prevent high nickel consumption, especially in people with allergies and nickel excess [4]. Enzyme-based chemical biosensors are based on biological recognition [5]. To function, an enzyme must be able to catalyze a specific biochemical reaction and must remain stable under the normal operating conditions of the biosensor. Biosensor design is based on knowledge of the analyte of interest and the complexity of the matrix in which the analyte needs to be quantified [6]. Biopolymer (BF) films on electrode surfaces can enhance performance and can be considered a

widely used alternative to highly toxic electrodes. Although more environmentally friendly and less toxic, BF is recommended. Fabrication of electrochemical platforms without the use of metal thin films could lead to non-toxic sensing technologies for future applications [7]. Therefore, we need an analytical method to detect low concentrations of metals in food and water. There are various methods for metal analysis such as atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), inductively coupled plasma mass spectrometry (ICP-MS), and electrochemical methods [8,9]. Obtaining detailed speciation analysis of metals requires prior extraction, which may result in slight changes in the chemical form of the metal during extraction, while also significantly increasing the potential for sample contamination. Electrochemical analytical methods appear to be an alternative with many advantages, including high sensitivity, low detection limits on the g/L scale, ease of use, and simple sample preparation [10]. In this study, we describe a method for the detection of nickel in foods using four biopolymers (alginate, agar, chitosan, and carrageenan) for immobilization of urease, and three biopolymer concentrations for optimization. We measured with linear sweep voltammetry the analytical performance characteristics of four biopolymers.

## MATERIALS AND METHODS

### *2.1. Reagents*

We used pure chemicals to test the efficiency of four biopolymers for immobilizing receptors on screen-printed electrodes: nickel sulfate standard solution (0.5–10 mg/L); ammonium buffer solution at pH=8 as electrolyte solution, urease (0.1%) as receptor and biopolymers: agar, carrageenan, alginate and chitosan (acetic acid solution as solvent (0.1M)) were obtained from Sigma Aldrich Chemical Company. We used three concentrations of all four biopolymers for the above reagents: 0.5%, 1%, and 1.5%. We fixed urease with each biopolymer on a disposable screen-printed carbon-based electrode (SPE) (DRP110, dimensions: 3.4 x 1.0 x 0.05 cm). All solutions were prepared with 18.2 M resistivity deionized water (Millipore, Direct-Q 3 UV).

### *2.2. Voltammetric measurements*

Estimating the amount of bound urease is important to determine the efficiency of the immobilization method since nickel ions are known not to interact with receptors at low levels/concentrations. We measured the voltammetric behavior of the urease-biopolymer interaction during immobilization linear sweep voltammetry. For LSV measurements, unlike potential step measurements, the current response is shown as a function of voltage rather than time. The sweep starts on the left side of the current/voltage graph, where no current flows. As the voltage moves further to the right (meaning higher oxidizing conditions), the current starts to flow and eventually peaks before falling. To explain this behavior, we need to consider the voltage's effect on the electrode surface's balance. For all experimental measurements, we measure voltammograms using a Metrohm Autolab bi-potentiometer μStat 300 and DropView 8400 software. Voltammetric analyses were performed at a temperature of 22 ± 2°C with a 50 μL sample solution containing the supporting electrolyte and nickel standard solution. We used four types of biopolymers (agar, alginate, carrageenan, and chitosan) to fix urease receptor. These four biopolymers were used for the experiments also by cyclic voltammetry in a previous study [11] with the same measurement conditions.

### *2.3. Procedures*

Biosensors with four biopolymers and three concentrations were measured using linear sweep voltammetry (LSV) over a potential range from −0.1 to 0.9 V, with a step potential of 0.002 V and a sampling rate of 0.05 V/s. We tested the concentrations of four receptor combinations to achieve functional biosensing interactions with nickel. A control experiment in which urease was immobilized with a biopolymer was performed under the same measurement conditions but without nickel. We use LSV techniques here because they work well in previous work [12]. Here we use these techniques to evaluate biosensor performance for different types and concentrations of biopolymers on carbon electrodes, which are inexpensive than silver electrodes.

### *2.4. Immobilization of urease on SPEs*

In contrast to the agar, alginate, (b) ırrageenan which were prepared in deionized water; chitosan was prepared in 0. :etic acid solution. 1 µL of urease was pipetted onto the SPE surface and fixed with 1 µL of biopolymers at 22°C for 3 hr, and each biopolymer was measured by LSV.

### *2.5. Biosensor performance*

Characteristics of performance for developed biosensors are measured by sensitivity, detection limit, and selectivity. The selectivity of the biosensor was measured at 10 mg/L samples using nickel-generated current since this concentration was found to be the maximum in food. Selectivity was evaluated for each biopolymer used in the biosensors at different LSV concentrations. Minerals analysed using the same technique and conditions were: Zinc, Cadmium, Copper, Iron, Magnesium, Sodium, and Calcium.

## RESULTS

### *3.1. Voltammetric behavior of urease fixed in four biopolymers*

Linear sweep voltammograms resulting from agar immobilized with urease and a nickel standard solution are presented in Figure 1. The LSV detection method illustrates the strength of capacitive currents for the biosensor combinations we tested.

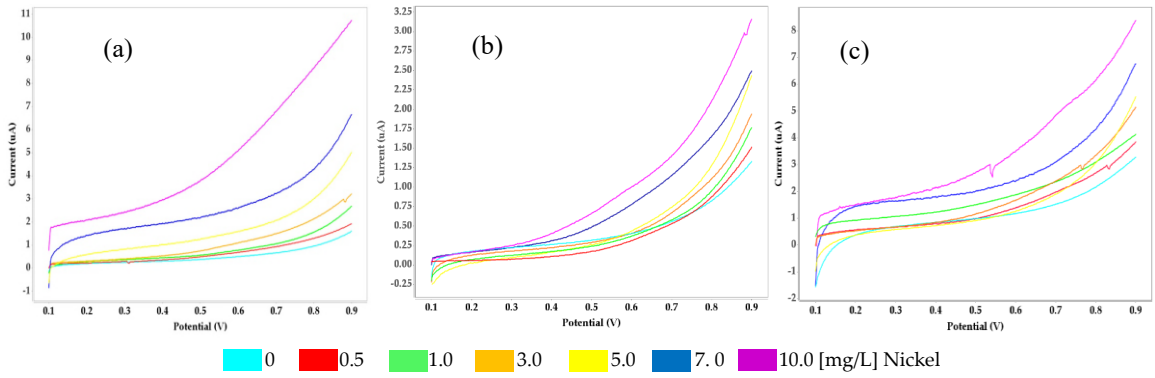



**Figure 1**. Voltammograms for three concentrations of agar (a) 0.5%, (b) 1%, (c) 1.5%.

Calibration curves for voltammograms for the three concentrations of agar are presented in Figure 2.

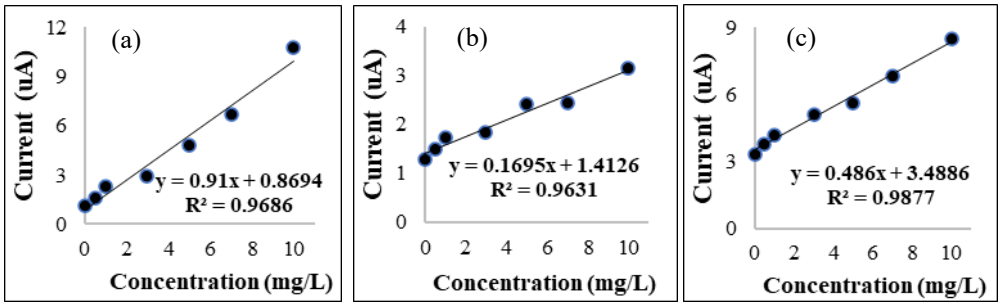



**Figure 2.** Calibration curve obtained with agar (a) 0.5%, (b) 1%, (c) 1.5%

The linear sweep voltammogram results of alginate and immobilized urease biosensor are shown in Figure 3 and calibration curves in Figure 4. This voltammetry assumes that higher concentrations of biopolymers are more easily detected than lower concentrations of biopolymers. Also, it showed slight peaks at two higher alginate concentrations (10 mg/l) (1 and 1.5%).

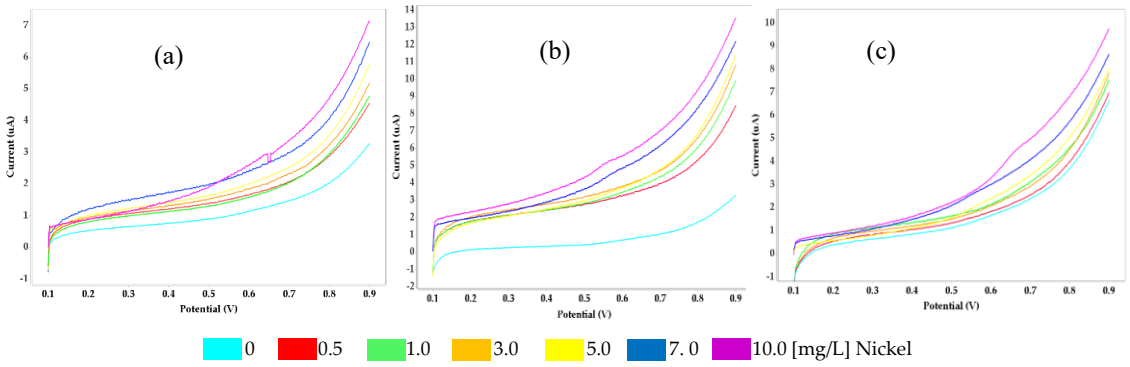



**Figure 3.** Voltammograms for three concentrations of alginate (a) 0.5%, (b) 1%, (c) 1.5%.

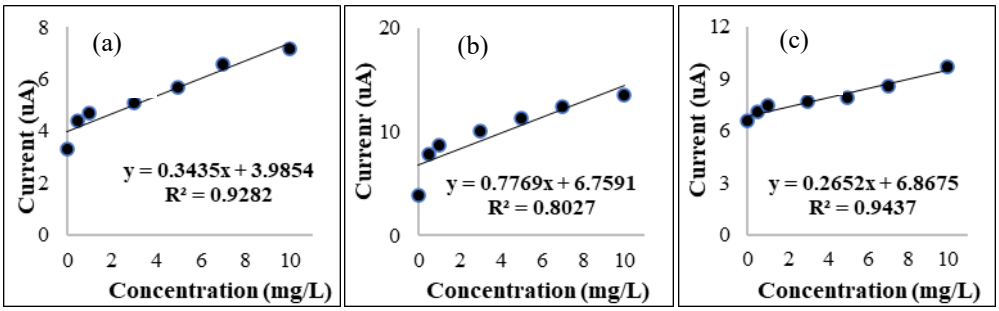



**Figure 4.** Calibration curve obtained with alginate (a) 0.5%, (b) 1%, (c) 1.5%

The LSV shows again that capacitive currents strongly dominate the generated LSV current (Figure 5) and calibration curves indicate a linear regression between 0.87 and 0.95 (Figure 6). The results for chitosan differ in linearity, but also in performance, and they are presented in Figure 7 (LSV voltammograms) and Figure 8 (calibration curves).

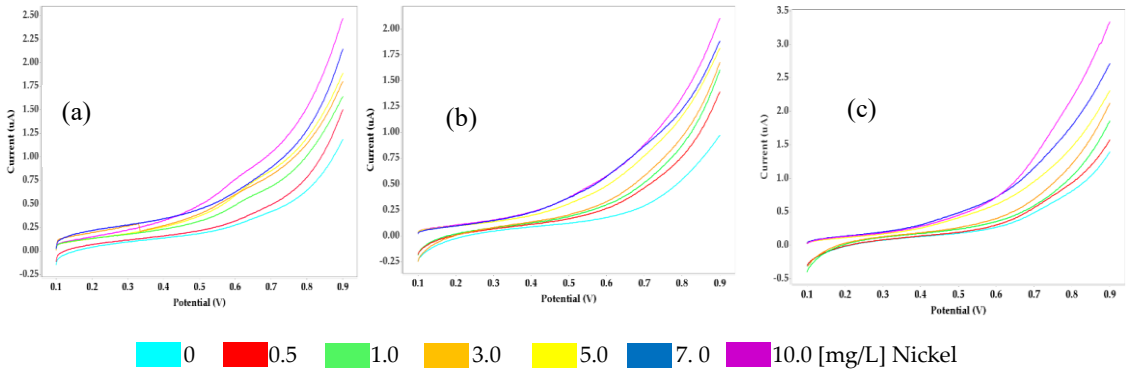



**Figure 5.** Voltammograms for three concentrations of carrageenan (a) 0.5%, (b) 1%, (c) 1.5%.

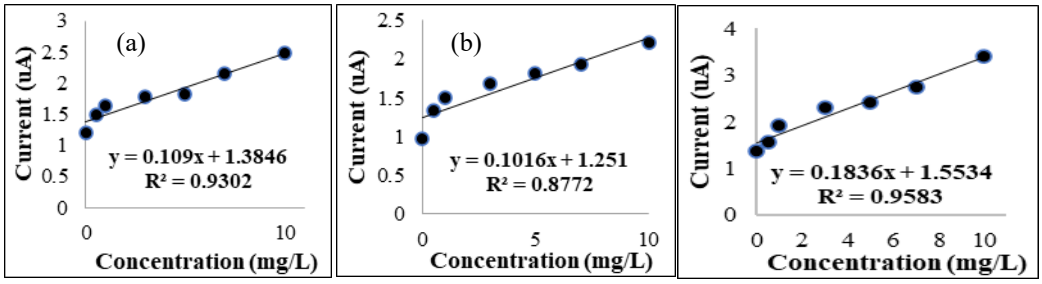



**Figure 6.** Calibration curve obtained with carrageenan (a) 0.5%, (b) 1%, (c) 1.5%

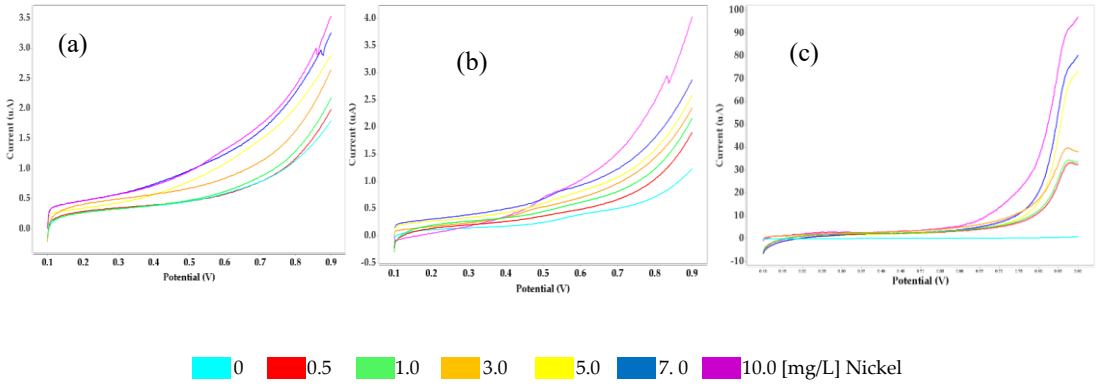



**Figure 7.** Voltammograms for three concentrations of chitosan (a) 0.5%, (b) 1%, (c) 1.5%.

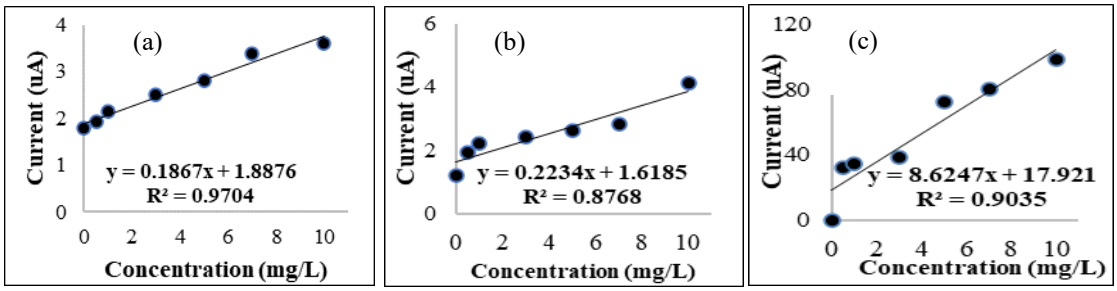



**Figure 8.** Calibration curve obtained with chitosan (a) 0.5%, (b) 1%, (c) 1.5%

*3.2. Biosensor performance*

Table 1 lists agar, alginate, carrageenan, and chitosan-based biosensors and results of calibration curve $R^2$, sensitivity, and detection limit. The limit of detection is inversely related to sensitivity, meaning that the same biopolymer, technique, and biopolymer concentration are effective in detecting nickel. The most effective biosensors were determined by the highest sensitivity and lowest detection limit, at concentrations of 0.5% and 1% for carrageenan and urease, respectively.

**Table 1.** Analytical performance of biopolymer-based biosensors with three concentrations

| Biopolymer | Concentration [%] | $R^2$ | Sensitivity [μA $Mm^{-1}$ $cm^{-2}$] | Limit of detection (LoD) [mg/L] |
|---|---|---|---|---|
| Agar | 0.5 | 0.9686 | 0.8474 | 0.098 |
| | 1 | 0.9631 | 4.5238 | 0.016 |
| | 1.5 | 0.9877 | 1.6179 | 0.076 |
| Alginate | 0.5 | 0.9282 | 2.1514 | 0.034 |
| | 1 | 0.8027 | 0.8226 | 0.091 |
| | 1.5 | 0.9437 | 2.8333 | 0.018 |
| Carrageenan | 0.5 | 0.9302 | 6.7967 | 0.008 |
| | 1 | 0.8772 | 6.8729 | 0.005 |
| | 1.5 | 0.9583 | 4.1546 | 0.018 |
| Chitosan | 0.5 | 0.9704 | 4.1390 | 0.025 |
| | 1 | 0.8768 | 3.1245 | 0.044 |
| | 1.5 | 0.9035 | 0.0834 | 0.079 |

We analyzed the effect of competing minerals on the electrochemical detection of nickel content using a biosensor based on four biopolymer-immobilized urease.

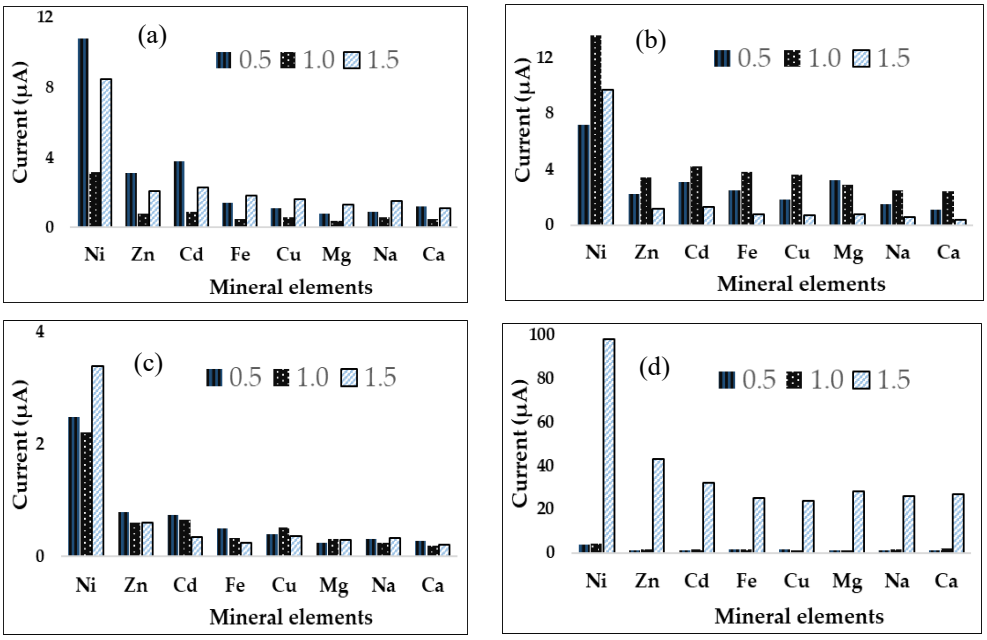



**Figure 9.** Selectivity evaluation against the interference of mineral elements with (a) agar, (b) alginate, (c) carrageenan, (d) chitosan;

The experiment was performed by recording the change in voltammetric behavior before and after the addition of different metal ions $Zn^{2+}$, $Cd^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Mg^{2+}$, $Na^{+}$, and $Ca^{2+}$ in an ammonium buffer solution. The concentration of $Ni^{2+}$ was 10 mg/L, as were other ions (Fig. 9a–d). In general, selectivity assessments for most biopolymers

showed good values, but carrageenan had the highest selectivity assessment, indicating that its analytical performance was superior to the other biopolymer candidates we tested.

## DISCUSSION

Previous studies regarding the use of these biopolymers with cyclic voltammetry showed better results on voltammetric behavior because indicated irreversible anodic peaks compared with LSVs, where the capacitive current dominates the generated LSV current but the performance characteristics are higher in the present study [11]. Carbon-based SPEs are widely used in electroanalytical research for the development of sensors for the determination of organic and inorganic analytes due to their low cost, good electron transfer, and biocompatibility [13]. In a study for developing nickel biosensors, it was reported that urease attached to SPE could effectively detect nickel in food samples [12]. Using the same dynamic range as previous studies, we advanced the work by optimizing receptor fixatives with four biopolymer types and concentrations. It was performance improvements at different concentrations of fixative, from which the best-performing biosensors could be discerned. Based on performance characteristics, the highest values were obtained with LSV for carrageenan at 0.5% and 1% concentrations, with sensitivities of 6.7967 and 6.8729 $\mu A\ Mm^{-1}\ cm^{-2}$, respectively. This was followed by the 1% agar (4.5238 $\mu A\ Mm^{-1}\ cm^{-2}$) and 0.5% chitosan (4.1390 $\mu A\ Mm^{-1}\ cm^{-2}$) combinations with the urease biosensor. Regarding the limit of detection values with LSV, they were highest for the same biopolymers and concentrations (0.008 mg/L at 0.5% and 0.005 at 1% for carrageenan, and 0.016 mg/L of 1% agar). These results suggest they are good candidate biopolymers in biosensors that are designed for detecting nickel in sample analytes.

## CONCLUSION

Biopolymer matrices have significant advantages such as: being easy to prepare, biologically relevant, inexpensive, having high immobilization capacity, and having good mechanical stability, making them suitable for various biomedical, biotechnological, and agricultural applications. Biopolymers are ideal substrates for the immobilization of urease and they can be used in the development of biosensors for nickel detection. Biopolymers are also well suited to create composites with various functional materials, thereby improving their properties. In voltammetric testing of biopolymer-based biosensors, a current is generated by sweeping an applied potential between a reference electrode and a biopolymer-modified electrode over a range relevant to the redox reaction of the analyte. For LSVs, the capacitive current dominates the generated LSV current but sensitivity is improved. In conclusion, we found that a carrageenan biopolymer conjugated to urease on screen-printed electrodes has a potential to adaptively measure nickel in samples.

## ACKNOWLEDGEMENTS

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# SECTION

# MICRO AND NANO TECHNOLOGIES

# NOVEL MATERIALS WITH MICROENCAPSULATED ESSENTIAL OILS, POTENTIAL APPLICATIONS FOR SKIN CELLULAR REGENERATION DRESSINGS

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## ABSTRACT

Biopolymers have gained notoriety due to their unique physical, chemical, and mechanical characteristics and performances. Due to their regenerability, compostability, biocompatibility, non-immunogenic, non-toxic, or non-allergenic properties, they have surpassed the boundaries of the food industry and have become fundamental components in biomedicine or the pharmaceutical industry. Thus, the most well-known applications are as drug delivery systems, tissue engineering, or wound dressings. In addition to all these characteristics, biopolymeric films have proven to be excellent matrices for incorporating various biologically active substances: natural compounds, essential oils, antioxidants, etc. The present study aimed to develop sodium alginate films, with the addition of tea tree and chamomile essential oils capsules. After development through the casting method, the materials were tested for evaluate the physical properties (thickness, retraction ratio, color, transparency, opacity), chemical properties (antioxidant characteristics), mechanical properties (tensile strength and elongation), as well as solubility (water activity index, swelling index, water absorption capacity). The results indicated that alginate based - materials with the addition of essential oils capsules can be used in the pharmaceutical and cosmetic industries for developing films with regenerative properties. Similarly, other biologically active substances can be incorporated, and the development technology can be easily scaled up to an industrial level.

**Keywords:** biopolymers, tea tree, chamomile

## INTRODUCTION

Biopolymers are natural polymers, from regenerable resources, based on natural compounds, with good physico-chemical, mechanical and swelling characteristics and complete biodegradability. The facile biodegradability of the polymers is due to oxygen and nitrogen atoms from its backbone. After biodegradation, biopolymers are converted into $CO_2$, water, biomass, and other natural compounds [1,2].

One of the main characteristics of bio-based polymers is the higher biocompatibility which make them suitable for biomedical applications such as regeneration of tendons or ligaments, tissues, scaffolds or wound dressings [3]. Nowadays, the most known dressings are bio-based ones. The easy of manufacturing into foams, hydrocolloids, aerogels, hydrogels, nanogels or nanofibers, the controlled release and good stability promote their use in medical and pharmaceutical field. Most of the biopolymer-based wounds contain antibiotics or natural substances that accelerates the healing [4]. Sodium alginate (SA) has important features compatible with cellular and skin regeneration, due to its hemostatic properties or the ability to reduce bacterial proliferation. Furthermore, SA is a hydrophilic biopolymer which keep the moisture of the environment, thus promoting the wound healing [5]. Besides other natural compounds embedded in biopolymer-based matrix, essential oils (EO), such as eucalyptus, lavender, peppermint, thyme, pine, fennel, frankincense were used to accelerate the healing time of burns, cuts, common wounds, to treat the infections with *Staphylococcus aureus* and *Escherichia coli*, or to reduce the pain and inflammation [6]. The EOs' volatility, environmental conditions instability, and susceptibility of degradation make them difficult to handle and to use [7]. In this regard, their immobilization through loading into biopolymeric matrices represent a better alternative [8]. The most suitable process to keep the EO properties is their encapsulation.

Encapsulation is the process by which a certain liquid, solid, or gaseous substance is embedded in a matrix, usually made of biopolymers. Microcapsules can be obtained through various methods, including extrusion, emulsification, spray drying, coaxial electrospray system, lyophilization, coacervation, in situ polymerization, supercritical fluid technology, and fluidized bed coating. Among all methods, extrusion, emulsification, and spray drying are the most commonly used techniques for the encapsulation of oils [9,10]. In this process, the encapsulated substance is usually called the active or core, and the polymer used for coating it is called wall, matrix, or envelope. The biobased matrix does not react with the encapsulated compound and must possess excellent film-forming and barrier properties against oxygen, water, pH, heat, light, or pressure [11,12].

Encapsulation of substances in biopolymeric matrices has numerous benefits, such as: *i)* increased stability, efficacy, and shelf life – due to the coating, the encapsulated active substance is protected from exposure to environmental conditions; *ii)* controlled release - depending on the encapsulation technique and the selected material, the release mechanism can be triggered by various factors. Depending on the development mode, the capsule can be designed to release at a specific processing, storage, or consumption stage, or in a targeted area within the body; *iii)* ease of handling and precision – by modifying certain properties of the encapsulated product, it can be easier to handle and use in the industry; *iv)* increased acceptability of food products by providing new colors, flavors, or masking unpleasant odors; *v)* prevention of interactions with other ingredients in a complex food formula [13–15].

The present study aims to develop biopolymeric films with microencapsulated tea tree and chamomile EOs, with potential use as skin regeneration dressings. Tea tree EO present excellence antimicrobial potential, with antiprotozoal, antifungal, and antiviral effects [16], helps reduce inflammation and enhance wound healing [17]. Chamomile is abundant in bioactive substances and it is used as analgesic, antiviral, anti-inflammatory, antiseptic, antidiabetic, antiproliferative, and antibacterial agent [18].

## MATERIALS AND METHODS

Sodium alginate, glycerol, and calcium chloride were purchased from Sigma Aldrich, and tea tree and chamomile EOs from Ingen Laboratories. First step was the development of biopolymeric capsules with 0.5% EO using extrusion technique. As such, 5 g of sodium alginate and 150 ml water were used to form the coating. The mixture was kept at 60°C under stirrer (450 rpm) for 20 minutes. After 20 minutes, the temperature was reduced until 40°C and the EO was added. The mixture was kept 10 more minutes under stirring and transferred into a wide container. The caviar box was loaded and the capsules were obtained after their release into 10% calcium chloride solution and maintained for three minutes. After that, the capsules were transferred into clean water and the calcium chloride excess was removed.

Biopolymers-based films were developed under the same condition, but using casting method. After film-forming solution development, the capsules were added and the mixture was maintained 10 minutes at 40°C and 450 rpm. After that, it was poured onto a silicone foil and maintain until complete drying and test in order to evaluate the thickness, retraction ratio, color, transparency, opacity, antioxidant properties. The tensile strength and elongation, as well as water activity index, swelling index, and water absorption capacity were tested.

For thickness evaluation, an Yato (Shanghai, China) electronic micrometer, with 0.002 mm precision, was used and the result represent the average of the five readings in different areas of the film. The retraction ratio represents the difference between the thickness of the film-forming solution and the final thickness, related to the thickness of the film-forming solution, and multiplied by 100.

Color was evaluated using CIELab system and the parameters such as luminosity ($L^*$), $a^*$, and $b^*$ were read with Chroma Meter CR400 colorimeter (Konica Minolta, Japan). A blank standard illuminat was used as reference ($L^* = 94.12$, $a^* = -5.52$, and $b^* = 9.27$).

Transmittance and opacity were evaluated using Epoch (BioTek Instruments, Winooski, VT, USA) spectrophotometer. 1 cm x 3 cm samples were places into a cuvette and the transmittance was read at 660 nm (an empty cuvette was used as standard). For opacity determination, the value of absorbance read at 600 nm was divided at film thickness.

The mechanical properties such as tensile strength and elongation were tested using ESM Mark 10 texturometer with a 5 KN cell and special grips for thin films and foils attached. The STAS ASTM D882 (Standard Test Method for Tensile Properties of Thin Plastic Sheeting) was used as standard method [19] and the film was cut into 1 cm × 10 cm specimen. The travel speed was set at 10 mm/min. The tensile strength was determined after divided the maximum load at the area, and the elongation represents the ratio between final and initial length after test sample breakage.

Taking into account that these films are intended to be used as possible dressings, it is very important to establish their complete solubilization and water absorption capacity. A good dressing material is that one which is able to keep the constant moisture. The swelling ratio capacity is an important parameter because, according to these results, it is possible to establish the future application of the films: the time needed until final

solubility or until bioactive ingredients releasement. Thus, 3 cm × 3 cm film samples were weighed, immersed in water at room temperature (23 ± 0.4 °C) and maintained for 0.5-20 min. Then the samples were removed from the container, and the excess water was eliminated using filter paper; the sample were weighted. Water activity index was read using the AquaLab 4TE equipment (Meter Group, München, Germany). The determination was made in triplicate. For moisture content determination, the 3 cm x 3 cm samples were weighted, placed into a 50 ml water container and maintained for 8 hours. After that, the samples were dried at 110°C for 24 hours and weighted again.

The antioxidant activity was evaluated using ABTS radical scavenging capacity. ABTS radical solution was prepared by mixing 10 mL of 7 mM ABTS solution and 10 mL of 2.45 mM potassium persulfate solution and incubation for 16 h at room temperature and darkness conditions. 20 mm x 20 mm film sample was immersed in 2 mL ABTS reagent and incubated for 0.5/1 h at 37 °C, 500 rpm (darkness conditions). The absorbance was read at 734 nm using an Epoq spectrophotometer (BioTek Instruments, Winooski, VT, USA).

## RESULTS

The samples were completely dried after 48 hours maintenance at 22°C ± 0.9°C. After development, the films were stored under controlled temperature and humidity conditions. Results of the films' testing are presented in Table 1.

**Table 1**. Characteristics of the films tested after development

| | | Film with encapsulated tea tree EO | Film with encapsulated chamomile EO |
|---|---|---|---|
| Thickness, *t*, µm | | 82.60 ± 0.33 | 95.70 ± 0.66 |
| Retraction ratio, *RR*, % | | 58.40 ± 0.40 | 44.16 ± 0.74 |
| Color | *L** | 38.14 ± 0.74 | 45.47 ± 0.53 |
| | *a** | -0.41± 0.33 | -0.43 ± 0.18 |
| | *b** | 6.12 ± 0.01 | 5.92 ± 0.69 |
| Transmittance, *T*, % | | 45.18 ± 0.33 | 42.84 ± 0.33 |
| Opacity, *O*, A/mm$^{-1}$ | | 44.14 ± 0.18 | 56.39 ± 0.40 |
| Tensile strength, *TS*, MPa | | 0.218 ± 0.69 | 0.184 ± 0.24 |
| Elongation, E, % | | 9.81 ± 0.30 | 11.42 ± 0.33 |
| Water activity index, $a_w$ | | 0.49 ± 0.03 | 0.48 ± 0.02 |
| Water solubility, *WS*, % | | 31.73 ± 0.09 | 46.12 ± 0.25 |
| Inhibition, % | | 38.13 ± 0.66 | 35.71 ± 0.72 |

For swelling ratio testing, the samples were kept for 0.5, 1, 3, 5, 10, 15, 20 min into a container with water (Figure 1).

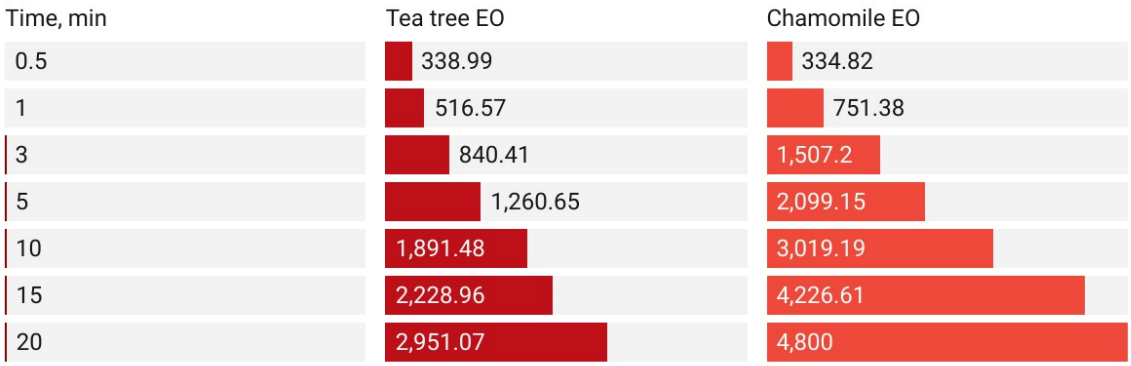



**Figure 1.** Swelling ratio evaluation

## DISCUSSION

The films with microcapsules added were homogeneous, with regular edges, and characteristic shine on the surface in contact with the drying foil. Contrary to films with the addition of non-encapsulated essential oil, which had the specific smell of the added substance, the present samples did not present any specific odor. The capsules were well incorporated into the biopolymeric matrix, and the films exhibited a similar thickness across the entire surface (Table 1, low values of standard deviation). The retraction ratio was with almost 15% higher for film with encapsulated tea tree EO than the sample with encapsulated chamomile EO. The results are directly correlated with the thickness, the values being inversely proportional to the thickness. The thickness influenced the luminosity of the films, as well. Thus, according to results presented in Table 1, the luminosity of the second film was lower than that of sample with tea tree EO. These results can be directly correlated with those of transmittance and opacity. Transmittance was higher for film with tea tree EO and lower for sample with chamomile. Inversely, the opacity was with 12 units higher for film with encapsulated chamomile EO. The water solubility was higher for samples with chamomile EO, as well. Even the difference is not so evidence, the results are important for industry. According to solubility degree, it is possible to develop a film or dressing suitable for different wounds or those that need specific moisture. According to swelling ratio results (Figure 1), even in the first 30 seconds the film with chamomile EO was more soluble, after longer maintenance in water, the film with tea tree EO was more resistant. After 3 minutes immersion, the results obtained for second sample was higher than the first one (1507 % instead of 840%). After 20 minutes immersing, the second sample has a 4800% swelling ratio index and the first one, 2951%.

## CONCLUSION

The aim of this study was to develop and evaluate the properties of biopolymeric films with encapsulated tea tree and chamomile EO added, in order to be used as dressing for cellular regeneration. The good mechanical and solubility tests results promote them as suitable materials for using as bandage materials. The dressings obtained from sodium alginate and EOs added may be a promising alternative to synthetic, conventional ones, with lower biocompatibility and waste-related issues.

Furter research must be focused on the antimicrobial activity and cellular regeneration tests. Similarly, other EOs can be tested and new dressings formulation can be obtained.

## ACKNOWLEDGEMENTS

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# PRELIMINARY TESTS ON FLY ASH POTENTIAL TO DEGRADE ORGANIC POLLUTANTS FROM AQUEOUS SYSTEM

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## ABSTRACT

Potential use of four types of fly ash waste as raw material for synthesis of photo-catalysts to be applied for wastewater treatment was investigated. In this respect the four selected fly ash types were subject to morphological characterization by dimensional analysis of particles and scanning electronic microscopy on to composition characterization by thermogravimetric analyses and X-ray fluorescence. Their potential to be used for advanced degradation of organic compounds via heterogeneous photocatalysis was investigated on a synthetic solution of methylene blue as model compound. Solutions containing methylene blue and fly ash (as catalyst) were subject to UV-VIS radiation and samples were collected at specific time intervals and were centrifuged in order to remove the fly ash. Methylene blue degradation efficiency was measured based on the adsorption at 662 nm via UV-VIS spectrophotometry. Obtained results proved the fly ash potential to be used as photo catalyst but in order to improve its degradation capability there is a need to develop new composite catalytic materials based on classic photo catalysts and fly ash.

**Keywords:** fly ash, photocatalysis, methylene blue, degradation

## INTRODUCTION

Water is the life source and a precious resource in all activities and continues to be loaded with different toxic pollutants, many of them which are not subjected to regulations [1]. As science evolves it is in our best interest to find solutions for treating water in order to maintain a healthy environment and a responsible use of our resources.
Flying ash is known as a solid residue generated in the process of burning different types of materials such as coal, biomass or solid waste within power plants and heating plants. Because of its powdery nature it's difficult to deposit on site and presents a high risk of contamination for water, air and soil due to its composition in soluble constituents and toxic heavy metals [2]. The global waste management data [3] shows only 37% of waste is stored in special locations of which only 8% are disposed of in sanitary warehouses equipped with gas collection systems, another 33% are irresponsibly dumped on the ground, while 11% are incinerated and only 19% are recycled.

The main composition of flying ash consists in $SiO_2$ and $Al_2O_3$ which makes it an accessible alternative for synthesizing adsorbent materials such as zeolites [4], or catalyst for degrading organic pollutants from aqueous system [2].
There are numerous studies which show great interest on developing efficient methods in photo degradation with UV-Vis radiation of organic pollutants [5] using different materials like fly ash –$TiO_2$ doped catalyst [6]. Materials such as zeolites with their large surface area combined with the increased capacity of $TiO_2$ for adsorption makes them efficient in the photocatalytic degradation [7]. The disadvantage of nanoparticles such as $TiO_2$ is the difficulty in separation from the aqueous system, for this reason fixation on a support material (zeolite, flying ash) facilitates the recovery rate of the catalyst [8]. The effective use of fly ash can improve waste management and aliviate pollution due to its composition, structure and porosity that is currently used as substrate material in different domains [9].

## MATERIALS AND METHODS

Morphological analysis was performed using Mastersizer 2000 – Malvern equipment to investigate particles dimensions and Scanning Electron Microscopy - Quanta FEG 250 with STEM to investigate the structure with high-resolution images. Thermogravimetric analyses were performed using an NETZSCH STA PC 409 which offers the possibility to analyse both TG and DTA. X-ray fluorescence using an EDXRF Rigaku NEX-CG equipment was used to determine the composition of analysed fly ashes. Photocatalytic treatment was performed using a Heraeus-type UV–VIS (reactor equipped with an immersed TQ-150-Z3 medium-pressure mercury lamp. Synthetic solutions of methylene blue (Merck) were used as model organic substance for degradation experiments.

## RESULTS

*Dimensional analyses of fly ashes*
Dimensional analyses revealed that specific surface area of the fly ashes follows the order FA1<FA2<FA3<FA4. That is also confirmed by the particle size distribution presented within Table 1.

**Table 1 Dimensional analyses of fly ashes**

| *Fly ash type* | *d (0.1), µm* | *d (0.5), µm* | *d (0.9), µm* |
|---|---|---|---|
| FA1 | 12.129 | 73.399 | 273.571 |
| FA2 | 7.489 | 60.711 | 266.061 |
| FA3 | 5.683 | 44.277 | 189.287 |
| FA4 | 3.324 | 36.598 | 256.333 |

*Fly ashes morphological characterization by scanning electronic microscopy*
Scanning electronic microscopy (SEM) images (see Figure 1) showed that fly ash particles are mainly spherical ones with high specific area.

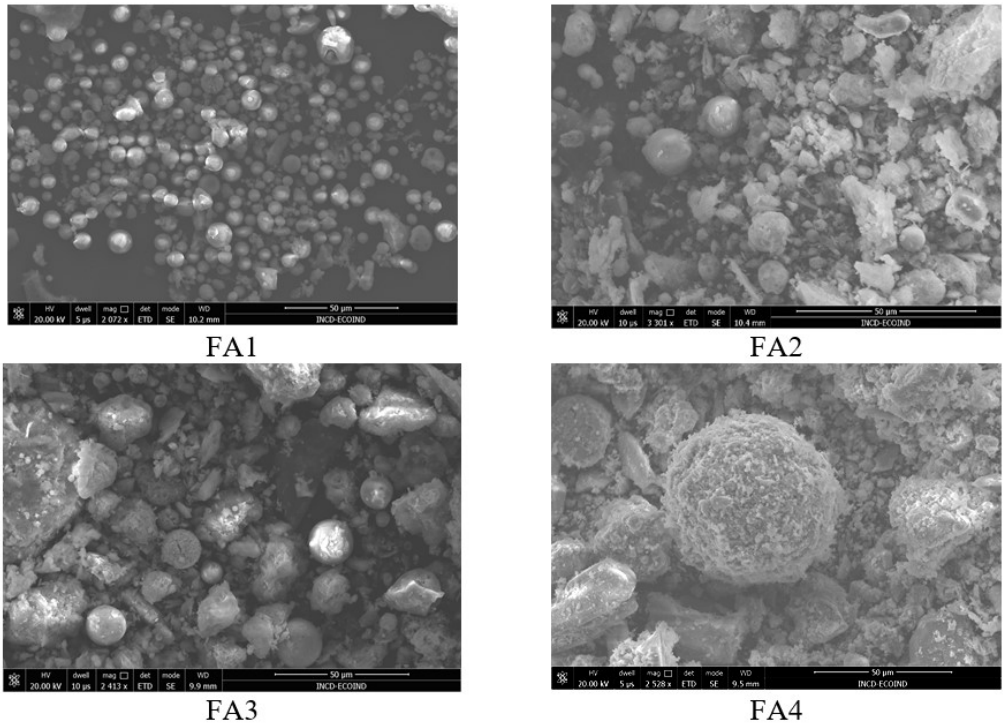



**Figure 1 Fly ashes SEM images**

*Thermogravimetric analyses of fly ashes*

Thermogravimetric analyses (presented within Figure 2) showed that FA1, FA2 and FA3 presents the behaviour of aluminium silicates and FA4 is acting similar to an iron based silicate.

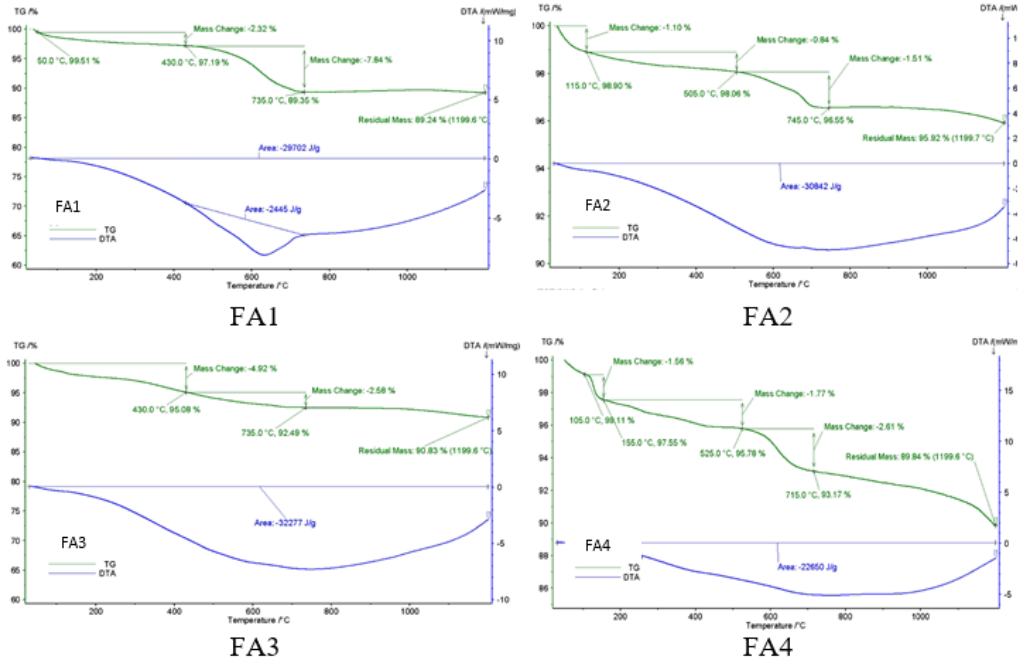



**Figure 2 Thermogravimetric analyses of tested fly ashes**

*Determination of fly ashes composition via X-ray fluorescence*
X-ray fluorescence (XRF) was used to determine compositional structure of fly ash and the obtained results are presented within Table 2.

**Table 2 Fly ashes composition**

| *Element (Oxide) %* | *FA1* | *FA2* | *FA3* | *FA4* |
|---|---|---|---|---|
| $SiO_2$ | 52.77 | 51.13 | 54.14 | 12.34 |
| $Al_2O_3$ | 26.67 | 27.89 | 24.49 | 3.51 |
| $Fe_2O_3$ | 8.83 | 7.56 | 8.20 | 71.04 |
| CaO | 4.24 | 5.73 | 3.73 | 2.23 |
| $K_2O$ | 1.99 | 2.32 | 3.15 | 0.57 |
| MgO | 1.73 | 2.24 | 2.49 | 0.70 |
| $TiO_2$ | 1.12 | 1.13 | 0.76 | 0.16 |

*Evaluation of fly ashes photocatalytic activity*
Evaluation of fly ashes photocatalytic activity was done using synthetic solution of methylene blue (MB) and UV irradiation. MB degradation efficiency was determined based on MB absorbance at 662 nm (wavelength corresponding to maximum absorbance. Correlation between MB absorbance and concentration was determined for the concentration domain of 5 – 22.5 mg/L and is presented within figure 3.

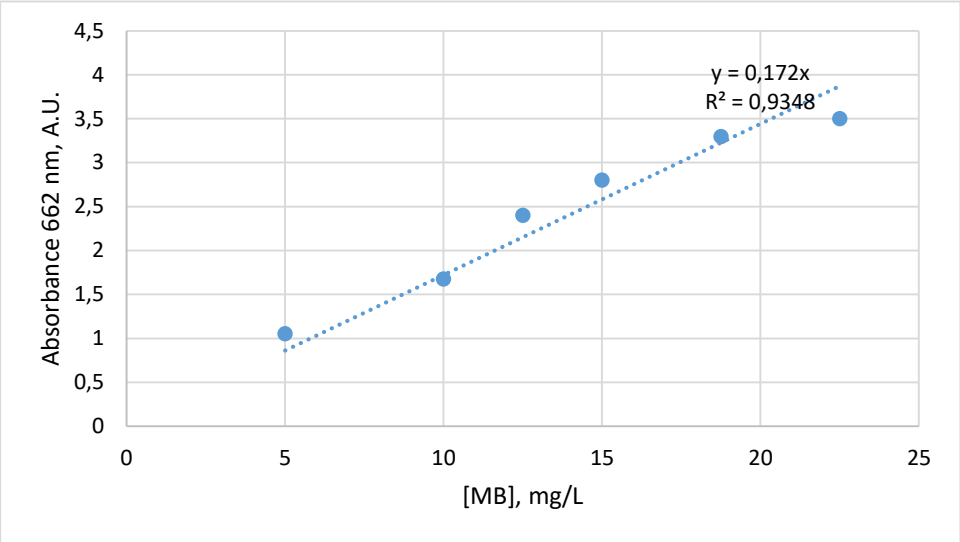



**Figure 3 MB absorbance at 662 nm vs. MB concentration**

The results obtained for photocatalytic degradation of MB after 30 minutes of UV irradiation at a fly ash concentration of 100 mg/L are presented within Table 3.

**Table 3 MB degradation efficiencies vs. fly ash type after 30 minutes of irradiation for fly ash concentration of 100 mg/L**

| *Photo catalyst* | *MB initial absorbance at 662 nm, A.U.* | *MB initial concentration, mg/L* | *MB absorbance at 662 nm, A.U.* | *MB concentration after 30 minutes, mg/L* | *MB degradation efficiency, %* |
|---|---|---|---|---|---|
| FA1 | 1.913 | 11.122 | 0.613 | 3.564 | 67.96 |
| FA2 | 1.947 | 11.320 | 0.557 | 3.238 | 71.39 |
| FA3 | 2.051 | 11.924 | 0.388 | 2.256 | 81.08 |
| FA4 | 1.797 | 10.447 | 0.604 | 3.511 | 66.39 |
| only UV | 1.733 | 10.076 | 0.623 | 3.622 | 64.05 |
| $TiO_2$ | 2.051 | 11.924 | 0.265 | 1.541 | 87.08 |

For the FA3 which presented highest efficiency the absorbance spectra of initial and after 30 minute of irradiation MB spectra are presented within Figure 4.

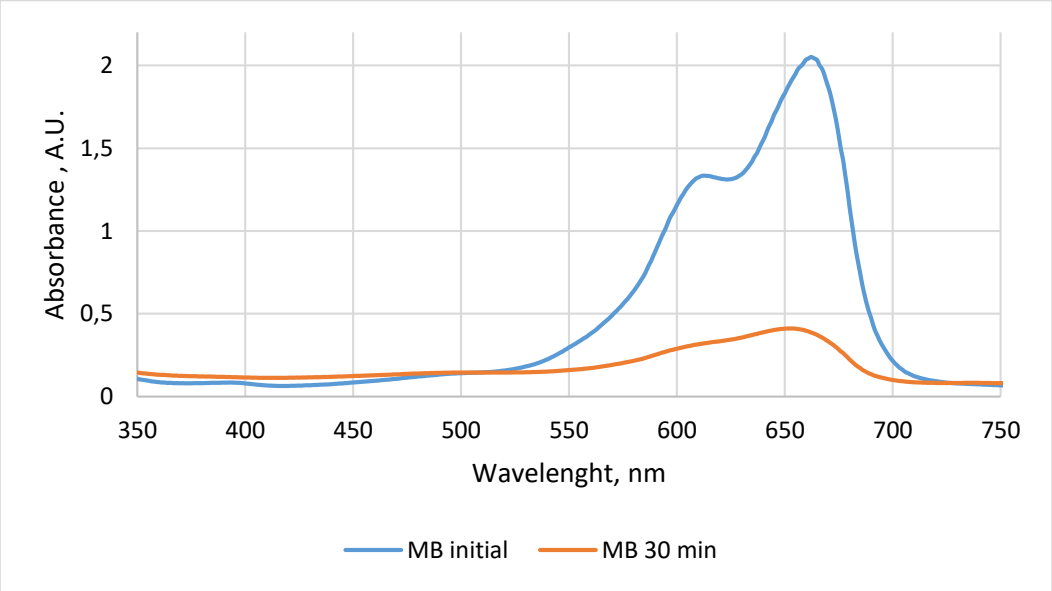



**Figure 4 MB absorbance at 662 nm for FA3**

## DISCUSSION

Dimensional analyses of all four fly ashes types revealed that particles dimensions are in the domain of tens µm to hundreds of µm with specific surface areas relatively high that can make them possible candidates for new photocatalytic materials.

Morphological characterisation by scanning electron microscopy proved that FA1, FA2 and FA3 are mainly present as aluminium silicates cenospheres with various dimensions, individually or forming agglomerates, and in generally are porous. On the

other hand FA4 morphological analyse confirmed the presence of iron oxides which forms various iron based silicates.

Thermogravimetric analyses results confirmed that that FAs are registering continuous mass losses as follows: between 40 and 450 ºC due to physically bound water, above 450 ºC a second mass loss can be due to chemically bound water, between 800 and 1000 ºC mass loss due to decarbonation of calcium and magnesium carbonates. The results are in accordance with other studies in the field [10].
XRF results proved that FA1, FA2 and FA3 presents an important content of $SiO_2$ followed by $Al_2O_3$, $F_2O_3$, CaO, $K_2O$, MgO and for FA4 the predominat oxide is represented by $Fe_2O_3$ followed by $SiO_2$, $Al_2O_3$, CaO, which was in accordance with SEM and thermogravimetric analyses and other data from the literature [11].

In relation to photo catalytic properties FAs potential followed the order FA3 > FA2 > FA1 > FA4 corresponding to MB degradation efficiency of 81.08%, 71.39%, 67.96%, 66.39% after 30 minutes under UV irradiation for an FA concentration of 100 mg/L. Compared to photolysis degradation efficiency highest potential for development of new catalysts is attributed to FA3 followed by FA2. FA1 and FA4 showed degradation efficiencies very close to the photolysis.

If compared with $TiO_2$ only FA3 seems to be a viable solution for new photocatalyst showing a MB degradation efficiency of 81.08% quite close to the one for MB using $TiO_2$ which was found to be 87.08% after 30 minutes of irradiation.

## CONCLUSION

Four types of fly ashes were investigated for their potential to be used for development of new photo catalysts. Their morphological characterisation via dimensional analyses and scanning electronic microscopy showed that all types of investigated fly ashes can be viable candidates for development of new catalysts. Compositional characterisation via thermogravimetric analyses and X-ray fluorescence showed that three types are belonging to the aluminium silicate group and the fourth is belonging to iron-based silicates. However, tests on their photocatalytic potential using methylene blue as a model compound showed that only one type – FA3 presents characteristics suitable for photo catalyses. Even if all four FAs proved to improve MB degradation compared with simple photolysis, only FA3 seems to be a good candidate for development of new composite catalytic materials based on classic photo catalysts and fly ash (MB degradation efficiency when using FA3 was quite close to that obtained by using classic $TiO_2$ catalyst). In this respect more work is needed in order to synthesize photocatalysts starting from FA3 and titanium dioxide precursors in various proportions, using various synthesis methods, in order to improve fly ash photocatalytic potential.

## ACKNOWLEDGEMENTS

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# SECTION GREEN BUILDINGS

# TECHNOLOGIES AND MATERIALS

# ENVIRONMENTAL BENEFITS OF MANAGING SECONDARY WASTE FROM INCINERATION PLANT IN CONCRETE - CARBON FOOTPRINT AND ABIOTIC DEPLETION

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## ABSTRACT

The use of secondary waste from municipal solid waste incineration plant in concrete has been studied for its potential to reduce the carbon footprint and abiotic depletion associated with traditional concrete production. Fly ash is a hazardous waste and it can be used as a substitute for cement in the production of concrete. Bottom ash can be used as a substitute for light natural aggregate, which was previously soaked in water for internal treatment of concrete. The composition of concrete includes: CEM I 42.5R, CSA, basalt, sand, zeolite, additives and admixtures. Studies have shown that concrete with secondary waste can decrease CO2 emissions and abiotic depletion compared to traditional concrete. In the article 4 scenarios are introduced (1 - concrete with fly ash, 2 - concrete with bottom ash, 3 - concrete with both secondary waste, 4 - reference concrete). The tests were performed for 1 kg of concrete, in the cradle-to-gate range, excluding transport. 4th scenario has the biggest carbon footprint and abiotic depletion influence. The least impact on environmental has 3rd scenario. Closing the loop by managing secondary waste in concrete fits into Circular Economy and Sustainable Development Goals, especially in SDG 12 – Responsible Consumption and Production and SDG13 – Climate Action.

**Keywords:** Concrete, Life Cycle Assessment, Circular Economy, fly ash, bottom ash, carbon footprint, abiotic depletion

## INTRODUCTION

The development of secondary waste management is consistent with the circular economy principle. The circular economy road map clearly indicates the problem of the depletion of non-renewable raw materials, the increase in their prices and Poland's

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dependence on their supplies from foreign markets, which may cause a risk of slowdown in the country's economic development. The challenges related to the transformation towards circular economy are both economic and environmental. Supporting the transformation towards circular economy is key to creating a low-emission, resource-efficient, innovative and competitive economy [1]–[4].

Life cycle thinking is an essential tool in the transformation towards the right circular economy. It requires a number of actions to be taken at all stages: design, acquisition of raw materials, processing, production, consumption and selective collection and management of waste (including preparation for its reuse). The communication published by the European Commission at the end of 2015 (Closing the loop - EU action plan for a circular economy) identified priority areas. These include plastics, food waste, critical raw materials, demolition and construction waste, and biomass and products made from biomass. So, among others: creating new building materials that will require the use of fewer natural mineral raw materials is an important element for the implementation of circular economy [5]–[8].

In life cycle assessment (LCA), the environmental category of abiotic depletion assesses the depletion of nonrenewable resources, such as minerals and fossil fuels, throughout a product's life cycle. This category quantifies the extraction and consumption of resources, including energy, and their potential contribution to environmental impact categories, such as resource base deterioration. The depletion of abiotic resources is a critical aspect of LCA, as it evaluates the availability of essential resources for future generations [9], [10].

On the other hand, the carbon footprint is a measure of the total greenhouse gas emissions caused directly and indirectly by a product, organization, event, or person. It is a key environmental impact category that assesses the relative contribution of different greenhouse gases to climate change, expressed as a factor of carbon dioxide equivalents. The carbon footprint is an important indicator in LCA for evaluating the potential impact of a product or activity on climate change [11]–[13].

These environmental impact categories, including abiotic depletion and carbon footprint, are essential for understanding the environmental implications of products and processes throughout their life cycles [14]–[16].

## MATERIALS AND METHODS

LCA analysis involves identifying the most important data that has an impact on the environment. The selected environmental categories include abiotic depletion and carbon footprint. The scope includes cradle-to-gate, which means from the extraction of raw materials to the moment of its use in industry. In the case of secondary waste, this ranges from production in the installation to use in the economy instead of storage. The calculation was made to produce 1 kg of concrete and did not take into account transport. Taking into account transport would probably be beneficial for scenarios 1-3 in which secondary waste is used, because the concrete mixing plant could be built on or within the plant, while the extraction of raw materials is usually associated with their further transport.

The IPCC 20113 GWP 100a (Intergovernmental Panel on Climate Change Global Warming Potential) methodology and SimaPro software were used. The IPCC 2013 GWP 100a method expresses greenhouse gas (GHG) emissions in kilograms of CO2 equivalent over a 100-year period [17], [18].

The composition of concretes adopted for the calculation of the carbon footprint and abiotic depletion for 4 scenarios is shown in Figure 1. The concretes were prepared using CEM I 42.5R, CSA, basalt, sand, zeolite, additives and admixtures .

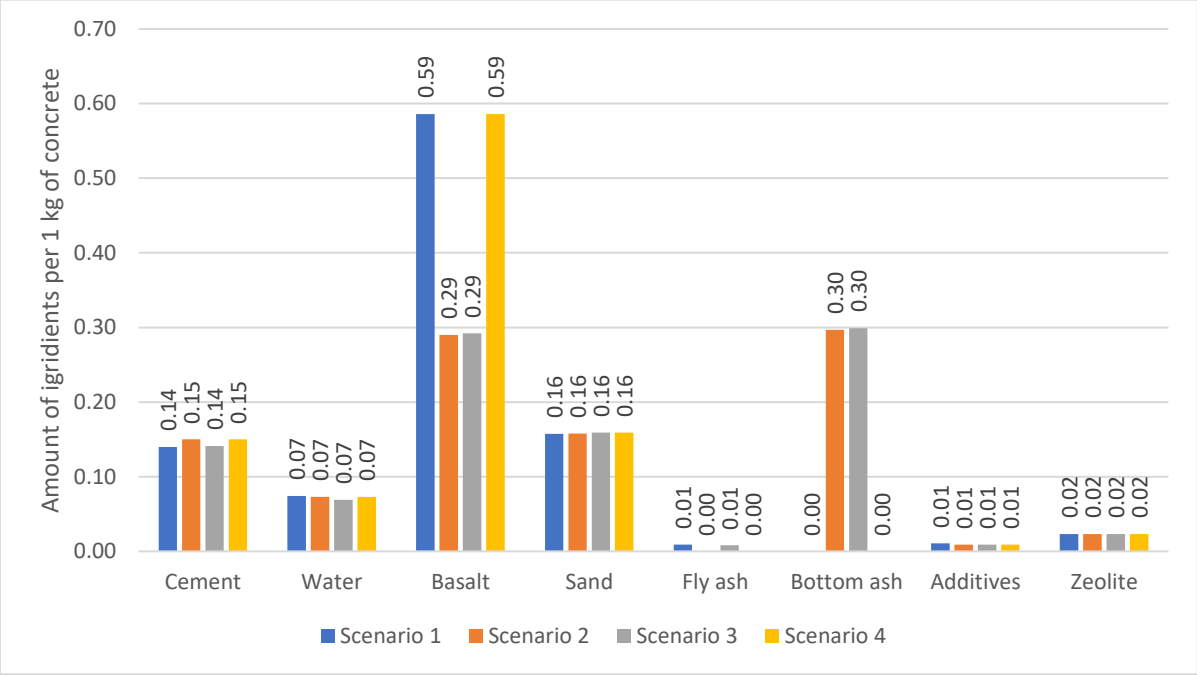



Figure 1. Ingredients of concretes for 4 scenarios.

## RESULTS

The greatest impact on the environment has the production of conventional concrete without the use of secondary waste from all analyzed scenarios. For 1 kg of conventional concrete produced, the emission is 0.0005 kg CO2 eq. The lowest CO2 eq emission is for scenario 4 and amounts to 0.00053 per 1 kg of concrete produced with fly ash and slag. Comparing scenarios 1 and 2, it is more environmentally beneficial to produce concrete with only slag than with only fly ash. The difference is due to the amount of waste used. However, in municipal solid waste incineration plant, almost 30% more slag is produced than ash, therefore scalability for slag and its larger share in concrete is necessary. The calculations show that the optimal environmental solution will be to create a mixture with the simultaneous use of two secondary waste, but as technological tests of concrete have shown, this will be a more difficult, but doable, process.




Figure Carbon footprint for 4 Scenarios.

As in the case of the carbon footprint, the environmental impact for the category of depletion of abiotic resources has the greatest impact on scenario 4, whose concrete composition does not take into account secondary waste from incineration plant and amounts to 2.5439E-08 kg Sb eq and 6.54E-01 MJ. Scenario 3 has the lowest environmental impact, which is 1.3317E-08 and 5.69E-01. The study also identified that cement is the most energy-intensive ingredient of concrete, while basalt has the highest kg Sb equivalent (4.11E-08 kg Sb eq per 1 kg of aggregate). The research confirms the correctness of the focus of this work, where the cement ratio is lowered by adding fly ash as an addition to the clinker and basalt is replaced with recycled aggregate, which constitutes slag.

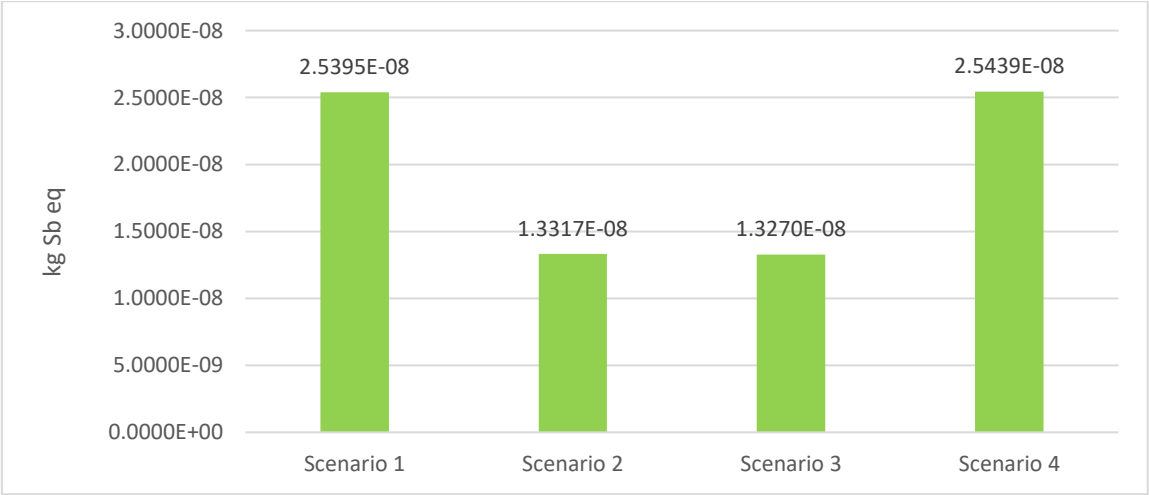



Figure Abiotic depletion for 4 Scenarios.

## CONCLUSIONS AND DISCUSSION

The environmental analysis examined the environmental impact in terms of abiotic resource depletion and climate change (carbon footprint). The work analyzed scenarios for the developed concrete mixtures. In the mixtures, attempts were made to use the highest possible share of secondary waste in order to maximize the positive environmental effect, while maintaining the appropriate mechanical, durability and strength properties contained in construction standards and regulations. Life cycle analysis studies in the above-mentioned two categories have shown that all analyzed scenarios for the produced concretes with the addition of secondary waste from municipal solid waste incineration plant are a source of environmental relief compared to the reference concrete. The research defined 4 scenarios for the production of 1 kg of concrete (1. Concrete with fly ash; 2. Concrete with slag; 3. Concrete with fly ash and slag, 4. Reference concrete). It was shown that the largest environmental relief occurs in scenario 3, and its difference compared to scenario 4 (reference) is 1.27E-05 kg CO2 eq per 1 kg of concrete (for the climate change impact category (carbon footprint)) and 1, 22E-08 kg Sb eq per 1 kg of concrete and 5.45E-02MJ per 1 kg of concrete (for the category of impact of depletion of abiotic resources).

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# APPLICATIONS OF THE TEMPERATURE STEP METHOD FOR DETERMINING THE SPECIFIC HEAT CAPACITY OF BUILDING MATERIALS

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## ABSTRACT

Properties of building materials include many properties, the most important are mechanical, thermal, chemical, and economic. The most important thermal properties that characterize the total heat losses and thermal inertia of materials are thermal conductivity and specific heat capacity. The first one describes the stationary heat transfer through the structures, but the second describes the ability to absorb and release the heat energy with temperature change dynamically, and it is related to a unit mass of the specimen.

The specific heat capacity can be measured directly using differential scanning calorimetry (DSC), the most common method of thermal analysis. DSC method uses the heat flow principle and homogeneous heating. Sensors with high sensitivity, short time periods and a small-sized chamber guarantee high detection sensitivity and stable, reproducible results. There are two main disadvantages which prevents these approaches from being widely used in the field of building physics – relatively small sample dimension and high equipment costs. Sample size limitation is very critical in the case of non-homogeneous building products.

A so-called temperature step method is being studied as an alternative approach for quick and cheap measurements of the heat capacity for building materials. Principle used in such measurements is a rapid temperature increase in a closed large-sized insulated chamber with the dynamic analysis of generated thermal information considering the thermal mass of the plates. In general, this method is based on measuring the heat amount required to heat a sample with known mass from one quasi-stationary temperature equilibrium state to another in an insulated chamber with calibrated heat losses.

This approach has been tested on several heterogeneous building materials, including samples with phase change materials with subsequent calculations of latent heat, showing good accuracy compared to the DSC method, as well as reducing the required measurement time. The use of this approach will make it easier and cheaper to determine the unknown or assumed only theoretically heat capacity property of building materials.

**Keywords:** heat capacity, temperature step method, building materials, phase change materials.

## INTRODUCTION

Building (construction) materials may vary in their physical, mechanical, and chemical properties. Thermal conductivity λ [W/(m·K)] and specific heat capacity $c_p$ [J/(kg·K)] describes the heat resistance and ability to absorb/release the heat energy accordingly.

Typically, the widely used parameter – thermal conductivity – is measured using *HotPlate* or similar equipment in laboratory conditions [1, 2]. The specific heat capacity property is measured using differential scanning calorimetry (DSC) method, which is based on the heat flow measurement principle for short time periods and a small-sized chamber, guaranteeing high detection sensitivity and precise results [3, 4]. The disadvantages preventing DSC approach from wide use in the field of building physics are small sample dimension and high equipment costs. Sample size can be the most critical limitation in case of large-scale or non-homogeneous building products.

As an alternative approach for quick and cheap measurements of the heat capacity properties for building materials is so-called temperature step (or ramp) method [5-8], which is based on the heat transition process within two isothermal steps for a large sample in the same measuring device used also for thermal conductivity measurement.

The application of such simple and cheap (thanks to the use of already existing equipment) temperature step method can also be used to determine the latent heat of phase change materials by choosing the temperature step values according to the known melting point temperature. In this case, the measured heat amount will consist of two parts - the heat capacity and the phase transition heat.

## MATERIALS AND METHODS

The principle used for the temperature step measurements is a controlled temperature increase in an insulated and calibrated *HotPlate* chamber with further analysis of thermal information considering the thermal mass of the plates. The main registered parameter is the amount of heat required to heat a sample and a chamber with known mass from one quasi-stationary temperature state to another with pre-calibrated heat losses from the empty chamber. Measurements were performed using *Netzsch HFM 446 Lambda Small* device (Fig. 1).

Initially, the top and bottom heat flow meter plates are kept at the same temperature. With the following temperature increase, the heat fluxes are measured until equilibrium is reached at the higher temperature (Fig. 2). The change in enthalpy (or absorbed heat) is calculated by integrating the heat flow measurements over time. For determination of specific heat capacity $c_p$ the following formula is used:

$$c_p = \frac{N_\lambda \cdot (Q - Q_E)}{m \cdot \Delta T}, \quad (1)$$

where $m$ is sample mass [kg], $Q_E$ is energy absorbed by the empty device (from the calibration) [J], $Q$ is energy absorbed during the sample measurement (includes device and sample parts) [J], $\Delta T$ is temperature difference [K] and $N_\lambda$ is heat flux sensor calibration factor [-].

As the energy absorbed by the empty device is subtracted from the final energy readings, measurement accuracy decreases significantly for small and light samples, the sample with masse below 20 g are hard to measure in general. Larger samples and a step of at least 10 K are recommended to improve the measurement accuracy.

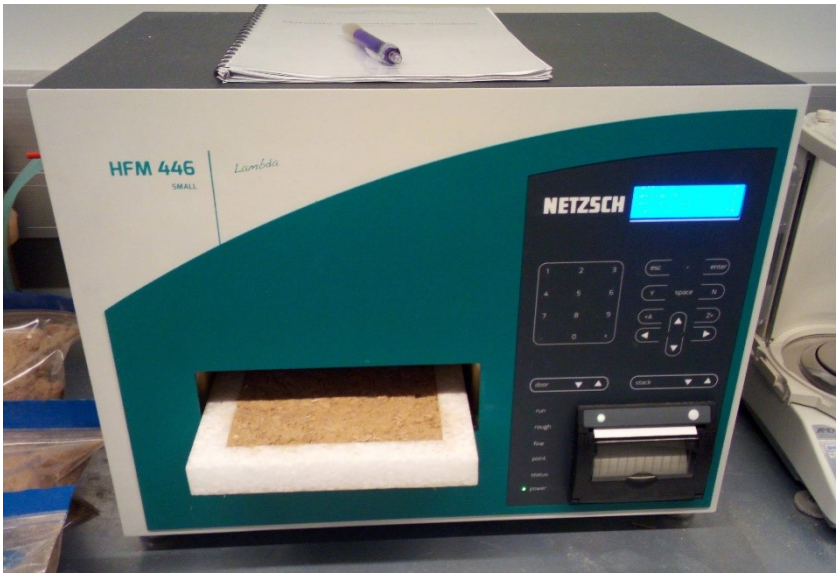



Fig. 1. Netzsch HFM 446 Lambda Small device with inserted sample.

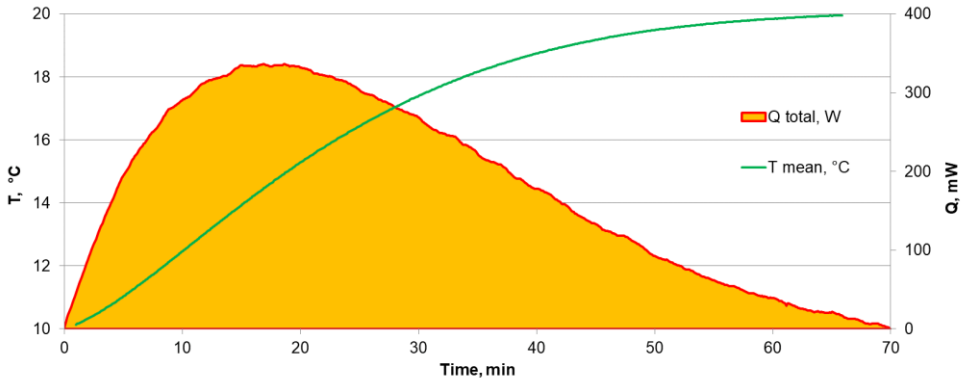



Fig. 2. Energy (orange area) supplied to increase the temperature from 10 to 20 °C.

The same temperature step method may be also used for determinations of latent heat for phase change materials (PCM), giving a quantitative value of the ability to store or release amounts of energy during the phase transition process [6, 9-10]. Supplying an energy to the sample with PCM in the solid state melts it, and during this phase change energy is added to the sample at constant temperature (melting point). The temperature will change only when the phase change is fully completed. The energy amount $Q_L$ [J] required to melt the sample with mass $m$ [kg] is given by the equation:

$$Q_L = m \cdot L, \quad (2)$$

where $L$ is latent heat value [J/kg].

https://doi.org/10.5593/sgem2023V/6.2/s26.63

In case of PCM an estimation of the latent heat can calculated indirectly by the evaluation of the heat flux increase over the temperature range that includes the phase change compared to a linear interpolation between points without phase change. For this purpose, three measurement series are required (see Fig. 3) - for the temperature range below the melting point (10 to 20 °C), the temperature range including melting point (20 to 30 °C for a PCM with melting point at 23 °C) and the temperature range above melting point (30 to 40 °C). The difference between the experimentally measured energy in the middle temperature range and energy calculated for the linear interpolation is the latent heat needed to melt the PCM in the sample. By applying this difference to a temperature interval of 10 °C and a known mass of melted PCM, the latent heat of fusion (or enthalpy of fusion) $H_f$ [J/g] describing PCM properties [11, 12] can be easily calculated.

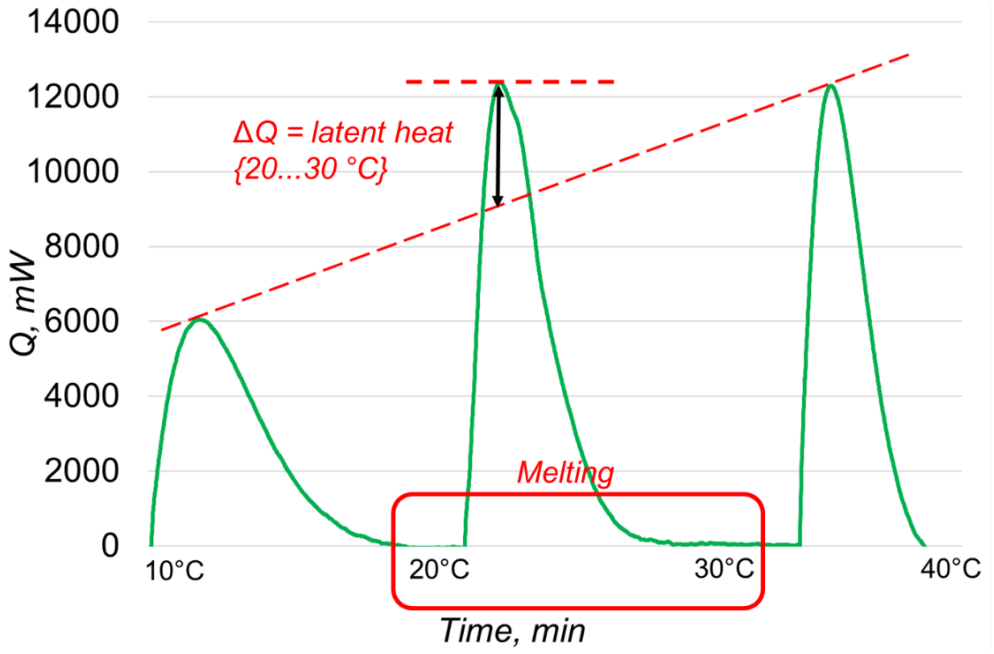



Fig. 3. Latent (phase transition) heat as difference between interpolation points.

## RESULTS AND DISCUSSION

This temperature step approach has been tested on several heterogeneous insulation, buildings and ecological materials, including samples with phase change material admixtures with subsequent calculations of latent heat of fusion, showing good accuracy compared to the literature data from DSC measurement [12-15].

The experimentally determined heat capacity and latent heat values are within the limits of literature data and differences do not exceed 10% (see Table 1), which is a very good result for the described indirect method.

**Table 1. Measured values of heat capacity and latent heat of fusion.**

| Sample | Heat capacity $c_p$ [J/(kg·K)] | | Latent heat of fusion $H_f$ [J/g] | |
|---|---|---|---|---|
| | Measured | Literature data | Measured | Literature data |
| Polyurethane foam | 1400 | 1400 … 1600* | - | - |
| Hempcrete** | 1400 | 1300 … 1500* | - | - |
| Plywood | 1400 | 1300 … 1400* | - | - |
| Straw | 1600 | 1600 … 1700* | - | - |
| Cornstalks | 1700 | 1600 … 1700* | - | - |
| Wood wool | 2000 | 1300 … 2100* | - | - |
| PCM S50 (bio-based) | - | - | 152 … 163 | 140 … 180 |

* *depending on density and composition*

** *hemp shives with a lime-based binder*

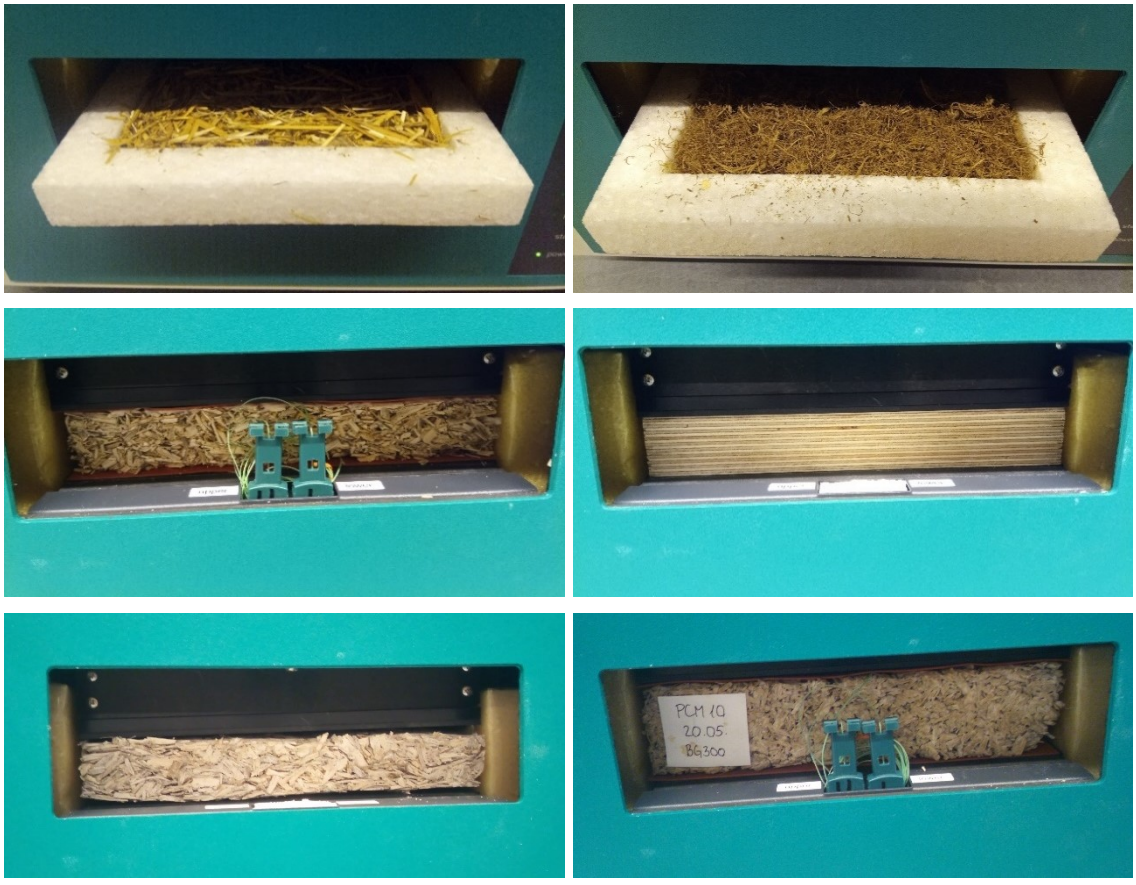



Fig. 4. Different material samples prepared for measurements.

## CONCLUSION

Described temperature step method for heat capacity determination has been successfully tested on heterogeneous building materials (incl. ecological materials like hemp, straw

and corn), loose fill insulation materials and materials with admixture of phase change materials with further indirect calculations of latent heat. All results are in good agreement with literature data and more accurate DSC measurement data. The main advantages of the described method are summarized below:

- good accuracy compared to the DSC method, but significantly cheaper than DSC;
- simple sample preparation, short measurement time;
- existing *HotPlate* thermal conductivity measuring devices can be used;
- the only method than can be used for large-scale and heterogeneous samples;
- can be successfully used to indirectly estimate the heat of fusion of phase change materials (if their total mass is known exactly).

The use of this approach will make it easier and less expensive to determine the unknown or only theoretically assumed heat capacity of building materials, opening the possibility for comprehensive measurements of heat capacity of materials used in construction.

## ACKNOWLEDGEMENTS

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# BRAZING OF $MoNbTaTiZrW_{0.2}$ HIGH ENTROPY ALLOY USING ECOLOGICALLY COATED RODS

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## ABSTRACT

Among the newest and best performing metallic materials are high-entropy alloys, which is why more than five thousand articles related to them have been published in the last decade. However, there are few articles that refer to the brazing behavior of new types of metallic materials. In this publication, the brazed joint between two biocompatible alloy components from the MoNbTaTiZrW0.2 system were performed using an ecological Cu-Ag-Zn alloy as filler material. The optical and SEM microscopy analyzes were performed for highlighting the microstructure of a cross-section of the brazed joint. The mutual dilution effects between the high-entropy alloys and the filler metal were quantified by EDS analyses, as well as the penetration mode of the filler metal through the gap between the parts. The soldering process between two HEA plates was performed without protection, in the atmosphere, using an oxy-gas flame with a neutral character. The analyzed joint between the high-entropy alloys had a good brazing behavior, achieving adequate wetting and adhesion of the Cu-Ag-Zn type brazing alloy on the surfaces of the joined parts. It was found that the chosen brazing alloy is very compatible with the base material, no unwanted intermetallic compounds were formed, the penetration into the capillary interstices between the samples was done correctly, without interruptions or cracks.

**Keywords:** brazing, ecologically rod, high entropy alloy

## INTRODUCTION

High entropy alloys are new metallic materials that contain at least five main elements with equimolar participations [1]. The important level of mixing entropy results by the concentration of each element varying, from 5 to 35 at. %. The high entropy in multicomponent alloy systems leads to the formation of solid solution type microstructures without brittle phases that have amazing mechanical, physical or chemical properties [2]. Ti, Mo, Zr and Ta are chemical elements considered biocompatible, because the toxic effects on the implant tissues are very reduced [3]. In the case of cast Mo alloys, processing at hot temperatures (more than 1000 $^{o}$ C) in the presence of oxygen (heat treatment, welding, or brazing) must be avoided. Molybdenum

oxide films segregated on the grain boundaries reduce intergranular cohesion. To limit this effect, strongly oxidizing elements like C, Al, Zr, Ti are added [4]. In the Ti-15Zr-Mo and Ti-20Mo-Zr alloys, the addition of molybdenum causes an increase in the elastic modulus through the precipitation of the ω phase [5]. Zhang et al. showed that solid solutions form in alloys that meet the criteria for $\Delta H_{mix}$ (mixing enthalpy) $-20 \leq \Delta H_{mix} \leq 5$, $\delta$ (atomic diameter) $\leq 6.4$ and $\Delta S_{mix}$ (mixing entropy) $12 \leq \Delta S_{mix} \leq 17.5$ [6]. Some elements added to the HEA matrix can improve the mechanical properties. Thus, it was observed that by increasing the Nb content, a linear increase in the compressive yield strength and Vickers hardness was obtained [7].

Brazing is an old and conventional joining method that allows the joining of ceramics or metals, that uses filler materials with low melting temperatures, without putting pressure on the parts. The filler material melts at a lower temperature than that of the base materials [8]. To avoid the pores and inclusions formation, the surfaces of the brazed parts must be mechanical, and chemically cleaned. If it is possible, the process must take place in an environment protected against oxidation [9]. The interaction between the molten filler material and the base metal can be hindered if there is an oxide film on the surface of the parts [10]. The fluxes used in brazing interact with the oxide films on the surfaces of the components, favoring the wetting effect and their mutual adhesion [11]. The diffusion of chemical elements through the brazing interface is important, because it ensures good adhesion of the joined parts [12]. However, the interdiffusion between the filler metal and the base material can modify the local chemical composition and implicitly the mechanical strength or corrosion resistance in the joint area [13]. Unlike traditional filler materials, those made from high-entropy alloys do not cause the formation of additional intermetallic particles in the braze seam [14]. Cadmium-free solder bars are an ecological solution for joining components made of high-entropy alloys intended for medical applications [15]. High surface wetting efficiency, good chemical compatibility and high corrosion resistance in various environments can be achieved using a special coating that contains elements with chemical activation and catalytic effects [16].

The paper presents the results obtained when brazing a high-entropy alloy from the MoNbTaTiZr system with addition of W, using an ecological Cu-Ag-Zn alloy as filler material. These high-entropy alloys exhibit high yield strength (900-1600 MPa) at ambient temperature, but also considerable compressive strength. To highlight the microstructural aspects, optical and SEM analyses were performed on a cross section of the brazed joint. The diffusion effects of chemical elements through the interface between the base metal and filler material were evaluated using EDS analyses. The cross-sectional examination of the brazed joint did not reveal the appearance of cracks, lack of adhesion or the formation of brittle compounds. In the specialized literature, no references were found regarding the obtaining of brazed joints between components from this alloy, which is still being studied and considered a potential new biomaterial.

## MATERIALS AND METHODS

The new high entropy alloys from $MoNbTaTiZrW_{0.2}$ system were obtained using the MRF ABJ 900 vacuum arc remelting equipment. High purity raw materials (99.5%) were used for performing the samples of about 40g/load. The designed percent composition for the high entropy alloy $MoNbTaTiZrW_{0.2}$ was as follows: Mo = 22.13%;

Nb = 13.69%; Ta = 21.12%; Ti = 11.86%; Zr = 23.3%; W = 7.90%. Tungsten was added in this alloy to improve the mechanical properties and has a molar participation equivalent to 0.2 at%. To obtain a good homogeneity of the chemical composition, the ingots were successive rotated to be remelted, six times on each part. After obtaining the samples were milled and then they were polished with 600-grit sandpaper. The dimensions of the brazed parts were 14x9x2mm. Prior to brazing, the samples were cleaned with ethanol, followed by pickling in borax powder.

The filler material used for brazing was an ecologically coated rod with the following chemical composition: Ag=45.4wt%; Cu=27.2wt%; Zn=24.2wt%, Sn=0.2wt%. The coating contains slugging ($H_3BO_3$, $Na_2B_4O_7$, $B_2O_3$), deoxidizing and pickling elements (KCl, NaF, $Na_2iF_6$, $Na_3AlF_6$, $KBF_4$, KF,). FH 10 flux type was used for the brazing, with the recommended temperature range of 550 °C - 800 °C.

The cleaned samples were superimposed on each other and were fixed in a rigid device. Brazing was performed using a neutral oxy-gas flame and a coated rod of Cu-Ag-Zn. To ensure good wetting and deep penetration into the gap between the parts, pre-heating of the samples was first performed by swinging the gas flame on the outer surfaces for 5 seconds. In the process the flame was oscillated between the base material and the filler material, to avoid overheating the parts to be brazed. After brazing, the parts were washed with warm water, then the excess solidified flux was removed with a wire brush and sandpaper.

## RESULTS

After cooling, cross-sections were cut from the brazed pieces, then their surfaces were ground and polished for metallographic analysis and microhardness measurements. The grinding was performed using grit paper (360, 500, 1000 grit) and then alumina alpha power (0.7 to 0.1µm grain size) was used for polishing. No reagent was used for etching. The metallographic analysis was carried out on cross-sections (Figure 1).

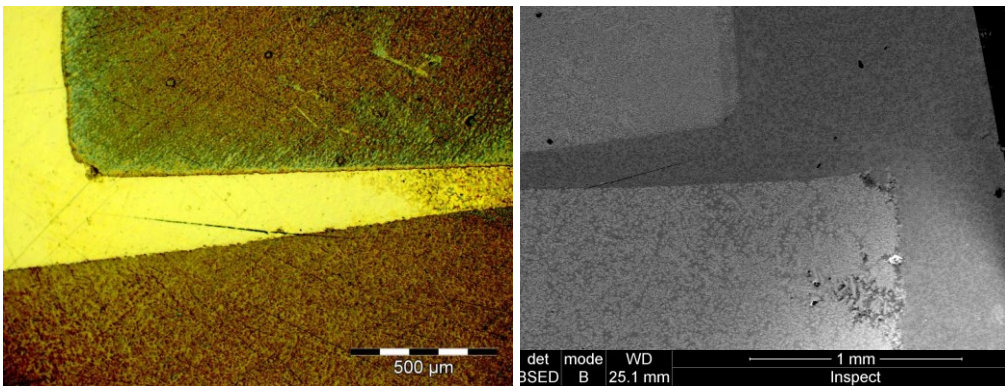



Figure 1. Cross section through the brazed joint: a) optical microscopy; b) SEM image.

Figure 2 illustrates the dendritic microstructure of both the MoNbTaTiZrW alloy and the Cu-Ag-Zn filler metal. The filler metal interface with HEA is smooth and continuous, without imperfections, showing a good adhesion between the two materials (Figure 2b). Regarding the filler metal, the concentration of the elements in the alloy is of 50 wt% Ag, 30 wt% Cu and 20 wt% Zn. The filler metal microstructure (Figure 2c) consist of dendrites Ag-rich and needle-like phases dispersed in a matrix Cu-rich.

In the case of HEA, the Rietveld analysis indicated the presence of Ta as the main phase, which is crystallized in a hexagonal system. Along with it, in smaller proportions, it appears as a solid MoNbTa solution, crystallized in the c.v.c. system. and the compounds Ti0.879Nb0.121, and Ti4Nb, both crystallized in the orthorhombic system. From the compositional chemical mapping of the HEA, it can be seen that Zr, Nb and Ti predominate in the interdendritic region of the alloy. Tungsten also accompanies Ta in the dendrites, which are mostly composed of Ta and Mo.

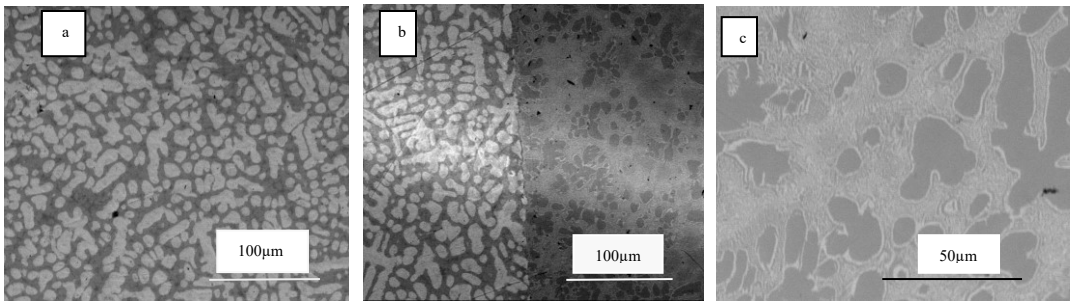



Figure 2. Microstructure of HEA alloy and filler metal. a) $MoNbTaTiZrW_{0.2}$ alloy; b) interface between HEA and filler metal; c) Cu-Ag-Zn filler metal.

Between the two materials, mutual diffusion of the elements was achieved through the brazing interface, as illustrated in Figure 3. It can be observed how Cu and Zn progressively enter the HEA on a distance of about 40 microns, while the concentration of Ag drops rapidly at a distance of 10 microns from the interface. At the same time, Ta exhibits the most significant diffusion effect to the filler metal of all elements in the HEA, penetrating more than 40 microns deep from the brazing interface.

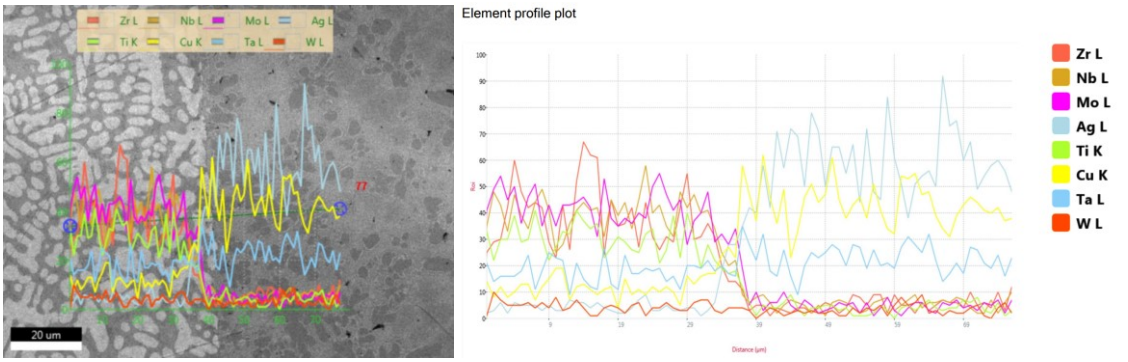



Figure 3. Elements profile plot along a transversal line on the interface of 77 microns length.

The hardness of the characteristic areas of the joint was measured with the Shimadzu HMV 2T apparatus from the LAMET laboratory, NUSTPB. A force value of 4.903 N was used and the test time was 10 seconds. 10 successive, in-line measurements were made on the central and lateral areas of the transverse surfaces of the samples, keeping distances of approximately 500 µm between the indentations. The measurements were carried out in cross-section, on both sides of the brazing gap, before and after joining. Before brazing the microhardness, average value, was of 538 HV0.5 while in brazed state was of 563 HV0.5.

## DISCUSSION

Current recipes of Ag-based bonding alloys are used for bonding dental prostheses made of biocompatible stainless steels. They are based on the Ag-Zn-Cu ternary system, to which small percentages of Ni or Sn are added [17]. Unfortunately, both Cr and Ni are not considered biocompatible elements, which may cause adverse reactions in some patients.

The paper analysed for the first time the behaviour of an Ag-based alloy as a filler material with ecological coating to brazing two components from the new MoNiTaTiZrW alloy, as a potential substitute for stainless steel. At the same time, the studied alloy has as a novelty compared to other similar alloys the presence of W in the metal matrix. During brazing, the coating of the filler rod is first melted. It contains mineral substances that react chemically and deoxidize the surfaces of the parts, thus ensuring a good wetting of the molten alloy on the solid surfaces of HEA. The melting of the alloy took place after the temperature rose to about 750 - 800ºC, and the liquid metal was absorbed into the very small gap between the parts (about 0.15 mm), due to the capillarity effect. The analyses carried out showed that the brazing alloy adhered and wetted the HEA surface very well, penetrating the gap between the components at distances greater than 4 mm, enough to ensure the shear strength of a joint between parts with a thickness greater than 10 mm. The elements that migrated more from the HEA to the filler metal were Ta and Mo. From the filler metal, the diffusion over the greatest distances was recorded in the case of Ag and Cu elements.

## CONCLUSION

Biocompatible MoNbTaTiZrW0.2 high entropy alloy behave well at brazing process. The high oxidation tendency of these alloys requires the use of brazing enclosures filled with inert gas to protect from ambient atmosphere. During heating, a thin layer of oxide forms on the surface of the brazed parts, which affects the mechanical integrity of the surfaces and changes the corrosion resistance. The Cu-Ag-Zn filler metal with ecological coating chosen for brazing the high-entropy alloy had a good adhesion to the surface of the parts, penetrated through capillarity along the length of 1/3 of the thickness of the parts, and did not cause the formation of brittle compounds. At the interface between the two materials, the mutual diffusion of the chemical elements occurred, which allowed obtaining of the monolithic joint. Although the maintenance time in the field of high temperatures is short during brazing, the diffusion of chemical elements through the interface was fast, without forming unwanted accumulations or segregation of hard compounds. This increase in hardness is the effect of mutual diffusion of chemical elements through the joining interface and the quenching effect associated with rapid cooling in air after brazing.

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# CARBON FOOTPRINT REDUCTION ON MANUFACTURING SMES FROM DIGITAL TECHNOLOGIES

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## ABSTRACT

Europe must avoid losing manufacturing SMEs. However, presenting distinct maturity levels, these companies have a hard time fulfilling the necessary ecological sustainability level. Controversially, despite the intense debate about digital technologies, there is still a need for knowledge in the industrial world about the impact of these technologies to make manufacturing SMEs environmentally friendly.
According to data made available by Stone Portuguese Federation, in 2019, the Portuguese Ornamental Stone sector (OS.Pt): (i) exported to 116 countries; (ii) ranked 9th in the World International Stone Trade; (iii); was the second country in the world in international trade per capita; (iv) exports covered imports by 660%; (v) 45% of exports were to countries outside Europe and (vi) had a total turnover of EUR1,230 million. Mainly comprised of small and medium-sized enterprises (SMEs), the OS.Pt sector is relevant for the Portuguese Economy, representing over 16,600 direct jobs, and is one of the main generators of private employment in inland regions has registered an average annual growth rate in turnover of 5.13% over the last ten years and has generated around 500 new jobs from 2016. By using the Portuguese stone sector case study, considering the investment to improve the operations towards digital, a framework was designed and used in five groups of forty stone manufacturing SMEs, each representing a digital level. Through a quantitative methodology based on five years after the investment, depending on the technology used and level of investment in digital technologies, it was found a potential reduction of CO2 emissions between 10.7% and 35.1%.

**Keywords:** Digital technologies, Decarbonization, Manufacturing SMEs, Ecological Footprint, Stone Industry

## 1. INTRODUCTION

According to the European Commission, in 2022, the number of active small and medium enterprises (SMEs) in the EU-27 represented 99,8% of all enterprises in the non-financial business sector in Europe [1], providing job opportunities to more than 84,9 million people and act as suppliers of goods and services to individuals and large organisations

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[2]. Despite their relatively small size, these 24.3 million SMEs represent a substantial contribution to the global energy-related CO2 emissions [1], which, according to the International Energy Agency (IEA), grew 0.9% in 2022, reaching a new high of over 36.8 Gt [3].

In response to this scenario, the AEC companies' investments in digital technologies, for instance have grown in the past few years, reaching more than EUR 560 billion in 2022 [4]. However, despite this massive investment, some studies show that no significant impact on carbon emissions is still visible globally [5]. Therefore, **what impact do digital technologies have on reducing carbon footprints in manufacturing SMEs?**

Given this question, this research aims to formulate an empirical framework rooted in key performance indicators (KPI) to evaluate the impact of digital technologies on carbon footprint reduction. By relating the industrial firms' digital level to their energy efficiency, the digital rank (DR) concept will be part of this framework, representing the level at which a company uses digital technologies. Once formulated, this framework will be applied to a representative sample of manufacturing SMEs ranked in different digital levels, from which the KPIs will be assessed from the data collected. From these results, conclusions about the impact of digital technologies on manufacturing SMEs' carbon footprint reduction will be inferred.

## 2. Theoretical Background and Research Methodology

Signed by 195 nations, the Paris Agreement is a landmark international agreement to address climate change and its negative impacts. The agreement aimed to substantially reduce global greenhouse gas emissions to limit the global temperature increase to 2° Celsius above pre-industrial levels by 2050. Rooted in the Paris Agreement, the carbon footprint emissions in AEC are assessed mainly by frameworks using the life cycle assessment (LCA) for manufacturing products (ISO 14040:2006). The LCA focuses on the life cycle of a product, from the extraction of raw materials through the stages of production, distribution, use and building demolition. The ISO 14040 series standardises the structure of the technique. Therefore, to achieve the objective as mentioned earlier, anchored on the ISO Standard 14040, a comprehensive framework for assessing the impact of digital technologies on the carbon footprint will be designed and used, following the LCA methodological steps: (1) the description of the context (2) framework designing, (3) environmental impact assessment, and (4) results interpretation.

### Empirical Context, Population, and Sample

The first methodological LCA stage involves presenting the purpose and extent of the study by establishing its boundaries [6]. This requires selecting a representative sample from the population to be studied.

Boosted by digital and connectable technologies, the current transition to a digital economy popularised by academics and practitioners as Industry 4.0 (I4.0) [7]. Behind the I4.0 technologies, with yet unpredictable impacts in AEC, the Building Information Modelling (BIM) is a promising but poorly explored carbon mitigation digital technology. With the tendency to be globally mandatory in the short to medium term and oriented to zero-waste and neutral ecological footprint [8] from the BIM generalisation, a procurement model oriented to low-carbon products in AEC seems to emerge [9].

Portugal, for example, is considered a world-class producer of stone products, fully integrated into a very competitive and global supply chain that is AEC. Made up of manufacturing SMEs, the Portuguese stone Sector (OS.Pt) directly employs over 16.600 people, is the leading private employer in the interior territories and the second one on value-added per working hour in the country [10]. Despite its traditional historical roots, since 2004, the OS.Pt has recorded an average annual growth rate in turnover of 5.13% and generated over one thousand new skilled jobs over the last ten years [11]. In anticipation of the mandatory BIM, the OS.Pt, probably recognising that its future will depend on its ability to supply low-carbon products over the last fifteen years, has been investing strongly in digital technologies[1]. Although this is a general trend in Portuguese stone companies towards digitalisation, different realities and investment capacities have been stratifying the sector in terms of the digital level of the operations of each company that makes up the OS.Pt, fulfilling, therefore, the conditions to be used as the population to be studied.

Most of the OS.Pt companies are industrial SMEs incorporating downstream activities, transforming the raw materials into products for the building construction sector. Integrated into the AEC supply chain, in 2019, according to the Stone Portuguese Federation[2], in 2019, the Portuguese Ornamental Stone sector (OS.Pt) (i) exported to 116 countries, (ii) ranked 9th in the World International Stone Trade, (iii) made Portugal the second country in the world in international trade per capita, (iv) the exports covered imports by 660%, (v) 45% of exports were to countries outside Europe, and (vi) had a total turnover of EUR1,230 billion. From this population, a representative sample of 16,2% of the OS.Pt companies covering the eighteen Portuguese continental districts were surveyed in 2022.

**Framework Design**

The second stage of the LCA is framework designing, an indispensable tool for the quantitative assessment of environmental impacts. Therefore, this framework for assessing the impact of digital technologies on the SMEs' carbon footprint requires a prior evaluation of each company's digital level in the sample.

This evaluation must be carried out through an in-loco survey, from which, the digital rank of each company is established: (i) the zero digital rank (DR#0) assigned to the companies that have not started any digitalisation step yet; (ii) the DR#1 assigned to the companies using one digital production machine; (iii) the DR#2 assigned to the companies using two digital machines; (iv) the DR#3 assigned to the companies using at least three digital machines integrated with their Enterprise Resource Planning (ERP) and finally (v) the DR#4 assigned to the companies using more than three machines fully integrated in a collaborative way with the marketplace, fulfilling the I4.0 concept [12].

A critical aspect of this endeavour is developing a set of key performance indicators (KPIs) [13]that serve as indispensable metrics [13].For this framework, the carbon footprint reduction will be inferred from the improvements in energy consumption, raw materials yield, and additional rework.

[1]https://www.clustermineralresources.pt/

[2] https://www.assimagra.pt/pt/

The *Energy Efficiency KPI ($KPI_{EE}$)* – assessing the output units per one hundred kWh - evaluates the company's energy efficiency (Equation 1).

Equation 1. $KP_{EE} = Average \frac{\sum(\text{O_UNITS})}{\sum(\text{E_CONSUM})}$

The *First Time Through KPI ($KPI_{FFT}$)* – assessing the reworked units per output units this KPI evaluates the average ratio between the units not requiring any rework per total output units, thus representing the production effectiveness (Equation 2).

Equation 2. $KPI_{FFT} = Average \frac{\sum(FTT_UNITS_k)}{\sum(\text{O_UNITS_k})}$

The *Raw-Material Efficiency ($KPI_{RMY}$)* - assessing the output units per consumed material, this KPI evaluates the average raw material consumption per total output, thus representing the material yield achieved (Equation 3).

Equation 3. $KPI_{RMY} = Average \frac{\sum(\text{O_UNITS_k})}{\sum(\text{M_CONSUM_k})}$

The *Carbon Footprint Emissions ($KPI_{CO2\text{-}Eq}$)* – assessing the Kg CO2 equivalent emissions per 1000 output units this KPI evaluates the CO2 emission intensity (kg CO2/kWh) calculated as the ratio of CO2 emissions from public electricity production (as a share of CO2 emissions from public electricity and heat production related to electricity production), and gross electricity production (Equation 4).

Equation 4. $KPI_{CO2\text{-}Eq} = Average \frac{\sum(\text{E_CONSUM})}{\sum(\text{O_UNITS})} \; x \; 0{,}23314$

During 2022, 144 OS.Pt companies were visited to evaluate their digital rank and collect data. From these surveys directly involving the company managers, digital assets were evaluated in terms of the aforementioned digital ranks and the respective DR assigned. Moreover, annual data on each company's electricity consumption and total units was collected as well as average data on the rework and raw materials yield.

**Environmental Impact Assessment**

The third stage of the LCA consists of making it possible to assess the significance of environmental aspects for a group of actors representing a population. It requires collecting data from the SMEs and assessing the KPIs to comprehensively evaluate the impact of digital technology on their carbon footprint emissions.

From the in-site surveys, the DR#0 OS.Pt companies can produce 294 pieces per thousand kWh of electricity consumed, 72,9% of these pieces require additional rework, and a raw material yield of 65,7%; the DR#1 companies can produce 303 pieces per thousand kWh, 76,3% of these pieces requiring additional rework with a raw material yield of 63,7%. In turn, the DR#2 OS.Pt companies can produce 401 pieces per thousand kWh, DR#3 can produce 589 pieces per thousand kWh, and the DR#4 companies can produce 645 pieces per one thousand kWh, only 19,7% of these pieces requiring additional rework, and reaching a raw material yield of 84,9% (Table 1).

Table 1. KPIs: Results and average

| Digital Rank | $KPI_{EE}$ (units/1000kWh) | $KPI_{FFT}$ (units/units) | $KPI_{RMY}$ (output/raw_material) | $KPI_{CO2\text{-}Eq}$ (KgCO2-eq/Kunits) |
|---|---|---|---|---|
| DR#0 | 29,4 | 27,1% | 65,7% | 793,0 |
| DR#1 | 30,3 | 23,7% | 63,7% | 769,4 |
| DR#2 | 40,1 | 57,1% | 69,3% | 581,4 |
| DR#3 | 58,9 | 80,3% | 77,8% | 395,8 |
| DR#4 | 64,5 | 81,2% | 84,9% | 361,5 |
| Average | 44,6 | 53,9% | 72,3% | 580,2 |

## 3. Results Interpretation

This methodological stage evaluates the study's established objectives [6]. In this regard, Table 1 shows the evolution trend of the $KP_{EE}$ as the company moves to digital operations; the production output per unit of energy consumed follows a positive exponential trend line (dotted line).

The $KPI_{FFT}$ indicator shows evolutionary characteristics, similar to the energy efficiency indicator. However, linear trend gains of 16,48% and a standard deviation of 0,248 indicate that the gains will probably continue well beyond the time when the investment in digital technologies is made.

The $KPI_{RME}$ indicator shows a regular positive trend when companies climb the digital ladder. With a linear trend gain in raw materials of 5.05% waste, this indicator evolution shows positive implications far beyond carbon reduction, particularly productivity.

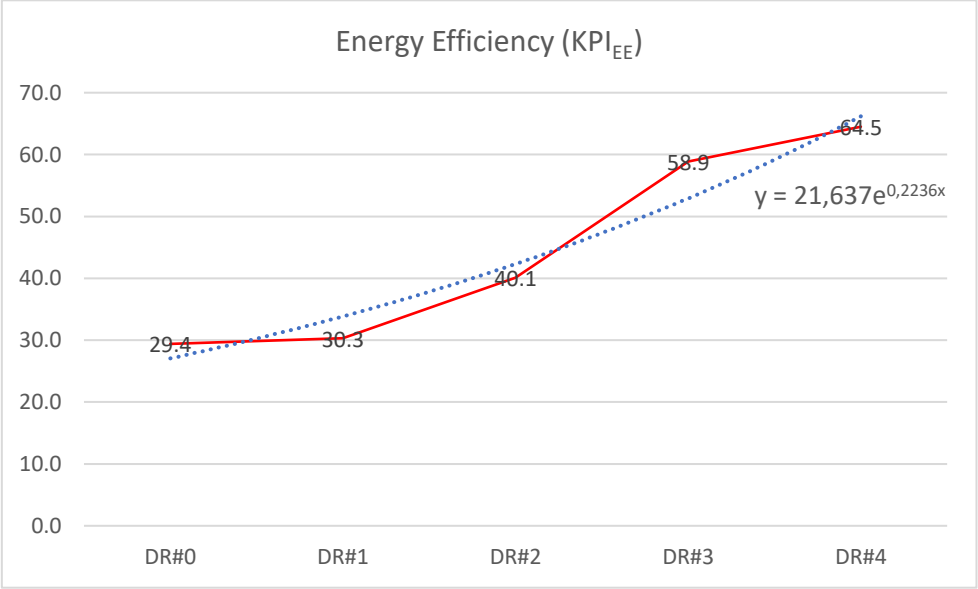



Figure 1. Energy efficiency ($KPI_{EE}$): Evolution Trend.

Finally, the $KPI_{CO2\text{-}Eq}$ shows an average sample carbon footprint of 58.0 Kg CO2 equivalent per thousand pieces, with a standard deviation of 18.0. Moreover, it shows that for the same output, the DR#0 OS.Pt companies' emissions are 2.19 times higher than the DR#4 companies.

By converting the energy reduction to carbon equivalent released[3], using the framework on the sample, based on five years after the investment in digital technologies, it was found that companies at the lowest digital level (DR#0) emit, on average, 793,0 Kg of CO2-eq per thousand output units, have to rework 72,5% of the pieces, and waste 34,3% of the raw materials consumed. Controversially, companies with the highest digital level emit an average of only 395.8 Kg of CO2-eq per thousand output units, must rework 19.7% of the pieces, and waste 22.2% of the raw materials consumed.

## CONCLUSION

The main objective of this research was to assess the impact of digital technologies on the carbon footprint reduction of manufacturing SMEs. Employing a quantitative methodology based on ISO Standard 14040, a framework was designed and used on a representative sample of Portuguese stone manufacturing SMEs. From the above results, conclusions can be drawn in the light of two scenarios:

**Moderate Scenario**: A company without digital equipment (DR#0) decides to invest in digital technologies to become DR#2. From this digital evolution, the company will obtain a potential reduction in its carbon footprint of 10.7%, corroborated by its potential reduction in rework of 30.0% and a 3.5% gain in the raw materials yield.

**Optimistic Scenario**: Considering the same starting situation (DR#0), if the company decides to invest in digital technologies reaching DR#4, it could reduce its carbon footprint by 35.1%, corroborated by a potential rework reduction of 54.1% and a 19.5% gain in the raw materials yield.

### Suggestions for future investigations

Despite this positive impact on carbon reduction, more than merely investing in digital technologies might be needed to achieve these benefits. Since the migration to digital requires a multidimensional approach, new research is suggested to explore this further.

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# FLAME-RETARDANT TREATMENT EFFEECT OF WOVEN FABRIC USING HANJI YARN FOR CAR SEATS

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## ABSTRACT

This research aims to contribute to the development of eco-friendly car seats using hanji yarn by examining the flame-retardant treatment effect of two types of hanji yarn fabrics woven with polyester warp yarn and two different types of weft yarn: hanji-polyester blended yarn (76:24) and 100-percent hanji yarn, respectively. In terms of the flame-retardant treatment, NICCA FI-NONE PD-38 was used as the flame retardant with the concentrations of 10, 20, and 30 percent, and a flame resistance test was conducted in accordance with MS 300-08, the flame resistance standard for car interior materials. The test results show that the fabric untreated with flame-retardant chemicals completely combusted while the fabric treated with a flame retardant exhibited self-extinguishing properties, regardless of the concentration of flame-retardant chemicals. In an analysis of the thermal stability of the two different types of hanji yarn fabrics, the fabric treated with flame-retardant chemicals exhibited thermal stability by forming a carbonaceous layer faster than the untreated fabric. In an analysis of the changes of functional groups, conducted by infrared spectroscopy to examine the chemical structures of the flame retardant used in the research, the P-OR ester peak and the P-O peak were observed in the spectral range from 900 to 1,000 $cm^{-1}$ and from 725 to 845 $cm^{-1}$, respectively. This suggests that the flame retardant used in the research is a phosphorus compound.

**Keywords:** flame-retardant treatment, woven fabric, car seat, hanji yarn

## INTRODUCTION

As car materials are regarded as important elements that allow automakers to gain a competitive edge through automotive differentiation and diversification, research on new materials is increasingly important. There is also an urgent need for further research on automotive seats since they occupy more than 60 percent of a car's interior space, thereby playing a critical role in deciding the ambience of the interior space [1]. In recent years, with the active development of eco-friendly vehicles, such as hybrid or electric cars, the use of biomaterials and other eco-friendly interior materials is increasing accordingly. Therefore, it is necessary to develop environmentally-friendly car seats, since the use of eco-friendly materials for car seats that come in direct contact with the user's body has the potential to satisfy the user both visually and psychologically through the diverse textile sensations and colors of natural fiber materials. Automobile brands are now enhancing new materials with the aim of

applying eco-friendly materials to their car seats [1] and constantly investing in the development of new materials and new technology. Car seat fabrics made with natural or recycled materials can not only be used as an effective marketing strategy but also benefit the health of drivers and passengers. It is known that modern consumers regard comfort and perception of luxury as the most important factors when choosing car seat materials [2]. Hanji represents an excellent eco-friendly material with high durability, absorbency, and classical elegance. In particular, Korea is an optimal location for cultivating paper mulberry, the raw material for hanji, and thus hanji made in Korea is widely recognized for its excellent quality. In this regard, this research focuses on the development of eco-friendly automotive seat materials using hanji yarn, which is made from the traditional Korean product. Hanji is classified as cellulose fiber, based on its component. Cellulose fiber has many strengths, such as high absorbency, breathability, and a soft and warm touch, but one of its biggest drawbacks is high flammability, meaning that it is more susceptible to combustion and unsafe from the risk of fire. Therefore, in order to develop car seat materials using hanji yarn, it is urgently necessary to research its flame-retardant treatment effect. There are several methods used to make cotton, another type of cellulose fiber, flame resistant, such as weaving fabrics with flame-retardant fiber and post-processing for flame retardancy [3,4]. Among them, the post-processing method is widely employed because the methods of processing natural fibers including cotton and wool are relatively simpler [5,6]. Flame retardants are broadly divided into organic and inorganic types. Organic flame retardants include brominated, phosphorus, and chlorinated chemicals, while inorganic flame retardants, including antimony oxide and aluminum hydroxide, are mostly used as auxiliary flame retardants [5,7].

The goal of this research is to examine the flame-retardant treatment effect of fabrics woven with hanji yarn for the development of eco-friendly car seat fabrics. For the research, two different types of fabrics were woven with polyester yarn as warp and blended yarn with a 76:24 mixture of hanji and polyester yarn (“hanji/PET yarn”) and 100-percent hanji yan as weft, respectively. These two types of fabrics were treated with flame-retardant chemicals and underwent a flame resistance test. The chemical structures of the flame retardant used in the research were analyzed by Fourier transform infrared spectroscopy (FT-IR), and the morphology and thermal characteristics of fabric treated with flame-retardant chemicals were analyzed with a scanning electron microscopy (SEM) and a thermogravimetric analyzer (TGA), respectively. It is hoped that the result of this research can contribute to the development of eco-friendly automotive seat fabrics.

## EXPERIMENT MATERIALS AND METHODS

### 1. Experiment Materials

Weaving: In order to weave fabrics for this research, 150 denier polyester yarn, which is used for car seat fabric and was offered by Hyosung TNC, was used as warp yarn, while hanji/PET blended yarn with 230 denier and 100-percent hanji yarn with 180 denier were purchased from Ssang Young Spinning and used separately as weft yarn. The polyester warp yarn and one of the two different types of weft yarn, blended yarn with a 76:24 mixture of hanji/PET yarn and 100-percent hanji yarn were plainly woven into two types of fabrics on a Rapier loom (STM400, Sambo Engineering, Korea) at Donga

SD, located in Daegu, Korea. The total number of warp ends was 2,300, and optimal fabric width was 20 inches. The characteristics of the woven fabrics are presented in Table 1.

**Table 1. The characteristics of fabrics**

| Samples | Properties | | | | |
|---|---|---|---|---|---|
| | Weave | Thickness (mm) | Density (ends/inch) | | Weight ($g/m^2$) |
| | | | Warp | Weft | |
| A | Plain | 0.37 | 60 | 64 | 156.04 |
| B | Plain | 0.35 | 60 | 72 | 144.88 |

A [warp: polyester yarn, weft: blended yarn with a 76:24 mixture of hanji yarn and polyester yarn (indicated as hanji/PET below)], B [warp: polyester yarn, weft: 100% hanji yarn (indicated as hanji below)]

Flame-retardant chemicals: In this research, NICCA FI-NONE PD-38 (Nicca Korea) was used as the flame retardant. Since self-extinguishing properties were not observed when a fabric was treated with a 5-percent concentration of the flame retardant in a preliminary test, the concentration was set at 10, 20, and 30 percent.

## 2. Experiment Methods

Flame resistance test: Flame retardancy refers to the property of slowing down ignition and reducing the intensity of combustion. In this research, flame resistance was tested in accordance with MS 300-08, a flammability resistance test standard for car interior materials.

The burning speed is calculated according to the following formula.

$$B = D/T \times 60$$

B: burning speed (mm/min), D: fire spreading distance (mm), T: burning time (second)

The test environment and conditions are presented in Table 2.

**Table 2. Test environment and conditions**

| Sample conditions | Environment | Laboratory conditions | Environment |
|---|---|---|---|
| Date | Dec. 27 - Dec. 28, 2021 | Date | Dec. 28, 2021 |
| Temperature | (21 ± 5)℃ | Temperature | (21 ± 5)℃ |
| Relative humidity | (55 ± 5)% | Relative humidity | (55 ± 5)% |
| Leaving time | 24h | Weather | - |

Morphology observation: The morphology of fabrics with and without flame-retardant

treatment was observed at 30-times magnification with a magnifying glass (KAI-P100, Toolis, Korea). And then, for a closer observation, the fabrics were coated with platinum through a vacuum deposition process and observed at 3,000-times magnification with a scanning electron microscope (JSM-6700F, JEOL, Japan).

Infrared spectroscopy measurement: The change in functional groups of fabrics with and without flame-retardant treatment was measured using attenuated total reflection (ATR) with FT-IR (Spotlight 300, Perkin Elmer, the UK) with a spectral range of 4,000-650 $cm^{-1}$.

Thermogravimetric analysis: The pyrolysis temperature and thermogravimetric changes of the fabric treated with flame-retardant chemicals were measured by TGA (TGA Q500, TA Instruments, the USA) with the temperature range from 20 to 600℃ in a nitrogen atmosphere at a heating rate of 20℃/min.

## RESULTS AND ANALYSIS

### 1. Flammability

Table 3 presents images of fabric samples with and without flame-retardant treatment before and after they went through a flame resistance test in accordance with MS 300-08, the flame resistance test standard. The test results show that the untreated fabric completely combusted while the fabric treated with flame-retardant chemicals exhibited self-extinguishing properties, regardless of the concentration of flame-retardant chemicals. Table 4 shows the results of the fire spreading distance (mm), the burning time (s), and the burning speed (mm/min) of the MS 300-08 test. The acceptance criteria for flammability resistance was that the tested sample extinguished itself within 60 seconds without burning at the burning speed of no more than 80 mm/min (maximum) or at the fire spreading distance of no more than 50 mm from the measuring point. This is defined as self-extinguishing. As shown in Table 4, it was found that the untreated fabric samples did not meet the acceptance criteria as the fire spreading distance was 254 mm from the measuring point, the burning time was 146 seconds, and the burning speed was 105 mm/min. The comparison between the fabrics using hanji/PET yarn as weft and the fabrics using 100-percent hanji yarn as weft indicated that the woven fabrics with hanji/PET yarn weft had a longer burning time and a faster burning speed, which made them susceptible to combustion. This is because thermoplastic synthetic fibers are more vulnerable to combustion than natural fibers. Meanwhile, it was found that flame-retardant treated fabrics were immune to burning, except only when the fabric using hanji/PET yarn as weft was treated with a 10-percent concentration of flame-retardant chemicals. The other flame-retardant treated fabrics did not burn at all regardless of their concentration of flame-retardant chemicals and their fire spreading distances were zero. Also, both their burning time and burning speed were all zeros, showing that there was no occurrence of combustion. In addition, it was confirmed that the fabric woven with hanji/PET yarn with a 10-percent concentration of flame-retardant chemicals as mentioned above also exhibited self-extinguishing properties. The flame retardant used for the flame-retardant treatment demonstrated a flame-retardant effect by interfering with specific combustion steps such as heating, thermal decomposition, and heat generation during the combustion process to suppress or alleviate combustion [8]. The flame retardant used in this research is a type of

phosphorus flame retardant, as shown in 3.2 IR Analysis. Phosphorus-based flame retardants react to flammable materials during the combustion process to form a carbonaceous layer on the surface of a polymer, which blocks the oxygen required for combustion, thereby causing a flame-retardant effect. In particular, phosphorus-based flame retardants react with elemental oxygen in a polymer and cause dehydration and carbonization, which displays a flame-retardant effect. Therefore, it has been reported that phosphorus-based flame retardants can serve as flame retardants in polymers containing elemental oxygen [8].

**Table 3. Test samples before and after flame resistance test in line with MS 300-08**

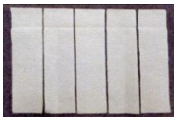
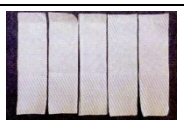
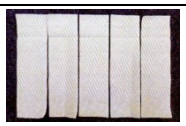
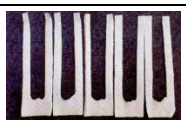
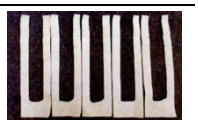
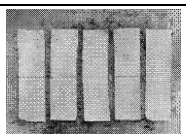
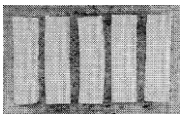
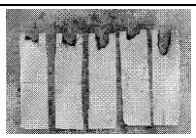
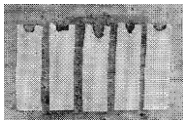
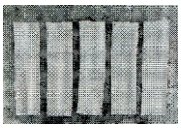
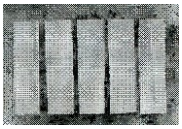
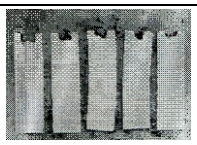
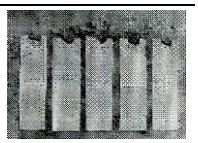
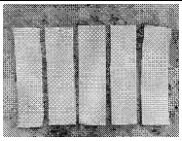
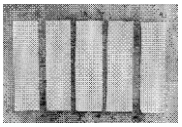
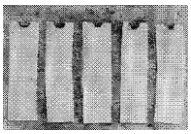
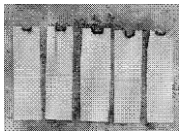
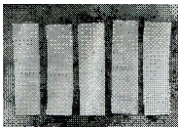
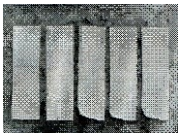
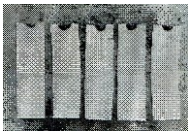
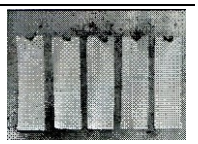
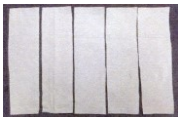
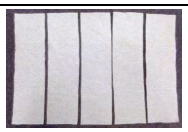
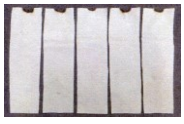
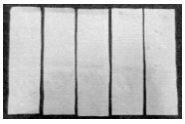
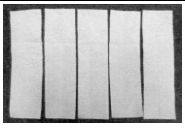
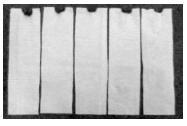
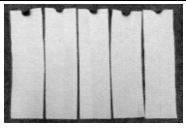
| Samples | | | Before test | | After test | |
|---|---|---|---|---|---|---|
| | | | Height | Width | Height | Width |
| Hanji/PET | | |  |  |  |  |
| Hanji | | |  |  |  |  |
| Conc. of flame retardant (%) | 10 | Hanji/PET |  |  |  |  |
| | | Hanji |  |  |  |  |
| | 20 | Hanji/PET |  |  |  |  |
| | | Hanji |  |  |  |  |
| | 30 | Hanji/PET |  |  |  |  |
| | | Hanji |  |  |  |  |

**Table 4. Results of flame resistance test in line with MS 300-08**

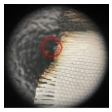
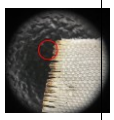
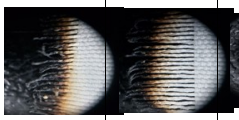
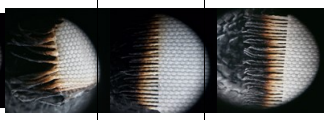
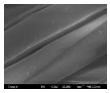
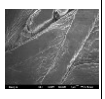
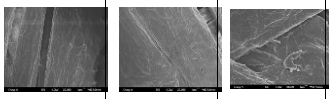
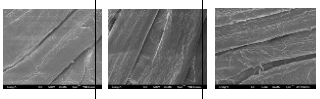
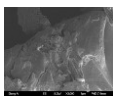
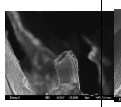
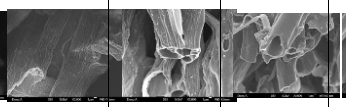
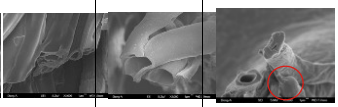
| Samples | | | | Fire spreading (mm) | Burning time (s) | Burning speed (mm/min) | Result |
|---|---|---|---|---|---|---|---|
| Hanji/PET | | | Height | 254 | 146 | 105 | Fail |
| | | | Width | 254 | 169 | 169 | Fail |
| 100% hanji | | | Height | 254 | 143 | 106 | Fail |
| | | | Width | 254 | 161 | 117 | Fail |
| Conc. of flame retardant (%) | 10 | Hanji/ PET | Height | 5.8 | 15.2 | SE | Pass |
| | | | Width | 2.4 | 3.8 | SE | Pass |
| | | Hanji | Height | 0 | 0 | SE | Pass |
| | | | Width | 0 | 0 | SE | Pass |
| | 20 | Hanji | Height | 0 | 0 | SE | Pass |
| | | | Width | 0 | 0 | SE | Pass |
| | | Hanji | Height | 0 | 0 | SE | Pass |
| | | | Width | 0 | 0 | SE | Pass |
| | 30 | Hanji/ PET | Height | 0 | 0 | SE | Pass |
| | | | Width | 0 | 0 | SE | Pass |
| | | Hanji | Height | 0 | 0 | SE | Pass |
| | | | Width | 0 | 0 | SE | Pass |

## 2. Morphology Measurement

Table 5 features photos of the samples measured and taken using a magnifying glass and a SEM to observe changes in morphology before and after the combustion of the untreated fabrics and the fabrics treated with flame retardants. As presented in the photos taken with the use of a magnifying glass in Table 5, the shape of the yarn was maintained after combustion due to its flame-retardant treatment, and the shape of the end of the yarn was also kept intact as the concentration of the flame retardant increased. In the case of hanji/PET yarn, however, it was possible to observe that the end tip of the yarn contracted and melted due to the influence of the thermoplastic PET. A closer look at the SEM photos for detailed observation shows that the untreated fabric

using hanji/PET yarn as weft had a smooth fiber surface. On the other hand, in the case of the flame-retardant-treated fabric using hanji/PET yarn as weft, the flame retardant coated the surface of the fiber and the thickness of the fiber also increased. In the woven fabric with weft hanji yarn, the fiber shape remained almost intact before and after the flame-retardant treatment due to the fine hairs on the surface of the fiber, but the thickness increased due to the flame-retardant treatment. Examining the fiber shape before and after combustion found that the fiber was greatly damaged as if the paper had been torn due to carbonization during combustion in the case of the untreated fabric woven with weft hanji/PET yarn. On the other hand, in the case of the fabric using the same yarn and treated with a flame retardant, it was found that the cross-section of the fiber was not damaged at all with a flame-retardant concentration of 20 percent or more. In the case of the fabrics woven with weft hanji yarn, it was confirmed that the fiber was carbonized by combustion and its shape contracted when the fabric was not treated with a flame retardant. In the case of the flame-retardant-treated fabric using the same yarn, however, it was confirmed that the fiber's cross-section remained intact even at the flame-retardant concentration of 10 percent, and as circled in the image in Table 5, a blowing effect was identified, which was caused by the expansion of the phosphorus-based flame retardant used in this research. This is consistent with the results of the study conducted by Ka et al. [9], according to which the blowing effect is caused when a phosphorus flame retardant emits non-flammable gases during the flame resistance test, whereby lowering the concentration of the surrounding oxygen and preventing the inflow of oxygen into the fiber prevented the fiber from contracting or being damaged.

**Table 5. SEM photo of samples before and after flame resistance test**

| Item | | | Untreated | | Treated with flame retardant | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hanji/PET | Hanji | Hanji/PET | | | Hanji | | |
| | | | | | 10 | 20 | 30 | 10 | 20 | 30 |
| Magnifying glass | After flame | ×30 |  |  |  | | |  | | |
| SEM | Before flame | ×3000 |  |  |  | | |  | | |
| | After flame | ×3000 |  |  |  | | |  | | |

### 3. IR Analysis

The FT-IR spectra of the fabric samples demonstrated the changes in functional groups according to each flame-retardant treatment. As shown in Figs. 1 and 2, it was confirmed that the fabric treated with a flame retardant had the P-OR ester peak in the spectral range of 900-1,000 $cm^{-1}$, and the P-O peak in the spectral range of 725-845 $cm^{-1}$. Judging from the aforementioned results, it is estimated that the flame retardant used in this experiment was a phosphorus-based compound. Phosphorus flame retardants promote char formation, suppressing the generation of flammable gases during material decomposition, while reducing the amount of heat generated during combustion by forming a nonflammable film on the glass of fiber surface [10]. Meanwhile, a phosphorus-based flame retardant consists of a halogen-free flame-retardant system that responds to environmental problems and is known to demonstrate good compatibility and weather resistance due to its stable chemical structure and easy processing of flame retardants [11]. Therefore, this research utilized the advantages of phosphorus-based compounds and identified outstanding flame-retardant effects as shown in Tables 3 and 4.

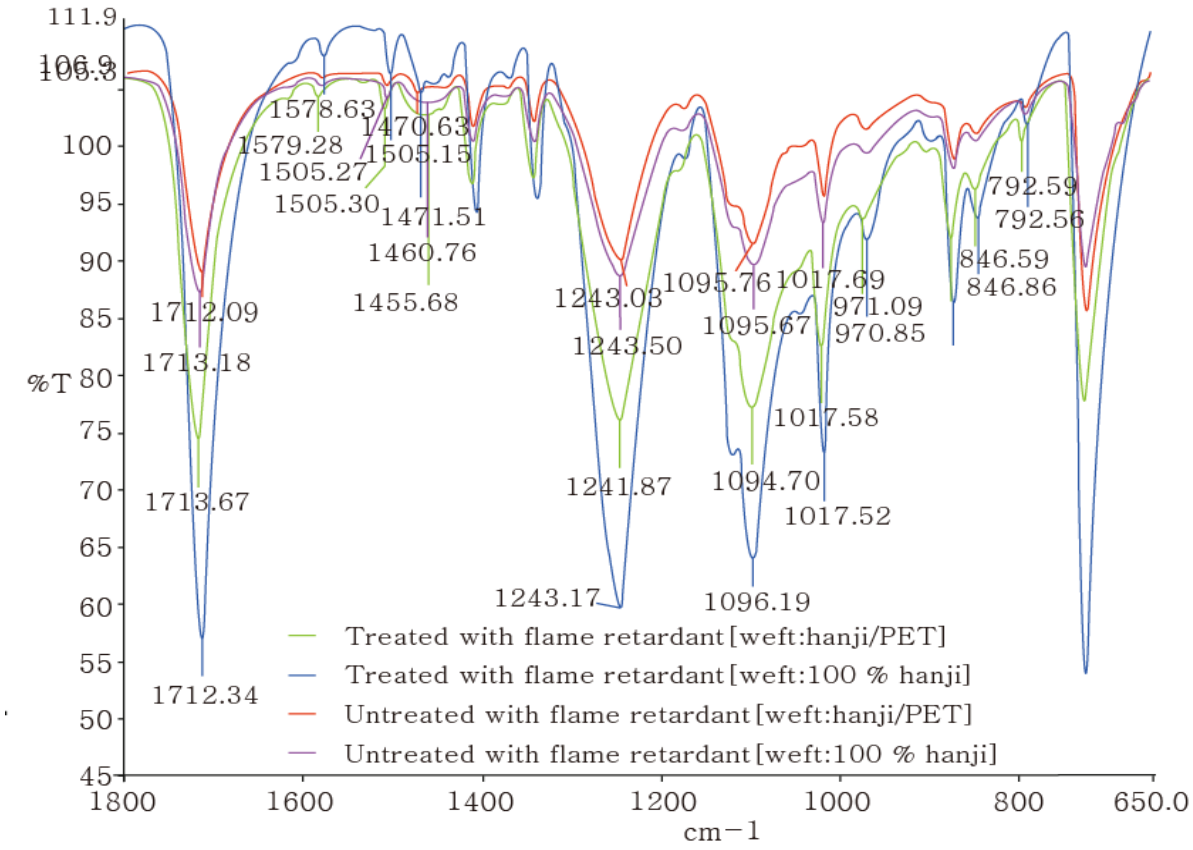



Figure 1. FT-IR spectra of warps of woven fabrics.

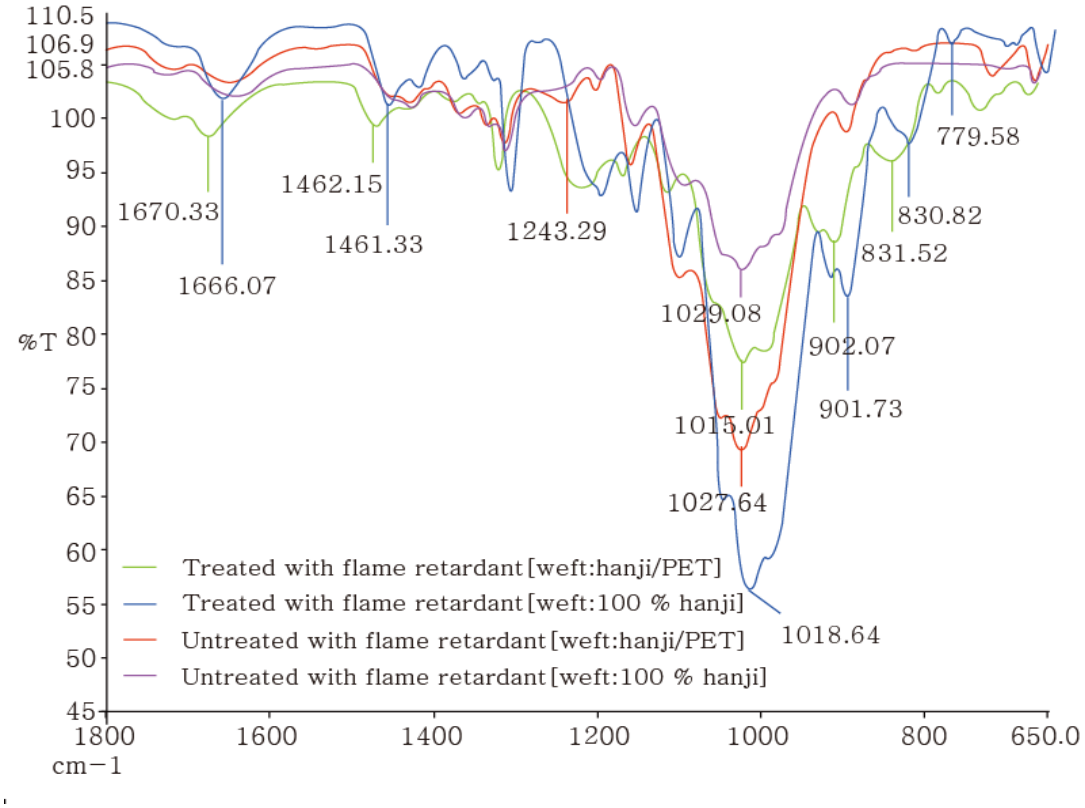



Figure 2. FT-IR spectra of wefts of woven fabrics.

## 4. Thermogravimetric Analysis

Figures. 3 and 4 show the results of thermal stability analysis on the woven fabrics with weft hanji/PET yarn and weft hanji yarn, respectively, depending on whether the fabrics were flame retardant treated or not. In the case of the fabrics treated with a flame retardant, as shown in Figs. 3 and 4, the thermal decomposition began at a temperature 30 ℃ to 100 ℃ lower than that of the untreated fabric according to its concentration of flame-retardant chemicals, and then the mass decreased slowly. This appears to be caused by the fact that the flame-retardant-treated fabric begins to burn more quickly than the untreated fabric, forms a carbonaceous layer, and then the carbonaceous layer protects the fabric from heat to prevent rapid mass loss, as explained in 3.1 Inflammability and 3.3 IR Analysis and identified in the study conducted by Ka et al. [9]. Moreover, the comparison of the use of hanji/PET yarn weft and the use of 100-percent hanji yarn weft found that, in the cases of the fabric using hanji/PET yarn, it was easier to form the carbonaceous layer due to the combustion of the flame retardant, thereby slowing down the mass loss. However, this difference was insignificant, and thus the thermal stability of the fabric possibly resulted from the prevention of mass loss at high temperatures due to the flame-retardant treatment rather than a difference in materials.

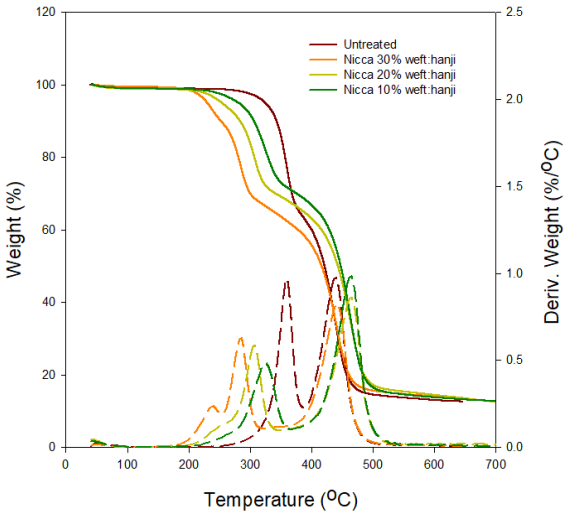



Figure 3. Thermogravimetric analysis for woven fabrics with hanji/PET as weft.

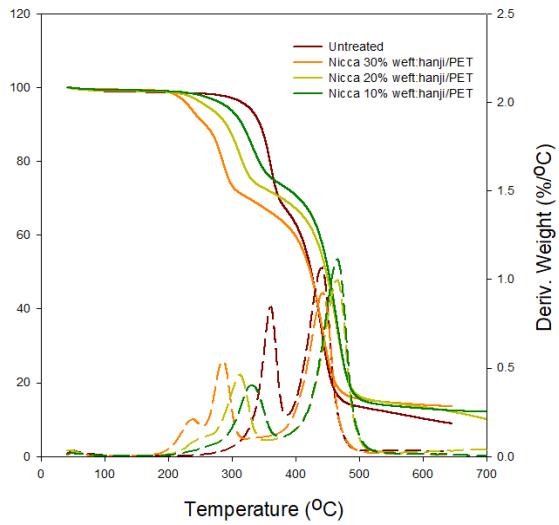



Figure 4. Thermogravimetric analysis for woven fabrics with hanji as weft.

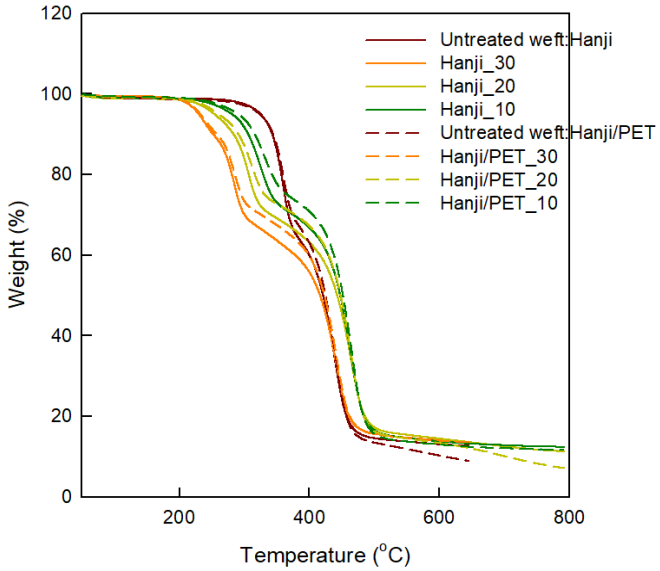



Figure 5. Thermogravimetric analysis for hanji/PET and hanji.

## CONCLUSION

This study aims to help the development of eco-friendly automobile seats using hanji yarn. For this study, two types of hanji fabrics were woven by using hanji yarn, and their flame-retardant treatment effects were examined. This research concludes as follows.

First, the flame resistance test showed that the untreated fabric was completely burned, while all the flame-retardant treated fabrics exhibited self-extinguishing properties

regardless of their concentration of flame-retardant chemicals. In addition, observing the shapes of the flame-retardant treated fabrics and the untreated fabrics before and after combustion through a magnifying glass and a SEM confirmed that the treated fabrics maintained their original shape even after combustion.

Second, based on the results of the FT-IR analysis to identify the changes of functional groups due to the flame-retardant treatment, the P-OR ester peak and the P-O peak were confirmed in the spectral range of 900-1,000 $cm^{-1}$ and in the spectral range of 725-845 $cm^{-1}$, respectively. Therefore, it could be estimated that the flame retardant used in this research was a phosphorus compound.

Third, it was discovered that the flame-retardant treated fabric thermally decomposed at a temperature 30℃ to 100℃ lower than that of the untreated fabrics depending on its concentration of flame-retardant chemicals, and then a carbonaceous layer was formed on the fiber more quickly than the untreated fabric, thereby providing thermal stability. In addition, it was found that there was almost no difference in thermal stability between hanji/PET and 100-percent hanji wefts at high temperatures.

As described above, the NICCA FI-NONE PD-38 flame retardant used in this research exhibits outstanding flame retardancy for fabrics woven with hanji yarn, and thus it is expected that it could be applied to fabrics for car seats.

## ACKNOWLEDGEMENTS

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# FOAM GLASS GRAVEL – GREEN LIGHTWEIGHT FILLER FOR NATURAL HYDRALUC LIME-BASED PLASTERING MORTARS

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## ABSTRACT

In view of the growing population and urbanization, it is necessary to ensure the development of advanced building materials capable of ensuring the quality of living conditions with as low energy consumption as possible. A proven way to achieve this is to create a composite material with a lightweight aggregate. However, the use of lightweight aggregate could also affect other material properties, such as mechanical strength or water absorption. In this study, the silica sand aggregate was partially and completely replaced with foam glass gravel in the composition of natural hydraulic lime plasters in order to reduce thermal conductivity of the material. The use of foam glass is also profitable with regard to the environmental aspect, due to its recyclability. The effect of application of glass gravel on the structural properties, mechanical strength, thermal properties, and water transport parameters was studied for 28-days cured samples. According to the results, the application of foam glass gravel significantly reduced the thermal conductivity of the plaster while maintaining its sufficient strength despite the increase in high porosity, even when the silica sand aggregate was fully substituted. Based on the data obtained, it was concluded that the use of foam glass as a filler is beneficial in improving the thermal properties of plastering mortars.

**Keywords:** light weight mortars, foam glass, natural hydraulic lime, thermal properties

## INTRODUCTION

With a growing population and increasing construction of residential buildings, providing comfortable housing is becoming a challenge as it leads to an increased demand for energy followed with an increase in greenhouse gas emissions [1]. One way to guarantee the thermal comfort of the occupants as well as reduce the overall energy consumption for heating is the use of materials with low thermal conductivity in building construction [2]. In addition to thermal insulation, lightweight mortars can also have this favourable property.

Due to their suitable properties, lightweight mortars have been studied more extensively in recent years. These mortars are produced using various lightweight fillers, such as expanded polystyrene, cork [3] or pumice [4], which effectively increase thermal insulative capability of mortar. There is also possibility of using waste materials, for example waste paper [5] and plastics [6], as a secondary raw material, which is considered as eco-friendly aggregate. Unfortunately, the use of alternative fillers can

have an unfavourable effect on other material characteristics such as mechanical resistance or water absorption. For this reason, the properties of the mortar with alternative filler must be sufficiently tested and verified.
The aim of this work is to design lime-based mortars with improved thermal properties containing porous foam glass gravel. The effect of sand substation by lightweight filler on mechanical resistance and water absorption of the mortars is also experimentally assessed.

## MATERIALS & METHODS

The studied samples consisted of natural hydraulic lime HL 3.5 (Zement- und Kalkwerke Otterbein GmbH & Co. KG, Germany), silica sand aggregate (Filtrační písky, s.r.o., Czech Republic) of size fraction 0/2 with a loose bulk density of 1670 $kg \cdot m^{-3}$, foam glass gravel (Refaglass, Ltd.,Vintířov, Czech Republic) with loose bulk density of 264 $kg \cdot m^{-3}$ and the same fraction as silica sand. The chemical composition of the dry raw materials, determined by X-ray fluorescence analysis (EDXRF spectrometer, ARL QUANT´X, Thermo Scientific) is shown in Table 1.

Table 1: Chemical composition of raw materials

| **Substance** | **Amount (wt.%)** | | |
|---|---|---|---|
| | **NHL** | **Silica sand** | **Foam glass gravel** |
| CaO | 84.3 | - | 12.4 |
| $SiO_2$ | 6.7 | 96.3 | 65.8 |
| $Na_2O$ | - | - | 11.9 |
| $Al_2O_3$ | 3.7 | 3.1 | 4.5 |
| $Fe_2O_3$ | 2.4 | - | 0.6 |
| MgO | 1.9 | 0.4 | 2.4 |
| $SO_3$ | - | - | 1.2 |
| $K_2O$ | 0.5 | - | 0.9 |
| $TiO_2$ | 0.2 | 0.1 | - |
| Σ | 99.7 | 99.9 | 99.7 |

* NHL = natural hydraulic lime

The composition of the studied mortars is given in Table 2. The reference lime mortar (M-Ref) contained only sand as filler. In case of the other two mixtures, silica sand was partially or fully replaced with porous foam glass gravel. The replacement ratio was 50% (M-SFG) and 100% (M-FG) by volume of the sand, respectively. Prior to mixing, the foam glass was immersed for 24 h in a portion of the batch water to eliminate water absorption at the grain surface that could significantly impair the workability of the mix. The water dosage for mixing was adjusted to achieve a normal consistency (spread diameter 160 ± 5 mm, according to EN 1015-3 [7]).

Table 2: Composition of the prepared plastering mortars

| Mixture | Composition in ($kg \cdot m^{-3}$) | | | |
|---|---|---|---|---|
| | NHL | $H_2O$ | Silica sand | Foam glass gravel |
| M-REF | 600 | 390 | 1660 | - |
| M-SFG | 600 | 440 | 830 | 140 |
| M-FG | 600 | 500 | - | 280 |

Table 3: Applied experimental methods and measured parameters

| Measurement | Method | Unit | Device | Expanded combined uncertainty | Used standard |
|---|---|---|---|---|---|
| Bulk density | Gravimetry | $kg \cdot m^{-3}$ | - | 1.4 % | EN 1015-10 [8] |
| Matrix density | Helium pycnometry | $kg \cdot m^{-3}$ | Pycnomatic ATC | 1.2 % | - |
| Porosity | Mercury porosimetry | % | Pascal 140+440 | - | - |
| Pore diameter | Mercury porosimetry | μm | Pascal 140+440 | - | - |
| Flexural strength | Three-point bending test | MPa | Heckert PF 100 | 1.4% | EN 1015-11 [9] |
| Compressive strength | Uniaxial strength test | MPa | Heckert PF 100 | 1.4% | EN 1015-11 [9] |
| Elasticity modulus | Ultrasonic pulse velocity | GPa | Vikasonic | 2.3% | - |
| Thermal conductivity | Heat pulse | $W \cdot m^{-1} \cdot K^{-1}$ | Isomet 2114 | - | - |
| Heat capacity | Heat pulse | $J \cdot kg^{-1} \cdot K^{-1}$ | Isomet 2114 | - | - |
| Water absorption coefficient | Capillary water absorption | $kg \cdot m^{-2} \cdot s^{-0.5}$ | - | 2.3% | EN 1015-18 [10] |
| 24-hour water absorption | Capillary water absorption | $kg \cdot m^{-2}$ | - | 2.3% | - |

After the mixing, the fresh mixtures were cast into plastic moulds (40 mm × 40 mm × 160 mm prisms) and covered with PE foil for 48 hours. The samples were then

demoulded and placed in a humidified chamber at (22 ± 2) °C and (95 ± 5) % relative humidity to cure for another 26 days.

The hardened samples were tested in the age of 28 days. Prior the testing of basic structural parameters of the studied mortars, the hardened samples were dried in a vacuum chamber at 60 °C. The applied experimental techniques and investigated parameters of the mortars under examination are presented in Table 3.

## RESULTS AND DISCUSSION

Figure 1 and Figure 2 shows structure of foam glass gravel and the surface structure of hardened samples respectively, acquired by scanning electron microscope Phenom XL G2. The image (a) of control sample shows porous structure of material matrix with non-porous silica sand filler. On the other hand, images of samples with partially substituted (b) and fully replaced (c) silica sand with porous foam glass gravel clearly shows their higher open porosity and size of formed pores.

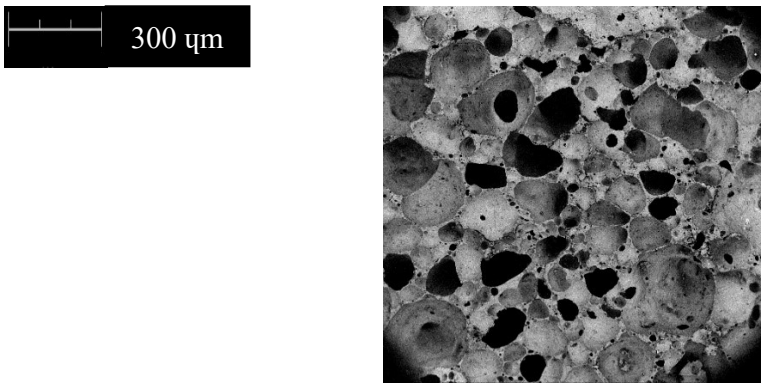
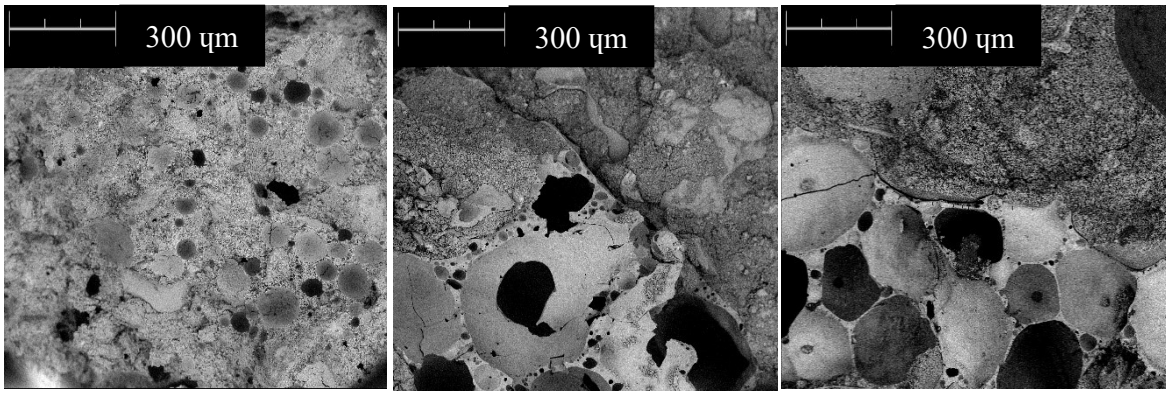



Figure 1: SEM micrograph of foam glass gravel.




(a) (b) (c)

Figure 2: SEM micrograph of M-REF (a), M-SFG (b) and M-FG (c).

The basic structural properties of the samples are summarised in Table 4. The partial and full replacement of silica sand with foam glass gravel resulted in a decreasing trend

of both bulk and matrix densities. The bulk density of M-FG mortar, containing only foam glass gravel as aggregate, was about 44.5% lower as compared to the value measured for the reference material. The total porosity $\Psi_{Hg}$ (%) and average pore diameter D (μm) measured by mercury intrusion porosimeter are also presented in Table 4. The average pore diameter increased when 50% of aggregate was replaced with foam glass gravel, and changed only negligibly with further increase to 100% replacement. M-FG achieved porosity more than 50% which meets the requirement of WTA Directive for repair mortars ($\Psi$ > 40 %) [11]. How significant was the increase of porosity is shown in Figure 3. The maximum volume of pores in reference mortar was in the range from 0.05 μm to 0.1 μm. In the mortar with only foam glass gravel aggregate, pores within the range 1-3 μm were also identified, which might affect the material strength and water absorption.

Table 4: Structural properties (28-days samples)

| **Sample** | $\rho_b$ (kg·m$^{-3}$) | $\rho_s$ (kg·m$^{-3}$) | $\Psi_{Hg}$ (%) | $D$ (μm) |
|---|---|---|---|---|
| M-REF | 1 681 ± 32 | 2 586 ± 50 | 27.6 | 0.072 |
| M-SFG | 1 430 ± 28 | 2 372 ± 47 | 37.2 | 0.097 |
| M-FG | 932 ± 18 | 2 165 ± 41 | 56.9 | 0.090 |

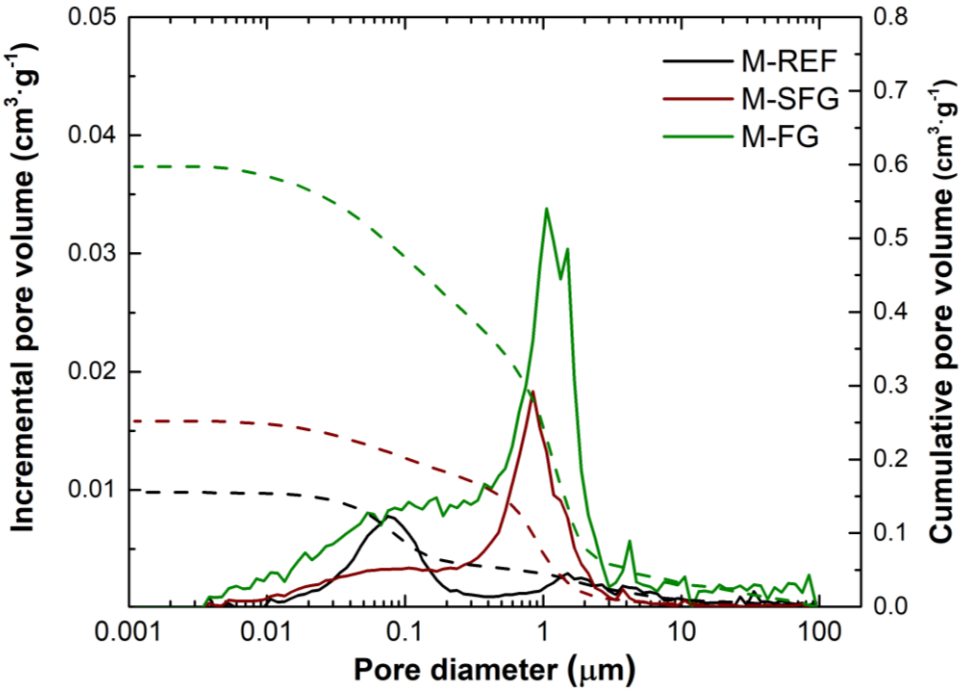



Figure 3: Pore volume of tested samples.

Parameters characterizing the mechanical strength and stiffness of the hardened mortar samples are presented in Table 5. As expected, the compressive strength decreased with incorporation of lightweight aggregate with higher porosity. The decrease was approximately 28% when the silica sand aggregate was fully replaced with foam glass gravel. The results of flexural strength show only small change between each mixture.

Taking into account the basic structural properties, the mechanical parameters deteriorated mainly due to the high porosity of samples and properties of ITZ between hydrated lime and foam glass particles. On the other hand, the mechanical strength is still sufficient with respect to the requirements of the WTA Directive [11], which sets the compressive strength of the repair mortar in the range of 1.5-5.0 MPa.

Table 5: Mechanical parameters (28-days samples)

| **Sample** | $f_c$ **(MPa)** | $f_f$ **(MPa)** | $E_d$ **(GPa)** |
|---|---|---|---|
| M-REF | 4.6 ± 0.06 | 0.8 ± 0.01 | 4.5 ± 0.10 |
| M-SFG | 3.6 ± 0.05 | 0.7 ± 0.01 | 3.9 ± 0.09 |
| M-FG | 3.3 ± 0.05 | 0.9 ± 0.01 | 2.2 ± 0.05 |

The thermo-physical performance of the mortars, described in terms of thermal conductivity and specific heat capacity, is shown in Table 6. Both parameters decreased when porous glass gravel was used as filler, the decrease in thermal conductivity was approximately 52.6% and 83.0% for M-SFG and M-FG, respectively. For the specific heat, the decrease was 15.2% and 26.3%. The porous nature of the alternative aggregate and its low density were responsible for this dramatic loss in heat transport and storage.

Table 6: Thermal parameters (28-days samples)

| **Sample** | $\lambda$ **(W·m$^{-1}$·K$^{-1}$)** | $C$ **(J·kg$^{-1}$·K$^{-1}$)** |
|---|---|---|
| M-REF | 0.836 ± 0.020 | 1095.1 ± 27.4 |
| M-SFG | 0.523 ± 0.012 | 928.0 ± 23.2 |
| M-FG | 0.142 ± 0.003 | 806.9 ± 20.2 |

The higher open porosity of the foam glass mortar samples also resulted in an increased ability to absorb and transport water. The mass gain, i.e. water ingress, is graphed in Figure 4. The results of testing the hygric parameters are given in Table 7. While the 50% sand replacement increased the water absorption coefficient by only 10%, the complete replacement of the filler with foam glass gravel increased the value by almost 70%. The WTA Directive [11] requires repair mortars to have 24-hour water absorption $W_{24h} > 0.3$ kg·m$^{-2}$, which all of the mortars tested safely met.

Table 7: Water absorption parameters (28-days samples)

| **Sample** | $A_w$ **(kg·m$^{-2}$·s$^{-0.5}$)** | $W_{24h}$ **(kg·m$^{-2}$)** |
|---|---|---|
| M-REF | 0.29 ± 0.007 | 0.805 ± 0.03 |
| M-SFG | 0.32 ± 0.008 | 0.822 ± 0.03 |
| M-FG | 0.49 ± 0.012 | 0.957 ± 0.03 |

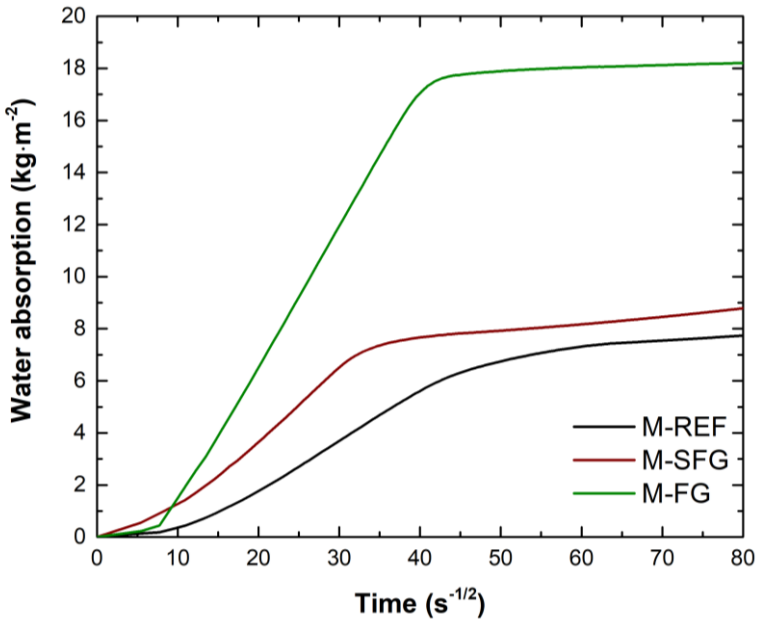



Figure 4: Water absorption of hardened mortars.

## CONCLUSIONS

The presented study shows the effect of partial or full replacement of commonly used silica sand with lightweight aggregate on the mechanical strength, thermal properties and water absorption of lime-based mortars. Although foam glass has high strength, replacing silica sand with foam glass gravel negatively affected the compressive strength due to increased porosity and pore size of the mortars when both partial and complete substitution occurred. The high open porosity of the foam glass gravel had a positive effect on the thermal insulation ability, unfortunately followed by increased water absorption due to the open porosity of the gravel and lime matrix itself.

It can be concluded that the use of foam glass gravel as an aggregate had a positive effect on the thermal properties of the mortars, while negatively affecting the mechanical strength, which remained still high enough for the application of the tested lightweight mortars in the repair and rehabilitation of existing buildings. The data obtained provide valuable input for the design and development of lime-based composites incorporating recycled secondary raw materials as fillers.

## ACKNOWLEDGEMENTS

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# FROM NEARLY ZERO ENERGY BUILDING (NZEB) TO ZERO-EMISSION BUILDING (ZEB) - CONCEPT ISSUES EMPHASIZED THROUGH A PARADOXICAL CASE STUDY

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## ABSTRACT

A case study for a public library which aim to become nearly Zero Energy Building (nZEB) is presented. Due to building's location in the city center of a medium sized municipality from Romania, important site limits have to be considered, as well as other pre-existent conditions. These conditions significantly limit the technological options that can be implemented to ensure nZEB compliance. Also, the destination itself of the building (library with free access to the shelves), imposes a specific energy profile (considering the intrinsic energy demand for its normal operation) and is limiting the inclusion into a very specific nZEB benchmark. Between obligations and possibilities, a cutting-edge package of technical solutions is detailed, which allows the building in operation to produce final energy almost as much as it consumes. However, a paradoxical situation is identified, in the sense that the building does not fit into the nZEB benchmarks, nor into the outlined ZEB benchmarks if the total primary energy is considered (both from conventional and renewable sources), although the implementation of the technical solutions actually lead the building to inclusion towards a $CO_2$ zero-emission building (with a calculated value for the CO2 emissions of 5,26 $kgCO_2/m^2$.year and significantly reduced final energy consumption). Thus, a structural problem in the nZEB and ZEB concepts (as defined in the EU framework) is identified, especially in the context of making these concepts mandatory in the EU member states.

**Keywords:** nearly Zero Energy Building (nZEB), Zero-Emission Building (ZEB), new buildings, total primary energy, $CO_2$ emissions

## INTRODUCTION

At the European level buildings consume about 43% of the final energy, with an identified stabilization of the final energy consumption in the recent years (2014-2019) [1]. This stabilization converges with the energy efficiency policies created within the European Union and transposed in the EU member countries national legislation and technical regulations [2], [3], [4], but it also indicates that the implementation rate of measures to increase the energy efficiency of buildings is still too slow. Currently in force in the EU member states, nearly Zero Energy Building (nZEB) concept comes with a paradigm shift in the way buildings must be constructed and renovated, with significant technical and financial implications into the society [5].

Furthermore, into the 2021 proposal to revise the EPBD [6], the pathway of energy efficiency in buildings is deepened through the introduction of Zero-Emission Building (ZEB) concept, to be mandatory for all new public buildings starting with 2027, respectively for all new buildings and for deep renovations of existent buildings starting with 2030.

Thus, considering the currently in force requirements that all new public buildings should be nZEB starting with 31 December 2018, as introduced by the European Performance of Buildings Directive [7] and the Romanian legislation [8], which was revised accordingly, this paper presents a case study of a new public building located in the city center of Cluj-Napoca, Romania, which aim to become nZEB.

The benchmarks used for nZEB are those in force in Romania within the year 2022 [9], [10], considering maximum values as targets for the primary energy (PE) and for the CO2 emissions, respectively a minimum 30% share of the total PE to be from renewable energy sources (RES). Further, the study compares the results with those obtained by using new nZEB benchmarks in force starting with February 2023 [11].

For the inclusion in the benchmarks, the analyzed building, which is a library with free access to the shelves, was considered, in extenso, as an educational unit.

The main objective of this study is to provide a perspective of the possibility of reaching the imposed targets, regarding maximum total primary energy consumption (both from conventional and renewable energy sources), maximum $CO_2$ emissions and minimum RES share, also simultaneously considering the minimum design requirements of the building, to ensure its comfort parameters (indoor temperatures for heating/cooling, controlled humidification, minimum ventilation flow rates, required lighting conditions etc.) taking into account its specific destination.

A second objective is to identify if nZEB or ZEB concepts, as defined currently within the EU framework, lead to coherent resources allocation, if considering the total primary energy as one of the criteria for defining nZEB or even the only criteria to define ZEB (as outlined in [6]).

## MATERIALS AND METHODS

The building analysed in the current case study has several particularities, but on the same time it is representative for the common conditions related with the new buildings located in the city centre of medium to large size municipalities (Cluj-Napoca has over 285.000 inhabitants).

The pre-existing conditions influence both the energy requirement and the applicable technologies for nZEB compliance of the building.

The general conditions refer to the limits imposed by the site, by not having additional available land (e.g., for the installation of ground-water heat pumps as a RES solution) and being very shaded by the surrounding buildings (e.g., with a direct effect in increasing the heating demand of the building).

The particular conditions are: the building was partially executed in the period 2016-2020, with the resistance structure currently completed – Fig. 1.a. Also, the nZEB

requirements were not applied and were not nationally defined in 2011, when the initial technical project was developed.

Another pre-existing condition for the project update refers to the initial architecture concept – Fig. 1.b, which must be respected.



Fig. 1.a. The building's current technical state

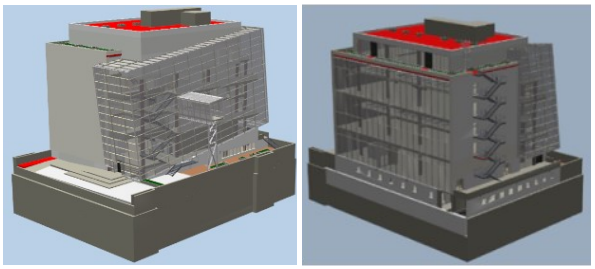


Fig. 1.b The architecture concept

In 2022 the project was resumed, so a holistic approach from the energetical perspective was considered. The proposed package integrates technical solutions for the building's architecture (regarding the thermal insulation of the building envelope and its air tightness) and for the building's services (Heating, Ventilation, Air Conditioning – HVAC systems, Domestic Hot Water – DHW and lighting), considering both conventional and renewable energy sources**.**

The energy consumptions calculations for each utility (heating, DHW, cooling, mechanical ventilation, and lighting) are based on the CEN European standards package for calculating the energy performance of buildings, using monthly methods for the energy calculations (including thermal balances to determine heating/cooling demand, calculation of DHW demand, calculation of mechanical ventilation minimum supply/return flow rates, calculation of lighting required power, due to consumer's comfort requirements – e.g., illuminance level, followed by the calculation of energy consumptions, including losses on distribution, storage and generation from the energy production equipment to the consumer).

Considering the specific destination of the building (library with free access to the shelves), several design minimum requirements are mentioned (Table 1), because it significantly influences the building's energy profile (the building's energy demand, regardless the types of equipment used to provide the energy). Thus, the building must be conditioned (heated/cooled) continuously and it is necessary to ensure controlled humidification due to the library destination. The building has a capacity of 226 maximum simultaneous readers and 21 staff members, for which domestic hot water and treated supply/return air flow rates within the mechanical ventilation system must be ensured. At the same time, higher visual comfort requirements for readers are also necessary, with a direct impact on increasing energy consumption to ensure the comfort requirements, specific to the building's destination.

https://doi.org/10.5593/sgem2023V/6.2/s26.68

Table 1. Building's specific design requirements which lead to energy demand/consumption in normal operation

| Heating | DHW | Cooling | Mechanical Ventilation | Lighting |
|---|---|---|---|---|
| **Ti.avg [ºC]** | **Hot Water Volume [liter]** | **Ti.avg [ºC]** | **Supply / Return Air Flow [$m^3$/h]** | **Specific Power [W/$m^2$] / Illuminance [lux]** |
| 18,4 ºC continuous | 5 liters/ day/user | 25 ºC continuous | 18000 / 16500 $m^3$/h humidification | Up to 26,7 W/$m^2$ Up to 500 lux |

The technical solutions proposed to lead the building to nZEB compliance, under normal operating conditions and considering all its pre-existing conditions, are centralized in Table 2.

Table 2. Holistic approach of the technical solutions considered for nZEB compliance

| **Building Management System (BMS)** | | | |
|---|---|---|---|
| **Architecture/Building envelope solutions** | | **HVAC, DHW, lighting solutions** | |
| External walls | 20 cm mineral wool (MW) | Heating | Air conditioning units with Variable Refrigerant Flow (VRF) technology and inverter, SCOP = min. 4,3 |
| Perimetral base | 15 cm extruded polystyrene (XPS) | Domestic Hot Water (DHW) | Electrical boilers; photocell batteries |
| Windows & glazed doors | Triple glazed, $R_{w.min}$ = 1,1 $m^2$K/W, ensuring air tightness | Cooling | Air conditioning units with VRF technology, ESEER = min. 6,4 |
| Curtain walls | $R_{cw.min}$ = 0,77 $m^2$K/W | Mechanical ventilation | Heat recovery efficiency = min. 75%; actuation systems with variable speed; psp ≤ 0,40 W/m3/h for fans |
| Terrace floors | 30 cm XPS or 15 cm PIR+15 cm MW | Lighting | Efficiency of the light source e = min. 130 lm/W; integrated sensors for presence / natural light, with dimmer |
| Lower exterior floor | 20 cm Polyisocyanurate (PIR) | **Of which/and Renewable Energy Sources (RES)** | |
| Unheated basement | 15 cm MW | Heating | Aerothermal heat pumps (VRF air conditioning units), SCOP = min. 4,3 |
| | | HVAC, DHW, Lighting | Photovoltaic panels (PVs) 82,5 kW, monocrystalline, efficiency min. 21,7% |

## RESULTS AND DISCUSSION

The nationally defined factors for the conversion from final energy into primary energy ($f_{Pnren}$ – non-renewable factor and $f_{Pren}$ – renewable factor), and further from primary energy into $CO_2$ emissions ($f_{CO2}$), considering the energy source or the fuel type, are detailed in Fig. 3.

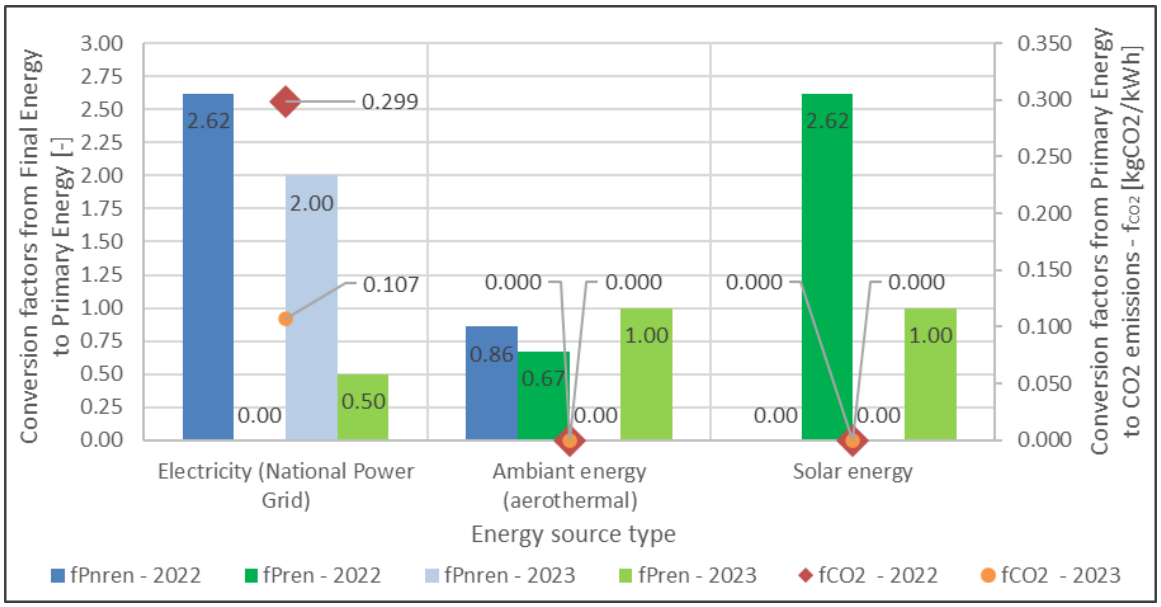



Fig. 3. Romanian conversion factors for the years: 2022 [12] and 2023 [11]

To identify and emphasize the impact of each nZEB criteria (total primary energy, $CO_2$ emissions and RES share) into the energy analysis results of the building, in Fig. 4, the final energy was converted into primary energy and $CO_2$ emissions, by using two distinct nZEB benchmark sets – one that was in force in Romania in 2022 and another one that is currently in force.

The obtained results of the energy analysis undertaken for the library with free access to the shelves are summarized below.

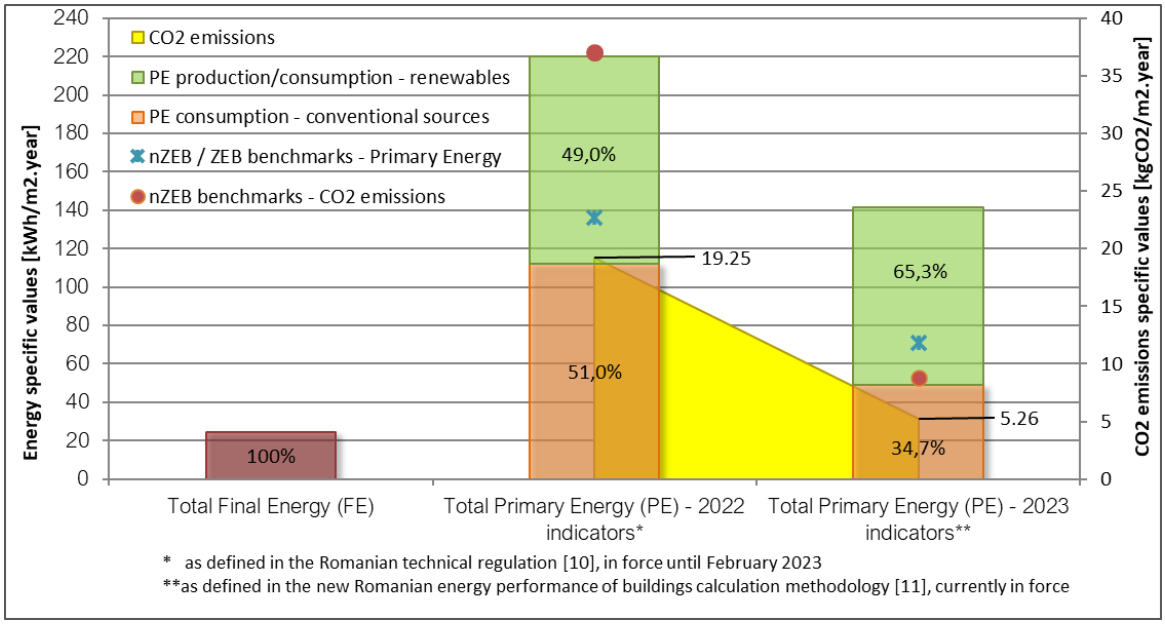



Fig. 4. Energy analysis results of the studied building

A relevant share of RES from the total primary energy consumption it is obtained (49%), with a value of total primary energy consumption from conventional sources of 112,13 kWh/m2.year, CO2 emissions 19,25 kgCO2/m2.year, which lead the building to nZEB according to the educational units nZEB benchmarks in force at the moment of the project update, back in 2022 (PE from conventional sources 136 kWh/m$^2$.year, $CO_2$ emissions 37 kg$CO_2$/m$^2$.year and 30% of the total primary energy from RES).

However, in February 2023, the Romanian nZEB benchmarks changed [4] and a projection of the results, considering the new benchmarks was undertaken. The new results are PE 141,76 kWh/m$_2$.year, CO2 emissions 5,26 kg$CO_2$/m$^2$.year and a share of 65,3% of the PE from RES. The new nationally used nZEB benchmarks for educational units are highly ambitious: PE from conventional and renewable sources 71 kWh/m$^2$.year, CO2 emissions 8,8 kgCO2/m$^2$.year and 30% of the total primary energy from RES.

For the new nZEB benchmarks the building will not achieve the new nZEB primary energy target but will achieve the $CO_2$ emissions target and the RES share target. However, it is stated that the final energy consumption of the building from conventional sources is 24,58 kWh/m$^2$.year, which is interpreted that the building will produce almost as much as it consumes. Also, it is emphasized the reduced value of $CO_2$ of 5,26 kg$CO^2$/m$^2$.year, obtained for the normal operation scenario, with the provision of all the comfort requirements due to its destination, and which actually lead the building towards a $CO_2$ zero-emission building.

Thus, starting from a particular case of a real building, several problematic aspects are identified regarding the nZEB and ZEB concepts:

- the design requirements of a building due to its destination significantly influence its energy demand, independent of the way in which the energy is produced in the building (with conventional, renewable, or mixed sources);

- the total primary energy (from conventional and renewable sources), as an nZEB / ZEB criterion, does not support buildings that have a higher energy demand to cover due to their actual destination, to provide the minimum design requirements, in order to ensure the occupants' comfort;
- although a package of innovative solutions (but available on the market, considering the need to comply with nZEB in mass) was used and considering common pre-existent conditions in real environment, the building cannot comply with nZEB / ZEB requirements (the total primary energy criterion is not reached);
- regarding the final energy consumed and the reduced $CO_2$ emissions that are obtained, these values can be interpreted that the building produces almost as much as it consumes, respectively that $CO_2$ emissions will be significantly reduced during operation, and both results can actually qualify this building as a building with $CO_2$ emissions towards zero, which is ultimately the main objective of the current energy transition and of the significant efforts made within the European Union.

## CONCLUSION

It is noticed that for certain categories of buildings located in the city center of medium to large size municipalities, important site limits have to be considered, which are pre-existent conditions that might significantly limit the technological options that can be implemented to achieve mandatory nZEB requirements.

The studied building, which is a library with free access to the shelves, was considered, in extenso, as an educational unit. However, the energy profile (based on the energy demand) of a library is very different from that of a school or a kindergarten, which might limit the inclusion into a specific benchmark.

Finally, between obligations and possibilities, a package of innovative technical solutions (but available on the market, considering the need to comply with nZEB in mass) is detailed, which allows the building in operation to produce final energy almost as much as it consumes.

Starting from a concrete case study (a real building), a paradoxical situation is identified, in the sense that the building does not fit into the new nZEB benchmarks, nor in the outlined ZEB benchmark, if the total primary energy (from conventional and renewable sources) is considered, although the implementation of the technical solutions actually lead the building towards a zero-emission building (with a calculated value for the $CO_2$ emissions of 5,26 $kgCO_2/m^2$.year and a final energy consumption of 24,58 $kWh/m^2$.year).

As long as the energy profile of the building requires an intrinsic minimum final energy consumption to ensure comfort conditions, regardless of how energy is produced at the level of the building or nearby, the use the total primary energy (from conventional and renewable sources) as criterion, is erroneous, because regardless of how the energy is produced, the energy demand will have to be provided to the building. At the same time, it is pointed out that such a European positioning is in contradiction with supporting the implementation of renewable energy sources on a large scale (e.g., if

considering a conversion factor of primary energy from renewable sources – solar, aerothermal etc. equal to the value of 1.00 – see Fig. 3).

Thus, a structural problem (related to the total primary energy criterion) in the nZEB and the outlined ZEB concepts as defined in the EU framework is identified, especially in the context of making this concept mandatory in the EU member states, with all the technical-economic consequences deriving from its implementation.

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# HEAVY METALS IMMOBILIZATION IN COAL COMBUSTION FLY ASH-PORTLAND CEMENT COMPOSITES

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## ABSTRACT

With the rapid growth of the population and urbanization, the demand for industrial production has continued to increase, and industrial solid waste has become more and more abundant. Industrial solid waste mainly includes fly ashes, slags, tailings, red mud, bottom ashes and other wastes, which account for approximately 80% of the total waste produced on Earth. Industrial wastes generally contain various heavy metal components such as Pb, Zn, Cu, Cd, Cr, As, Ba and Mn. The low recycling rate of solid waste results in significant land use for disposal, and the heavy metals and alkaline substances contained in solid waste spread pollution through weathering in the form of dust and leaching into soil and groundwater resources, leading to water contamination and land salinization. Heavy metal pollution not only has a negative impact on soil and water quality, but also gradually accumulates in the human body, causing serious health problems and diseases, such as Parkinson's disease, muscular dystrophy, and Alzheimer's disease, while some metals can even cause cancer. Contamination by heavy metals and other solid wastes poses serious consequences for the environment and sustainable development and, in the longer term, essentially threatens the very existence of an industrial and prosperous human society. Coal is the world's most abundant fossil fuel accounting for approximately 70% of the world's total proven fossil fuel resources. Coal combustion produces not only common pollutants such as SOx, NOx and dust, but also highly toxic heavy metal pollutants. As heavy metals in coal have the characteristics of low content, large total emission, easy to migrate and difficult to degrade, which cause a great harm to human body and environment, there is necessary to control the emission of heavy metals from coal combustion and treat them in a safe way. On that ground the use of fly ash (FA) from coal combustion in a cement-based composite intended for the construction industry, combined with the immobilization of heavy metals in the final product, may be a suitable solution to this problem. In this study, the immobilization of As, Ba and Cu contained in fly ash from coal combustion was experimentally investigated. Fly ash was used to replace 10 wt.% and 20 wt.% of Portland cement (PC) and 0-2 mm silica sand was used as an aggregate. The evaluation of the basic physical and mechanical properties was carried out. Special attention was paid to the evaluation of the immobilization of the studied heavy metals in the prepared composites; the leaching test was performed with a liquid/solid ratio of 10 L/kg. The concentration of As, Ba and Cu in the leachates was analysed by atomic absorption spectroscopy (AAS). The results showed that the concentrations of the studied heavy

metals in the leachates of the prepared composites were very low, which suggests that they were completely immobilized in the matrices. The mechanical resistance of the prepared mortars was slightly reduced by PC substitution by FA, but from the quantitative point of view it remained high enough for practical use of prepared composites in development of "green" building materials.

**Keywords:** heavy metals, immobilization, cement-based composites, coal combustion fly ash, atomic absorption spectroscopy

## INTRODUCTION

Fly ash (FA), a solid, fine powdery by-product of coal combustion, is produced in large quantities (estimated global annual production is 800 Mt) and contains hazardous components such as heavy metals. Significant amounts of FA are deposited in landfills and dumps, which can contribute to a number of environmental problems, and the global recovery of FA is still only up to 30% [1]. The mineralogical and chemical composition of FA is diverse, but all types of FA are rich in $SiO_2$ and $Al_2O_3$. This is advantageous for use in the construction industry, which is presently the main area of application for FA. The replacement of part of PC used in the construction with FA is beneficial from both an environmental and an economic point of view. However, for this type of utilisation, it is necessary to take into account the physical, chemical and mineralogical composition of the individual FA, in particular the content of heavy metals (HM) [1, 2].

For the safe management of waste containing HM, cementitious material treatment and thermal treatments are two types of effective immobilisation techniques, as they enable to convert the HM into a more stable and less soluble phase [3]. Within the cementitious treatment, Portland cement, magnesium phosphate cement matrix, and alkali-activated binders (alkali-activated low-calcium aluminosilicate materials, known as geopolymers, and alkali-activated high-calcium materials) are currently being widely studied [3-5]. Thermal treatment can be further subdivided into sintering (700-1200 °C), melting (1200-1600 °C) and vitrification (1100-1500 °C) based on the characteristics of the process product. However, secondary contamination from HM volatilisation during thermal treatment above 800 °C must be considered [6].

In this study, a coal fly ash was used for partial replacement of PC in the ratio of 10% and 20% by weight of PC, and silica sand was used as an aggregate. The main objective was to utilise the coal FA for the production of "green" building materials, together with the immobilization of the present HM as As, Ba and Cu. On the hardened samples, the basic structural properties and mechanical parameters were determined. The immobilization of HM was then evaluated on the leachates from prepared composites using atomic absorption spectroscopy (AAS).

## MATERIALS AND METHODS

The following materials were used in the preparation of cement-based composites: Portland cement CEM I 42.5 R (Českomoravský cement, Inc., Czech Republic, member of HeidelbergCement), brown coal fly ash (power station Vřesová, Czech Republic, milled for 1 hour in a ball mill), silica sand (size fraction of 0-2 mm, Filtrační písky

s.r.o., Czech Republic), and tap water. The chemical composition of PC and FA is shown in Table 1 and their specific density and particle sizes are given in Table 2. These were determined by X-ray fluorescence analysis (EDXRF spectrometer, ARL QUANT'X, Thermo Scientific), helium pycnometer (Pycnomatic ATC, Thermo Scientific), and a laser diffraction technique (Analysette 22 MicroTec plus, Fritsch), respectively.

Table 1: The chemical composition of PC and FA

| Material | $SiO_2$ | CaO | $Al_2O_3$ | $Fe_2O_3$ | MgO | $K_2O$ | $SO_3$ | $P_2O_5$ |
|---|---|---|---|---|---|---|---|---|
| | [mass %] | | | | | | | |
| PC | 21.3 | 61.5 | 5.1 | 3.6 | 0.8 | 0.9 | 2.3 | 0.1 |
| FA | 41.1 | 3.0 | 32.3 | 12.3 | 2.2 | 1.4 | 0.9 | 0.1 |

Table 2: The specific density and particle sizes of PC and FA

| Material | Density [g/cm$^3$] | $d_{10}$ [μm] | $d_{50}$ [μm] | $d_{90}$ [μm] |
|---|---|---|---|---|
| PC | 3.06 | 8.8 | 26.0 | 40.4 |
| FA | 2.38 | 4.8 | 22.7 | 44.3 |

The content of As, Ba and Cu in the fly ash (Table 3) was determined by a digestion with aqua regia according to the standard EN 13657 [7] followed by atomic absorption spectroscopy (AAS) performed on a SOLAAR S iCE 3000 apparatus (Thermo Scientific).

Table 3: AAS analysis of selected heavy metals content in FA

| Substance | [mg/kg $_{solid}$] |
|---|---|
| As | 77.6 |
| Ba | 717.5 |
| Cu | 113.1 |

The composites studied consisted of PC partially substituted by FA. The substitution ratio was 10% (referred to as FA 10) and 20% (referred to as FA 20) of the weight of PC. The pure cement composite was designated REF. The proportions of the raw materials are given in Table 4. The water to binder (PC, PC+FA) mass ratio w/b was kept at 0.5 for all mixtures. The specimens were cast into 40 × 40 × 160 mm prism forms and demoulded after 24 hours. Curing was then carried out under aerobic conditions in a humidity chamber at a controlled temperature of $T$ = (22 ± 3) °C and relative humidity of $RH$ = (95 ± 5) % for 27 days. The 28-day samples are shown in Figure 1.

Table 4: Composition of studied composites

| Mixture | m [g] | | | | w/b |
|---|---|---|---|---|---|
| | PC | FA | Sand | Water | |
| REF | 1000 | 0 | 3000 | 500 | 0.5 |
| FA 10 | 900 | 100 | 3000 | 500 | 0.5 |
| FA 20 | 800 | 200 | 3000 | 500 | 0.5 |



Figure 1: 28-day samples: REF, FA 10, FA 20 (from left)

Specific density $\rho_s$ [kg/m$^3$], bulk density $\rho_b$ [kg/m$^3$] and total open porosity $\Psi$ [%] were determined as the basic structural properties. The composites were dried in a vacuum dryer at 60 °C prior to testing. The specific density was obtained using a Pycnomatic ATC helium pycnometer (Thermo Scientific). The bulk density was measured using the gravimetric method. This was based on the dry mass and size of the composites. From the knowledge of the specific and bulk density values, the total open porosity was calculated. The relative expanded uncertainty was 2% for porosity, 1.4% for bulk density and 1.2% for specific density.

Flexural and compressive strength were tested according to the EN 196-1 [8]. Prismatic samples of 40 × 40 × 160 mm were used to measure the flexural strength $f_f$ [MPa]. The compressive strength $f_c$ [MPa] was determined on the fragments from the flexural test samples. A 40 mm × 40 mm loading area was used for the uniaxial compressive strength test. The dynamic Young's modulus $E_d$ [GPa] in the ultrasonic velocity test was achieved using the Vikasonic apparatus (Schleinbinger Geräte, Germany). The expanded combined uncertainty was 1.4% for both strength tests and 2.3% for the ultrasonic velocity test.

To characterize the immobilization of the examined heavy metals in the prepared composites, the leaching test according to the EN 12457-2 [9] was performed with the liquid/solid ratio of 10 L/kg. Leachates were obtained using distilled water and stirred at 175 rpm for 24 hours. The concentration of As, Ba and Cu in the leachates was quantified by atomic absorption spectroscopy (AAS) using a SOLAAR S iCE 3000 instrument (Thermo Scientific) using a furnace technique. The expanded combined uncertainty of this method was 10%.

## RESULTS AND DISCUSSION

The results of the basic structural properties tests are summarised in Table 5. It is apparent that specific density, bulk density and porosity were not affected by the replacement of cement with FA.

Table 5: Basic structural parameters of the studied composites

| Material | $\rho_s$ [kg/m$^3$] | $\rho_b$ [kg/m$^3$] | $\Psi$ [%] |
|---|---|---|---|
| REF | 2496 ± 30 | 2117 ± 30 | 15.2 ± 0.3 |
| FA 10 | 2501 ± 30 | 2102 ± 29 | 16.0 ± 0.3 |
| FA 20 | 2496 ± 30 | 2107 ± 30 | 15.6 ± 0.3 |

Table 6 shows mechanical properties of studied composites. Compressive strength was found to decrease with the increasing FA replacement. Compared to the reference sample, the decrease for FA 10 and FA 20 was 18.3% and 24.1%, respectively. As is clear from the literature, the resulting mechanical properties of a particular FA are directly related to its chemical and physical properties [2, 10]. Al-Shmaisani et al. [10] stated that it is typical for low calcium FA mixtures to exhibit a slower rate of increase in strength than a pure cement mixture at an early age. In this respect, the development in compressive strength in time can be expected. On the other hand, FA had a positive effect on the flexural strength and the dynamic modulus of elasticity. In the case of the flexural strength, the observed increase was 14.3% and 13.0% for FA 10 and FA 20, respectively. The dynamic modulus of elasticity was improved by 5.8% and 7.9% for FA 10 and FA 20 samples.

Table 6: Mechanical properties of the studied composites

| Material | $f_c$ [MPa] | $f_f$ [MPa] | $E_d$ [GPa] |
|---|---|---|---|
| REF | 65.2 ± 0.9 | 7.7 ± 0.1 | 34.4 ± 0.8 |
| FA 10 | 53.3 ± 0.8 | 8.8 ± 0.1 | 36.4 ± 0.8 |
| FA 20 | 49.5 ± 0.7 | 8.7 ± 0.1 | 37.1 ± 0.9 |

Table 7 presents the As, Ba and Cu concentrations measured in the leachates of the composites together with the maximum possible leaching and associated immobilisation ratios. The maximum possible leaching (mg/L) is the concentration of the single metal (present and added with FA) that could be leached from the composite if no immobilization took place. By relating the measured concentration of each metal to its maximum possible leaching, the immobilisation ratio was determined. It can be seen that the concentrations of all the metals analysed in the leachates were very low. For As, which was below the detection limit (n.d.) in all samples tested, the immobilization ratio was noted to be 100%. Cu immobilization of 99.6% and 99.0% was observed for FA 10 and FA 20. The lowest immobilization efficiency was found in the case of Ba, where the immobilization ratio was 76.2% for FA 10 and 87.7% for FA 20. However, it is

worth noting that the FA contained considerably higher amount of Ba than As and Cu (see Table 3). Furthermore, a not insignificant concentration of Ba was also detected in the leachate of the reference sample, suggesting that Ba was present in the raw materials used, which increased its amount in the FA samples.

Table 7: The concentration of studied metals in leachates and immobilization ratio

| Material | Element | Maximum possible leaching [mg/L] | Concentration [mg/L] | Immobilization ratio [%] |
|---|---|---|---|---|
| REF | As | - | n.d. | - |
| | Ba | - | 0.296 | - |
| | Cu | - | 0.001 | - |
| FA 10 | As | 0.172 | n.d. | 100.0 |
| | Ba | 1.594 | 0.379 | 76.2 |
| | Cu | 0.251 | 0.001 | 99.6 |
| FA 20 | As | 0.345 | n.d. | 100.0 |
| | Ba | 3.189 | 0.391 | 87.7 |
| | Cu | 0.503 | 0.005 | 99.0 |

## CONCLUSION

The objective of this work was to evaluate the use of coal fly ash containing As, Ba and Cu in Portland cement-based building materials. Samples were prepared using FA as a partial replacement for PC, with the FA content of 10% and 20% by weight of PC. Results showed that specific density, bulk density and porosity were unaffected by replacing cement with FA. The presence of FA resulted in a reduction in compressive strength. In comparison to the reference sample, the decrease for FA 10 and FA 20 was 18.3% and 24.1% respectively. However, the compressive strengths of 53.3 MPa and 49.5 MPa are acceptable for a number of building applications. On the contrary, FA had a positive effect on the flexural strength and dynamic modulus of elasticity. These parameters increased significantly compared to the reference. Leachates prepared from FA composites exhibited very low concentrations of As, Ba and Cu. In terms of immobilization of the studied heavy metals, the developed composites exhibited superior immobilization performance for both As and Cu. As, which concentration was below the detection limit in all samples, reached 100% immobilization and Cu immobilization of 99.6% and 99.0% was determined for FA 10 and FA 20, respectively. Lower fixation was observed for Ba, with immobilization ratios of 76.2% and 87.7% for FA 10 and FA 20. Given their structural parameters, mechanical properties and heavy metal immobilization efficiency, the developed coal fly ash-Portland cement composites appear to be promising “green” building materials, from both economic and environmental points of view.

## ACKNOWLEDGEMENTS

Authors greatly acknowledge the financial support received from the Czech Science Foundation under project No. 23-04744S - Research of heavy metals immobilization in alternative low-carbon composites. This research has been also partially supported by the Grant Agency of the Czech Technical University in Prague, grant No. SGS23/OHK1/3T-002/23 - Research and development of high performance building composites.

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# MANAGEMENT OF BUILDING MATERIALS TECHNOLOGICAL LOSSES IN CIRCULAR ECONOMY

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## ABSTRACT

This paper is structured in two directions. The first one presents issues related to technical losses within a construction project. The second one analyzes the integration of technical losses resulting from building materials in a circular economy, as they occur from 2 projects. Each project assesses costs and specific situations for each type of material based on the profile of each presented project. Benefits of technical losses management from building materials and waste are identified as they can be engaged into the circular economy, exemplified for the 2 analyzed projects. In the case of concrete, we bring to the attention of readers that its casting in the elements of site's evolution must be closely correlated with the casting of the elements of the site organization, for the 2 budgets to overlap and only one expense is made. For concrete steel it is important that its optimization is made from the shaped iron fasteners as they are part of the purchase cost, therefore they do not represent a supplementary cost for the project's budget. The importance of finding the optimal solution for reuse of used materials or technological losses brings us to the point in which the construction industry needs to change its approaching strategy on materials consumption.

**Keywords**: circular economy, building materials, waste, technological losses

## 1. INTRODUCTION

Circular Economy is a production and consumption model, requiring a pooling of existing materials and products, for use, rental, reuse, repair, refurbish and recycling for as long as possible. Thus, the product's lifecycle is prolonged. It involves in practice the waste minimization. When a product reaches its end of life, its constituent materials are kept in economy through recycling as much as possible. This approach differs significantly from the linear, traditional economic model, based on the principle *we use-we produce-we consume-we throw away*. To be effective, the circular economy requires industries and companies creating new business models aiming to *do more with less*, to rethink their provided products and to identify opportunities to extend products lifespan, by improving the ecological design and by materials selection (Marsh, A., Velenturf, A. and Bernal, S., 2022)[1] and (Braun, A. T., Peter -Moellhoff, P., Reichenberger, V. and Seiter, S. 2018)[2].

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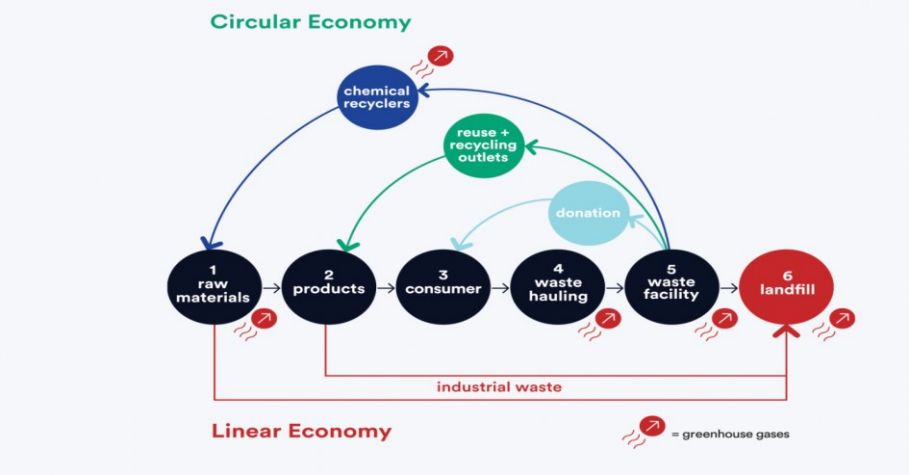



Figure 1.1. Linear Economy vs. Circular Economy (Recycle Track Systems, 2023)[1'] (https://www.rts.com/resources/guides/circular-economy/)

In a linear economy, natural resources are transformed into products that eventually shall become wasted. This process is often summarized by „*take, make, squander*" (Esa, M.R., Halog, A. and Rigamonti, L., 2016)[6]. The definition of circular economy in accordance with the opinion of Ellen MacArthur Foundation (EMF) describes "An industrial economy where materials never become waste and nature is regenerated by intent and design" (MacArthur, E., 2013)[8]. The use of regeneration as a term is important since circular economy is not only a preventive approach by reducing pollution (Benachio, G.L.F., Carmo Duarte Freitas, M., and Tavares, S. F., 2021)[7], but also it aims to repair previous damages by conceiving better systems within the industry itself (Murray, Skene and Haynes, 2017)[10].

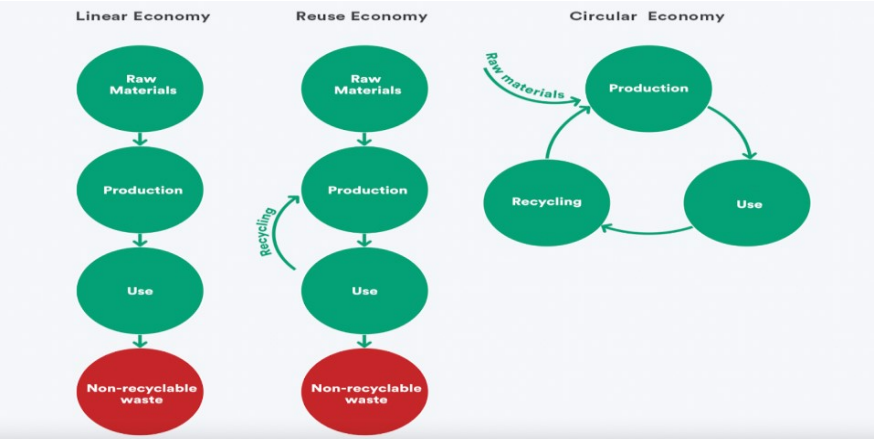



Figure 1.2 Organizational differences in diverse types of economies (Recycle Track Systems, 2023)[1'] ( https://www.rts.com/resources/guides/circular-economy/)

"Circular economy is an exceptional opportunity to transform the economy and make it more sustainable, to contribute to the achievement of climate objectives and preserve world resources, create local jobs, and generate competitive advantages for Europe in a world in deep change. The importance of the circular economy for European industry has been highlighted recently in the renewed EU industrial policy strategy" (de

Carvalho Araújo, C.K., Bigarelli Ferreira, M., Salvador, R., Kawane Ceciliano de Carvalho Araújo, C., Silva Camargo, B., de Carvalho Araújo Camargo, S. K., de Campos, C. I., and Moro Piekarski, C., 2022)[4] and (Cambria, D. and Pierangeli, D., 2012)[5]. As far as the construction and demolition waste is concerned, as reported by the Commission in January 2023, to the European Parliament (Esa, M.R., Halog, A. and Rigamonti, L., 2016)[6]. 20 Member States reported that they have already achieved the 70% recovery target set for 2020. This is a positive sign considering that from the point of view of weight, this is the largest waste stream in the EU. However, it should be remembered that the target includes refill (the recovery operation in which the appropriate waste is used for the restoration of areas or for engineering works of landscaping), a practice that does not preserve the value of materials in the economy and therefore does not lead to a circular economy (Building and Better Working World, 2023)[2'].

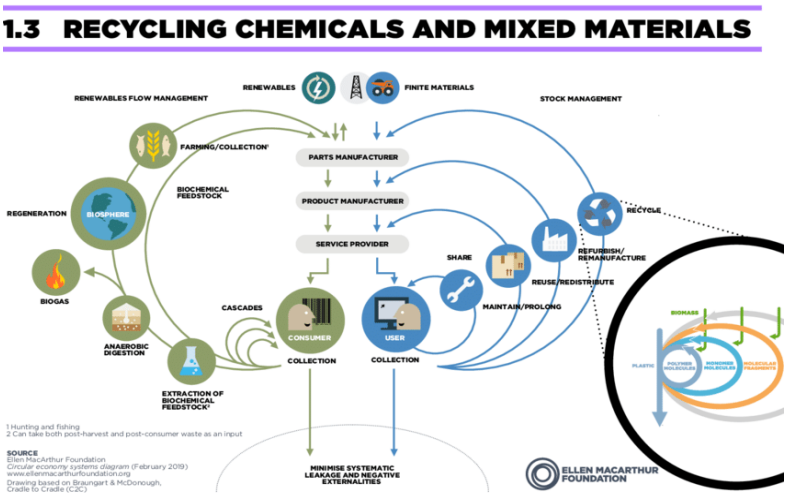



Figure 1.3 The Circular Economy System Diagram (Dittrich-Krämer, B., Kormann, J. C. C., Kormann, C. et all., 2019)[3'] (https://www.researchgate.net/figure/The-circular-economy-system-diagram-edited-for-the-purpose-of-this-white-paper_fig1_333603839)

Contribution of recycled materials to cover the total demand for materials is low. Trade in secondary raw materials is on the rise, both in the EU and in third countries. "In a circular economy, materials incorporated into products and components are recycled, when they reach the end of their life cycle, they are then injected back into the economy, as secondary raw materials. In the EU, the level of demand for raw materials exceeds the volume that could be supplied, even if all waste were converted into secondary raw materials. Therefore, the supply of primary raw materials will remain necessary. On average, recycled materials meet only around 10% of the EU demand for materials, despite a constant improvement since 2004" (Esa et all., 2016)[6].

## 2. Preliminaries - Circular Economy in the Construction Industry

### 2.1. Introduction of Circular Economy in the Construction Industry

The construction industry is responsible for more than 40% of the extraction of resources as well as for 25% of the solid waste generated in the world. The sector is adopting a model of „*take, make, squander*" (Taylor, B., 2021)[3] using materials for the

construction of buildings and their disposal at the end of the duration, since they are assembled for single use and do not store, not having established a potential for reuse. In this model, the phases that present this idea begin with the extraction of raw materials from the environment. which are then processed to become building materials and assembled in the construction site, in ways that cannot be disassembled or recycled, becoming obsolete at the end of the life of a building, being thrown into landfills or incinerated, together with all waste generated through the whole process (Mangialardo, A. and Micelli, E., 2018)[11]. In recent decades, however, there has been a change in basic assumptions in industry in general, with the adoption of a circular economy model. Waste generated by construction and demolition occurs throughout the life cycle of buildings even in the planning and design phases, because waste management and reduction in previous phases of a project are not taken into consideration (Esa et al., 2016)[6]. However, the phase with the greatest impact in terms of waste generation is the end of the service life, which represents 50% of the statistics (Kibert, C., 2008)[12]. This happens because most building materials are often removed at the end of their lifetime, as they have no potential for reuse (Akanbi et al., 2018)[13].

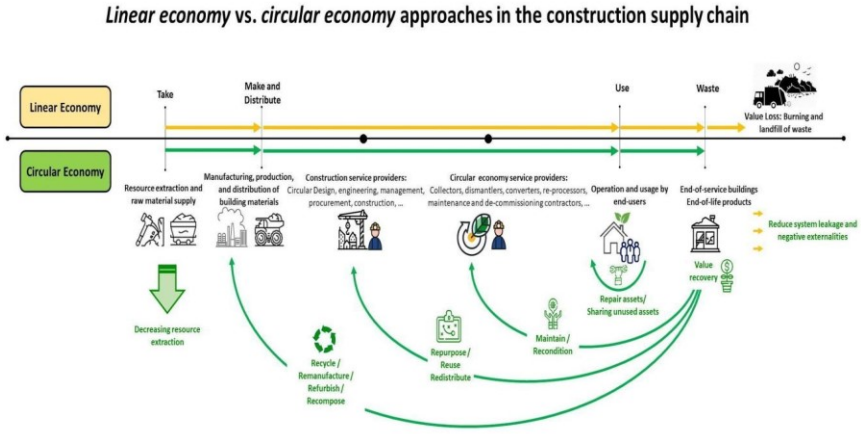



Figure 1.4. Linear Economy versus Circular Economy in the Construction Industry (https://en.wikipedia.org/wiki/Circular_economy#/media/File:Construction_supply_chain.jpg)[7'].

Uncertainties caused by fluctuating commodity prices, especially after the pandemic context Covid19, scarce materials, growing demand, consumer expectations, lack of adequate waste infrastructure and the use of wrong recycling technologies lead to complexities in the construction industry. The foundation of the Circular Economy is achieved through better resource management by lower consumption and replacement of the concept of „end of life" with the reuse, recycling, and recovery of materials and components (Cemex Ventures, 2023)[4']. From this point of view, the construction field has the best applicability due to its complexity and multiple uses of the so-called remnants of materials.

## 2.2 Perception of the Construction Industry on the Circular Economy

Industries perceive the circular economy as a method of integrating economic, societal, and environmental interests, by transforming linear economies into circular economies to get the best value of the product. Unfortunately, this integration comes at the cost of increased complexity in various manners. Current construction complexity

management techniques focus on reducing complexity at the local level rather than on recognizing the importance of the complexity of the transformation into a circular economy. This system perspective and the reality that every player needs to have that perspective and understanding to contribute effectively to a circular economy opens new possibilities for collaboration, but at the cost of increased complexity (Holcim, 2023)[5']. The circular economy is one of the secret weapons which the construction industry can implement to face one of the biggest challenges: to be one of the largest consumers of raw materials in the world. Because of the call to stop treating resources as unlimited, circular models are the way forward for the sector.

### 2.2.1 How the Circular Economy can drive Transformation and Remodeling the Construction Industry?

From water and electricity to steel and concrete, many resources are needed to build homes, offices, and roads (see Schneider, M., 2019)[9]. But at what cost? In Europe, construction accounts for more than a third of total energy consumption. And when buildings reach the end of their life, only 40% of construction waste is recycled or reused. Most of these recycled materials are recycled for second-class construction, such as roads, rather than new buildings. It is therefore no surprise that construction is most common in the circular economy strategies of European countries. They aim to minimize (or even eliminate) waste and pollution by improving efficiency and keeping products and materials in use. For construction, it includes everything, from the design phase to the construction, use and eventually deconstruction and recycling. However, the benefits of a circular approach far surpass the ecological one. It can also bring significant social and economic gains. Use of environmentally friendly building materials has a positive impact on well-being, for instance, and it has been shown to increase productivity by up to 10%. By innovating business models along the entire value chain, by innovating models one expects an additional global market of more than 600 billion by 2025, with an annual growth rate of 12%. Europe will play a leading role in this circular economy, with a market share of EUR 240 billion.

### 2.2.2 How do we integrate these new systems into existing ones without destabilizing the industry?

The construction comprises a long and varied value chain. Progress is already being made and we have identified numerous examples of pioneering approaches that fit our new market model. **Established players are diversified:** an international engineering firm applies the design of green buildings on a city scale to create more natural urban environments. A major architectural practice has created a new subsidiary for recycled building materials to meet the demand for more „circular products". **Creating new partnerships:** A leading recycling business forms valuable partnerships through a closed material recycling group. There are currently about 800 companies. Enter technology start-ups: a growing number of new technology players disrupt the construction industry. A company in the US has created services to track and accurately direct the use of energy in buildings, bringing energy savings of up to 50%. Technology now allows a German company to monitor the buildings and to detect malfunctions at an early stage to extend their service life. **New business models:** sharing economies and platforms also come in construction. Flexible office sharing can bring benefits both for tenants, as well as owners, as well as reduce space and wasted resources. Meanwhile, a digital ledger for building materials may prevent them from „disappear" as waste.

### 2.2.3 Creating Volumes and Profit Margins

This new circular economy ecosystem will enable the construction industry, especially in Europe, to discover new incomes and profit funds, with robust growth of up to 30% per year. In this regard, the scope of new business models will be bigger than others. Due to the size of the market, renewable and recycled materials will have the highest share. Currently they represent more than 70% of the new circular construction market. The rapid adoption of digital technology will fuel significant growth in new and innovative circular business models. We expect those in the planning and design phase to show 20% growth between 2020 and 2025. Operations models will grow by 13% globally and 18% in the EU in the same timeframe. So far, developed, the end-of-life phase will show the greatest growth over the next four years: 33% in Europe and 27% worldwide.

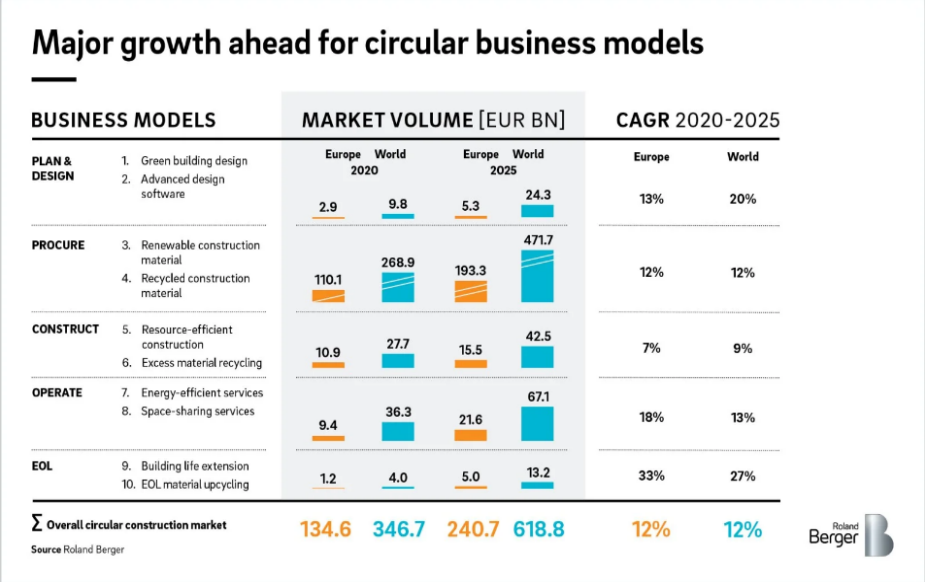



Figure 1.5. Overall Circular Construction Market, Source: Roland Berger (https://www.rolandberger.com/en/Insights/Publications/It%E2%80%99s-time-for-construction-to-embrace-the-circular-economy.html)[8']

## 3. Main part

In this section, a comparison is sought in terms of financial and time advantage the circular economy offers us within 2 investment objectives:
-Property 2S+Gf+MF+13F (generic named "Towers")
-Neighborhood of Individual Houses Gf+2F (28 units - generic named "Houses")

The study aims to analyze the following elements: concrete, steel concrete, as main building materials entering the circular economy process. What the paper is going to demonstrate with the study conducted on the 2 objectives, both individually and simultaneously is that *the circular economy is a management within the analyzed construction project, and the main player in this context is the project manager.* The latter has as his main objective optimizing resources in such a way that loss becomes raw material necessary for another activity. Why is it preferable to use this circular pattern in construction projects? The reasons are multiple, but the explanation in short is that the price of materials in the market it is impossible to keep throughout the entire project, but introducing in the circular model the losses generated by certain construction activities,

the manager can as part of a project whose duration exceeds 6 months (the period announced by the suppliers of the materials when they change prices of construction materials) recover from the disadvantages that have occurred as a result of rising prices. This is hard to predict, and most of the time, the final beneficiary bears all price increases. Comparisons are made using the same criteria for the same activity, considering the clear peculiarities of each project. What can be introduced into the circular economy in the construction business? Practically, anything. It only matters how it is done.

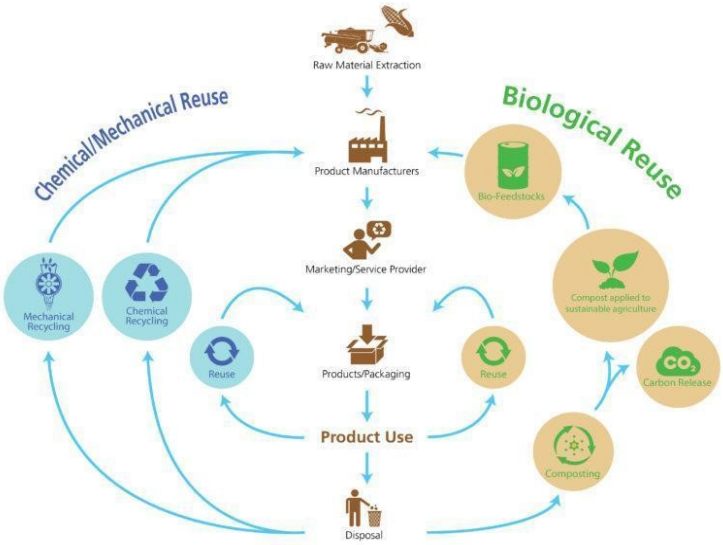



Figure 1.6. Chemical Reuse and Biological Reuse in Circular Economy [6'] (https://circulesolutions.com/circular-packaging/)

The paper pursues several examples.

**1. The defining Element of a Construction: Concrete**

In monolithic concrete pouring the amount required for concrete pouring can never be anything other than a multiple cubic meter or half a cubic meter. In 95% of cases, it is necessary to have a greater amount of concrete than the actual amount of concrete needed for the pouring. Why is this? If by calculation the casting needs 8.7 cubic meters of concrete, the manager orders 9m$^3$ (the station sells multiple 0.5 or 1m$^2$; whether the concrete is poured with auto pump or stationary pump one has to take into account that at pouring concrete it adds the machine hopper and the concrete on the column; this quantity cannot be redeemed in the place where concrete is poured unless water is introduced into the hopper and it is pushed on the column; but this damages the recipe of the concrete class, therefore in the correct management of concrete pouring is contraindicated what it was reported above); these 0.3 cm of concrete can be redeemed. How? The answer is simple but requires good specialty management and equally good Human Resources management. From 0.3 m$^3$ of concrete one can obtain 24 concrete slabs of 50cmx50cmx5cm. Their necessity? Within a construction site they are useful in making pedestrian alleys. The amount of 0.3 m$^3$ of concrete can mean 12 tiles of 50cmx50cmx10cm. With the latter one can pave a roadway inside a construction site.

https://doi.org/10.5593/sgem2023V/6.2/s26.70



Figure 1.7. Precast concrete slabs (own source)



Figure 1.8. Precast fence posts (own source)

Also, 0.3 cubic meters of concrete can represent 30 counterweights of the following sizes: 50 cm x 40 cm x 5 cm - necessary for the installation of chillers, outdoor units, supports for some eventual solar panels, supports for leveling materials or office containers, soles for metal fence posts used for fencing the site, cladding of eventual concrete channel elements, car wash ramp etc. Also 0.3 cubic meters of concrete can represent the following: 24 concrete fence posts of the following sizes 2.5 m x 7 cm x 7 cm. Their usefulness is given by their names: delimit 2 properties, delimit eventual platforms; delimit the construction site from the site management operations etc.
For example, 0.3 cubic meters of concrete can represent the thick reinforced concrete slab of a protection site for 1 m x 1 m x 30 cm facilities.



Figure 1.9. Precast hatches with lid (own source)

Now the paper analyses statistically how many concrete castings can occur in the case of a site for a 2BS+Gf+13F during a month. In 30 calendar days there are approximately 23 working days. If from the 23 days one considers that concrete is poured in 20 days, and the average pouring is 0.3 m of concrete saved for the elements mentioned above the following hypothesis is advanced:

-The area of the site organization is 300m$^2$ (30mx10m); for its fencing 32 pillars are needed, placed at 2.5m from each other; that means after 2 concrete pours the equivalent of fence pools were poured.

-Of the 300m$^2$, the area to be paved is 120m$^2$; for 120m$^2$, 480 tiles of 0.5 mx0.5mx5cm thickness are required; their production is carried out in 20 days.

Conclusion: In 22 working days in which concrete is poured a construction site organization of 300 sqm can be carried out as far as fencing and paving are concerned.

By analyzing the prices in the quantity lists, the paper will look at the following:

1. The cost of fencing and paving of the construction site organization is 25000 Romanian lei (5033 euro).
2. The cost of concrete waste disposal accumulated in one month: 32 pillars x 2.5 m x 0.07m x 0.07m = 0.40$m^2$.

480 tiles x 0.5m x 0.5m x 0.05m = 6$m^2$.

At a cost of 100 Romanian Lei (RON)/$m^2$ x 6.4$m^2$ = 640 RON/month/average.

Assessing that in the imposed graph of 2 projects:

-Property 2B+Gf+MF+13F,

-Individual housing neighborhood Gf+2 (28 units).

The volumes of cast concrete are approximated at:

-over 30.000$m^2$,

-over 20.000$m^2$.

We can appreciate that in a circular economy for the efficient use of volumes what cannot be managed in their first stage of life the following will occur:

-Within a project, the technological loss considered, due to the above-mentioned factors it is around 2%

-A. 30.000 x 0.02=600$m^2$

-B. 20.000 x 0.02=400$m^2$.

When we analyze within the budget:

A. Approx 600$m^2$x400RON/$m^2$= 240.000 RON,

B. Approx 400$m^2$ x 350 RON/$m^2$ = 140.000 RON.

To be able to recycle /discharge these quantities we find necessary from the budget point of view the following calculations:

600 $m^2$ ÷ 24$m^2$/container = 25 containers,

400 $m^2$÷24$m^2$/container= 17 containers.

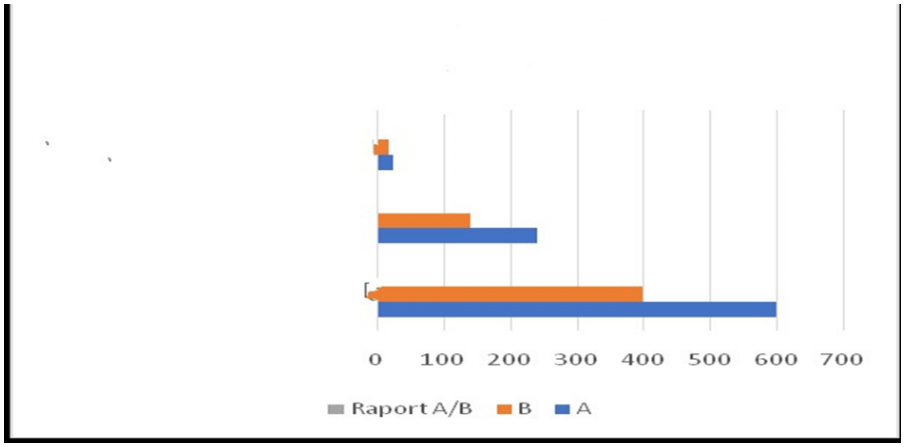



Fig. 1.10. Concrete loss management of the 2 projects: first set of values Recycled Volume (24$m^2$), second set of values budgets in thousand RON, third set of values technological loss ($m^2$)

The calculation is not relevant as the truck would exceed the maximum authorized tonnage by far (24$m^2$ concrete × 2.4 to/$m^2$ = 57.6 tons to which is added the mass of the truck (12 tons), reaching a total of about 70 tons.

To evacuate, it would be necessary 40 to - 12 to the truck mass=28to÷2.4 to/$m^2$=11.5$m^2$/ride. For:

A: 600m$^2$÷11.5m$^2$/ride = 52 rides x 500 RON (zone B authorization) + 600m$^2$ x 60 RON/m$^2$ = 62.000 RON,
B: 400m$^2$ ÷11.5m$^2$/ride=35 rides (there is no area tax to pay), but the cost of evacuation will be calculated: 400m$^2$ x 60 RON/m$^2$=24.000 RON.

If within the project, the project team organizes the work in phases embedded in a circular economy, the total amount that could be saved would be:

A: 240.000 RON (the acquisition cost of concrete) + 62.000 RON = 302.000 RON,
B: 140.000 RON (the acquisition cost of concrete) + 24.000 RON = 164.000 RON.

Total cost of concrete:

A: 30.0000m$^2$x400 RON/m$^2$=12.000.000 RON,
B: 20.000m$^2$x350 RON/m$^2$=7.000.000 RON.

To conclude relevantly what the real benefit is which the site has in this type of budget and resource management, the authors will add to the saved budget the budget allocated for concreting, fencing, and managing the site organisation:

**A:** Allocated budget S.O. (site organisation): 150.000 RON,

Allocated cost for the purchase of reinforced concrete slab of a protection site for external networks: 1.500 RON/protection site x 50 pieces = 75.000 RON,
Counterweights for chillers and other technical equipment: 20.000 RON.
Allocated or calculated total (as appropriate) is: 302.000+150.000+75.000+20.000=547.000 RON.




Figure 1.11 The cost of concrete for the property 2BS+Gf+MF+13F

**B:** Allocated budget S.O. (construction site organization): 90.000 RON.

Allocated cost for the purchase of reinforced concrete slab of a protection site for external networks: 1.500 RON/protection site x 150 pieces = 225.000 RON,
Counterweights for chillers/ photovoltaic panels and other aggregates are 50.000 RON.
The allocated or calculated total budget (as appropriate) is: 164.000+90.000+225.000 +50.000=529.000 RON.

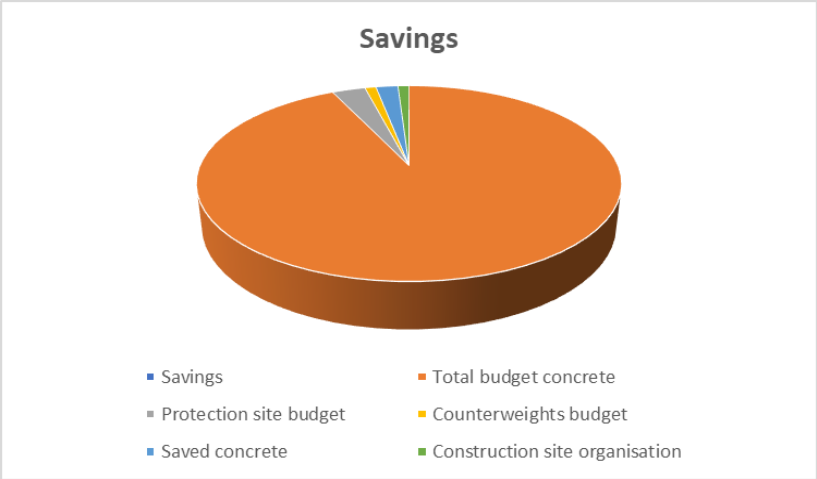



Figure 1.12 The cost of concrete for a neighborhood of 28 houses

Comparing the savings between the 2 projects

Table 1. Costs with the concrete in the analysis of 2 construction projects

| **Category** | **A** | **B** |
|---|---|---|
| Total cost of concrete [RON] | 12.000.000 | 7.000.000 |
| Volume of concrete [$m^2$] | 30.000 | 20.000 |
| Loss 2% [$m^2$] | 600 | 400 |
| Loss [RON] | 240.000 | 140.000 |
| Costs for logistics [RON] | 62.000 | 24.000 |
| Budget S.O. [RON] | 150.000 | 90.000 |
| Budget for protection sites [RON] | 75.000 | 225.000 |
| Budget counterweights [RON] | 20.000 | 50.000 |
| **Total** | **547.000** | **529.000** |
| *Concrete Savings Report* | *96.71 %* | |

With a brief look at the table above, the following can be noticed: Although the related loss for Project A is 50% higher, the ratio of money saved in the first project to the second is almost 1, which helps us to understand that the circular economy depends heavily on the profile of the investment goal in this case and less of the volumes we have available.

## CONCLUSION

With good organization, the total budget allocated to concrete can be saved and at the same time save production and logistics times with other activities in the natural cycle of a construction. Four percent up to 7% of the budget allocated can be saved depending on the type of construction and the need for concrete elements in its frame.

https://doi.org/10.5593/sgem2023V/6.2/s26.70

## 2. Reinforced Steel

One of the materials that can be used alongside concrete is steel. The latter can be used in various forms and methods, but in the authors opinion, it can be used as follows:

a. reinforcement of fence posts, mentioned above,
b. reinforcement to precast masonry bushes,
c. reinforcement of foundation plates and elevations for the reinforced concrete protection sites for facilities.



Figure 2.1. Precast elements for protection sites (own source)



Figure 2.2. Precast plates of reinforced concrete (own source)

Conducting anchors for handling/manipulating supports of all above mentioned materials



Figure 2.3. Metallic anchors for handling of precast reinforced concrete elements (own source)

The authors will not treat as cost the benefits of using concrete steel in the circular economy as it is found everywhere concrete is used in the making of precast elements, already dealt with in point 1, at the same time, the cost of disposing of the waste/surplus of concrete steel covers part of its acquisition by its pick-up by authorized type centers such as REMAT (Recycling Materials Centers). **Note:** Interestingly, it would have been the discussion on the subject of concrete steel in the idea where all this waste could have been melted and used into the process of realization of light metal profiles such as those used in the realization of plasterboard walls and ceilings, cable cots, C-bar brackets for extinguishing, plumbing, thermal and electrical installations, etc., but although the idea seems quite good the energy costs consumed for melting and making the finished product it exceeds several hundred times the purchase price. So, the authors analyze the concept stage and its use within the various tasks in the daily life of the construction site; among these, the authors mention the following uses in addition to those outlined above:

- Making leeks for various anchors: from angles to fence pillars and others,
- Supports for mounting fence panels,
- Anchors for managing all precast elements,
- Coupons made when aligning formwork elements,
- Current hand for balconies, stairs and technological gaps necessary for safe work.



Figure 2.4. Metallic anchors for fastening and putting into position of the fence panels (own source)

1. The circular economy influences construction sites if there is a project manager whose thinking is based on cyclicity.
2. In the case of concrete we bring to the attention of readers that its pouring into the elements of the construction site evolution must be in close correlation with the casting of the elements of the site organization, for the 2 budgets to overlap and there is only one expense.
3. For reinforced concrete, it is very important that its optimization is made of the elements of fastening the shaped iron (e.g.: balers rails, brand separators) whereas they, as mentioned in the examples, are part of the acquisition cost, so they do not represent an additional cost to the project budget.

4. Analyzing the information presented in the case study, the importance of finding the optimal solution for reuse of used materials or technological losses, it brings us to a situation where the construction world needs to change its strategy of approach to consumption.
5. There are, however, cases where the management of the elements does not take place according to the reported ones, and this is due to various specific factors of the yard, as well, therefore, the paper emphasizes once again the importance of a project manager who understands the profile of the project to be implemented and the peculiarities that they need to implement in order to ensure the implementation of the circular economy within the project it manages.

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# NATURAL FIBERS AS A SUSTAINABLE ALTERNATIVE FOR REDUCING CONCRETE MICROCRACKING

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## ABSTRACT

The incorporation of natural fibres as reinforcement of concrete is attractive from a sustainable perspective. In fact, natural fibres improve the deformation capacity and increase the ductility of concrete. In addition, natural fibres are lightweight, renewable, less expensive than man-made fibres and locally abundant. However, in order to determine their use in different concrete applications, it is necessary to know the impact on the mechanical properties and the benefits that can be obtained by incorporating the fibres. In this regard, the objective of the present study is to determine the impact of incorporating 0.5%, 1.0% and 1.5% eucalyptus fibre on the workability, compressive strength of concrete and the ability to control the development of micro-cracks. The results indicate that as fibre percentages increases, the workability decreases. However, for all the cases evaluated, the reduction in workability was not significant. Additionally, in all cases, higher compressive strength was observed at 7 days of age compared to the control sample without fibre. This fact may represent constructive advantages from a construction time optimisation perspective. Finally, the samples with fibres decreased the length, width and number of cracks generated in the concrete. Therefore, eucalyptus fibres are suitable as an alternative to control micro-cracking, which can increase the service life of the structures and save maintenance costs.

**Keywords:** *Eucalyptus globulus*, microcracking, natural fibres, reinforced concrete, sustainable concrete

## INTRODUCTION

Concrete is one of the most widely used materials in the world. Indeed, its versatility, low cost and durability have made it a relevant material in the construction industry, which is evidenced by an annual consumption of 30 billion tons [1-2]. However, although concrete is a material known for its high compressive strength, it is also predisposed to shrinkage cracking, rapid crack propagation and brittle failure [3].

These limitations can be minimised by incorporating fibre into the concrete mix. In effect, the incorporation of fibre alters the brittle failure of concrete to a ductile one.

Therefore, it improves the deformation capacity, reduces cracking and presents higher dissipation energy, which represents an improvement in the post-cracking behaviour of the concrete [4-5].

Although fibers can originate from human manufacture, natural fibers are especially attractive for incorporation into concrete. This is because natural fibers are less expensive, renewable, lightweight, biodegradable and locally abundant (sometimes as waste) [6].

Therefore, from a sustainable perspective, natural fibers are a solution with less impact on the environment. This is especially important in the construction industry, which is responsible (in Europe alone) for 30% of the emissions of carbon dioxide gases [7].

However, the incorporation of natural fibers can significantly decrease the compressive strength of concrete, which can produce situations where the practical possibilities to massively use fibre reinforced concrete are very limited [8]. This fact is especially relevant when the natural fibre is obtained from industrial waste where the fibre can be incorporated to the concrete in small percentages. Therefore, the extensive use of this waste depends on the reliability of the concrete mix properties for different engineering applications.

In this regard, the present work focuses on evaluating the impact of incorporating *Eucalyptus globulus* bark fibers (EGBF) into the concrete mix. These fibers are produced from bark *E. globulus* a highly available by-product in the forestry industry. In Chile, E. Globulus is one of the main commercial species in this industry and it is estimated that approximately 1,5 million m3 of bark are generated per year. Additionally, these plantations are present in other parts of the world such as Australia, Spain, Portugal, and Keyna [9]. In all these countries, companies have difficulties managing this by-product, mainly due to the low heating value, a complex morphology to transform and transport it, and a high ash content [8]. Even so, their primary use is as fuel, still being a by-product of low-added value. Therefore, it is essential to develop alternatives for the reuse of this by-product in order to minimize the problems of disposal and management of eucalyptus bark in different geographical areas.

The objective of the present study is to determine the impact of incorporating 0.5%, 1.0% and 1.5% EGBF on the workability, compressive strength of concrete and the ability to control the development of micro-cracks.

## MATERIALS AND METHODS

The experimental program consists of the evaluation of the impact of incorporating 0, 0.5%, 1.0%, and 1.5% of EGBF in concrete and mortar mixes. In this respect, the E. Globulus bark was provided by the company Forestal Collicura from Bio Bio Region in Chile, and converted mechanically in fibers with a size between 2-5 mm with a hammer mill by the Unidad De Desarrollo Tecnológico UDT (Technological Development Unit).

- Preparation of cylindrical concrete samples

The cylindrical concrete specimens were made according to NCh 1037:2009 [10], each specimen was designed for a compressive strength of 30 MPa. Additionally, the consistency of the specimens was evaluated by the Abrams cone slump method according to ASTM C143/C143M [11].

- Preparation of concrete slab samples

In order to study the micro-cracking behaviour of concrete with incorporated EGBF, samples of concrete slabs of 40X36X10 cm of dimension were elaborated. Due to the comparative nature of the study phenomenon, moulds were made based on the ASTM C1579-13 standard but with slight adaptations [12]. In particular, three triangular wooden structures were placed at the inner base of the mould (Figure 1), which contribute to induce early cracks in each sample.



Figure 1. Concrete slab mould.

The mixture placed in the mould was placed in the oven at a temperature of 36°C, according to the method for the evaluation of plastic shrinkage of fiber-reinforced concrete [12]. Finally, 24 hours after manufacture, the sample is removed from the oven and exposed to room temperature where the generation of micro-cracks is evaluated.

The samples were designed for a compressive strength of 30 MPa, which was evaluated according to NCh 1037:2009 [10]. Additionally, the consistency of the samples was evaluated by the Abrams cone slump method according to ASTM C143/C143M [11].

- Preparation of mortar slab samples

The 14X16X14 cm mortar slabs were designed for a compressive strength of 20 MPa, according to ASTM C 270 [13]. This strength contributes to stimulate earlier cracking compared to the 30 MPa concrete samples. Therefore, these samples contribute to the identification of the influence of the fibers as a potential micro-cracking control.

Additionally, the consistency of the specimens was evaluated by the reduced cone slump method according to Nch 2257/3:1996 [14].

The moulds used consist of those defined by the Réunion Internationale des Laboratoires et Experts des Matériaux, systèmes de construction et ouvrages (RILEM) [15]. However, in order to form the slab geometry, the central spacings were removed. Additionally, sandpaper was placed on the inner bottom of the mould in order to increase the internal stress and generate more restraint to deformation (Figure 2).

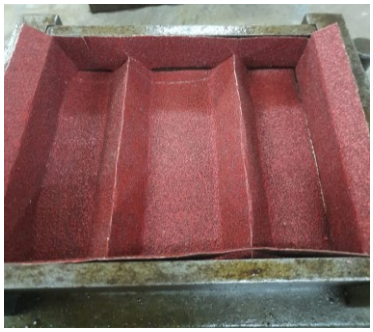


Figure 2. Modified RILEM mould for mortar slabs.

The mixture in the moulds was placed in an oven for 5 hours at a temperature of 36°C, according to ASTM C 1579-13 [12]. The samples were then exposed to room temperature for 24 hours. Afterwards, a second heating cycle was carried out in the oven at 36°C, which contributes to the production of more microcracks. Finally, the samples were again exposed to room temperature for 24 hours and the microcracks generated were evaluated.

- Microcracking evaluation

The micro-cracks produced in the concrete and mortar slabs were studied by means of a 4-step process. First, pictures of the cracked sections were taken with a Celestron microscope. Then, the micro-cracks were measured every 0.5 mm by means of the MicroCapturePro software integrated in the microscope. Then, for each photograph, the simple average of the crack lengths and widths was calculated. Finally, the values obtained from each photograph comprising a slab sample were averaged. In this way, an average value of width and length per sample is obtained.

## RESULT AND DISCUSSION

- Fresh state behaviour

Table 1 presents the consistency according to the Abrams cone method for concrete samples.

Table 1. Consistency in concrete and mortar samples.

| | Fiber addition percentage | | | |
|---|---|---|---|---|
| | Control | 0.50% | 1.0% | 1.50% |
| Concrete samples consistency (cm) | 5.3 | 4.5 | 4.0 | 4.0 |

It is observed that for all the mixtures in which fibers was added, the value of the cone decreased. However, all values are within the design range. Therefore, the changes in workability are not significant with the levels of additions worked.

The fact that this behaviour is present in both types of specimens is very relevant from a practical point of view. Indeed, according to the results, the addition of fibers should not lead to significant changes in the construction methods traditionally used in concrete.

- Compressive strength

Table 2 presents the compressive strengths of the concrete samples obtained at 7 and 28 days of curing.

Table 2. Compressive strength of concrete samples at 7 and 28 days of curing.

| | Fiber addition percentage | | | |
|---|---|---|---|---|
| | Control | 0.50% | 1.0% | 1.50% |
| Compressive strength, 7 days (MPa) | 19 | 23 | 22 | 22 |
| Compressive strength, 28 days (MPa) | 34 | 33 | 30 | 28 |

The results indicate that for all three percentages of fiber addition, the early compressive strengths are higher than the control specimens. In fact, the 7-day specimens reached 76% of the design strength when 0.5% fiber was added. Similarly, when incorporating 1.0% and 1.5% fiber, the 7-day strength reached 74% of the design strength. On the other hand, at this age, the control sample without fiber reaches 62% of the design strength.

For the samples aged 28 days, the compressive strengths for all three types of fiber additions were lower than the control sample. However, for 0.5 and 1.0% fiber addition, the strengths obtained are higher than the design strengths.
For the specimens with 1.5% fiber incorporated, it is observed that the resulting strength was lower than the design strength. This could be due to the fact that the strength values recorded for the samples in this category presented large differences, influencing the average compressive strength value. The existing dispersion between the compressive strengths results was attributed to some construction error, which led to the shortfall with respect to the design strength.
The fact that high compressive strength values have been achieved at early ages with the use of added fibers is particularly relevant considering that compliance with the design strength is only required at 28 days. However, in concrete construction practice, it is strategically important (technically and economically) to quickly reach strengths that allow to increase construction yields, e.g., by demoulding earlier, and to continue construction, where it is even possible to load the elements with eucalyptus bark fiber concretes.

- Micro-cracks in concrete slabs

Table 3 presents the average crack widths and lengths for the concrete slab samples.

Table 3. Average crack dimensions for concrete slab samples.

| Fiber addition percentage | Crack width (mm) | Crack length (mm) |
|---|---|---|
| Control | 0.26 | 271.37 |
| 0.5% | 0.14 | 152.22 |
| 1.0% | 0.00 | 0.00 |
| 1.50% | 0.00 | 0.00 |

The results show how the use of fiber as an admixture in concrete decreases the width and length of the cracks generated. Moreover, it is observed that for the cases of 1.0 and 1.5% fiber incorporated, no cracking occurs in the samples. On the other hand, for the case of 0.5% fiber incorporated, the cracking produced is less than in the control slabs (Figure 3).

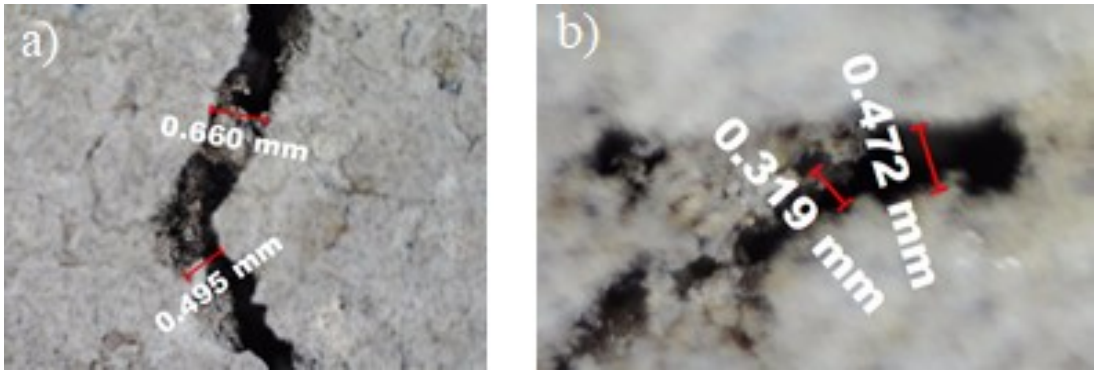



Figure 3. Crack width in concrete tile sample a) control sample b) sample with 0.5% fiber incorporated.

- Micro-cracks in mortar slabs

Table 4 presents the average crack widths and lengths for the mortar slab samples.

Tabla 4. Average crack dimensions for mortar slab samples.

| Fiber addition percentage | Crack width (mm) | Crack length (mm) |
|---|---|---|
| Control | 0.173 | 222.587 |
| 0.5% | 0.116 | 38.712 |
| 1.0% | 0.115 | 37.364 |
| 1.50% | 0.072 | 8.429 |

The results indicate that as the amount of fiber added to the mortar mix increases, the width and length of cracks produced decreases (Figure 4). This occurs even though in this case adaptations were made to stimulate greater crack production.

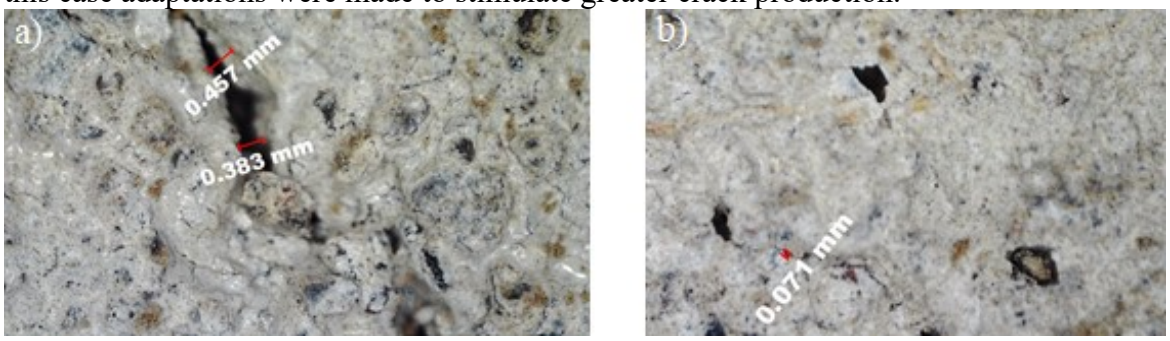



Figure 4. Crack width in mortar slab sample a) control sample b) sample with 1.5% fiber incorporated.

In addition, the three cases of added fiber percentages show more favourable results than the control sample. In this respect, a higher amount of fiber in the mix resulted in a lower production of micro-cracking (Figure 5). This trend was also observed in the concrete slabs samples, reducing the amount of cracks generated as the percentage of fiber incorporated in the mix increased.

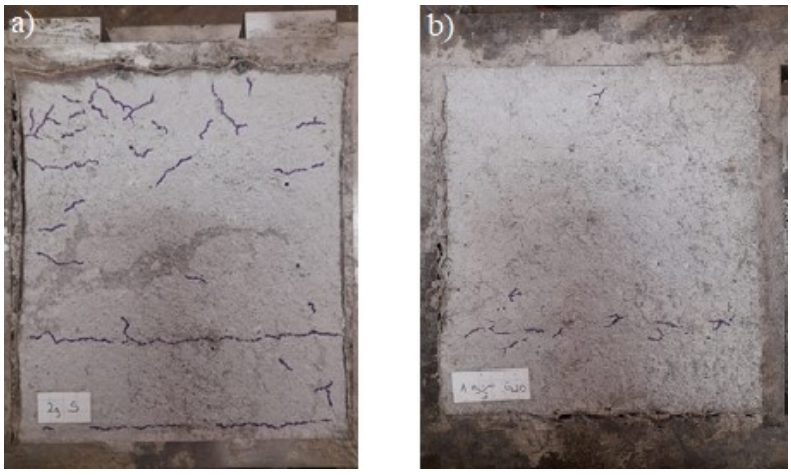



Figure 5. Crack mapping in mortar slab sample a) control sample b) sample with 1.5% fiber incorporated.

## CONCLUSION

The results obtained contribute to the understanding of the properties of concrete reinforced with Eucalyptus fibers. In effect, considering the obtained workability values, it can be established that in general the addition of fiber does not alter the fresh

concrete mix significantly. However, the slump values indicate that as the percentage of incorporated fiber increases, then the mixture gets drier.

For early ages (7 days), the compressive strengths obtained in all cases of specimens with fiber addition presented higher values compared to the control specimen. In fact, the percentages of strength achieved with respect to the design strengths were higher than 70%. At 28 days, the compressive strengths were lower compared to the control specimen. However, with additions of 0.5 and 1.0% fiber, the value of the design strength was exceeded. In the case of the specimens with 1.5% fiber addition, the compressive strength was lower than the design strength by 1.8 MPa. This may be due to a construction error, therefore it is recommended that the effect of this error be re-studied and that the percentage of addition not be discarded.

The remarkable early age behaviour is particularly relevant in the construction industry, as it allows for increased construction performances, faster demoulding times, earlier loading of concrete elements, or quicker commissioning. Examples of cases where these results can be beneficial are building in general (and high-rise construction in particular) or the cutting of joints and opening of concrete pavements to traffic.

Moreover, the good performance in compressive strength represents the possibility of using this solution in various applications. Indeed, considering that satisfying an adequate compressive strength is a requirement in different constructive uses with concrete material, ensuring an acceptable behaviour of this property in fiber concretes is particularly important to massify the reuse of a forest waste as a component of the concrete mix.

The evaluation of micro-cracks in the slab samples revealed that increasing the percentage of fiber addition decreases the width and length of cracks. In the case of concrete slabs, the cases with 1.0% and 1.5% fiber addition did not generate cracks. For the condition with 0.5% fiber addition, it showed a better behaviour in controlling crack production compared to the control sample. This fact exposes the potential benefits that can be obtained by incorporating the eucalyptus fiber as an alternative to reduce cracks in concrete. Furthermore, in order to confirm that the crack reduction was due to the fiber, mortar slabs were developed which, in a similar way, showed that by increasing the percentage of fiber, micro-cracking decreased.

In general, the addition of fibers in the concrete and mortar mix has generated good results in the reduction of micro-cracks, a factor that directly influences the durability of the structures. Therefore, it is presumed that the use of this residue would increase the durability of structures without significantly sacrificing compressive strength. Furthermore, the increased durability represents a decrease in major maintenance during the service life of the infrastructure, which means cost savings in repair work and maintenance shutdown times.

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# THE EFFECT OF CALCINATION TEMPERATURE AND DURATION ON THE COMPOSITION AND MORPHOLOGY OF SYNTHESIZED $C_3A$

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## ABSTRACT

Green building materials are gaining attention for achieving carbon neutrality in civil engineering. To reduce $CO_2$ emissions from cement, using low-cement or cement-free materials with local supplementary cementitious materials (SCMs) is promising. Blended cements with SCMs enhance long-term strength, but low-clinker blends often have low early strength. To address this, understanding the hydration of ordinary Portland cement is crucial. While $C_3S$ has been studied, the role of $C_3A$, the most reactive component of cement, has been explored less. This study aims to produce $C_3A$ in laboratory conditions, assessing the effects of calcination temperature and time on its composition and morphology. The $C_3A$ samples are prepared using the empirically computed amounts of $CaCO_3$ and $Al_2O_3$. The proposed design aims to produce pure cubic $C_3A$, with up to 3 wt.% of unreacted CaO and no mayenite $C_{12}A_7$ as side products. The results obtained demonstrated that the duration for which the maximum temperature is maintained before extracting the samples from the furnace is a crucial factor that exerts the most significant influence on the presence of mayenite in the $C_3A$ structure. The synthesized $C_3A$ will then be added into blends, and its impact on early strength will be evaluated. The findings from this study have the potential to provide valuable insights for designing and producing more environmentally friendly cements, with a specific focus on achieving superior early strength performance.

**Keywords:** tricalcium aluminate, $C_3A$, calcination temperature, mineral composition, morphology

## INTRODUCTION

In recent decades, green building materials have gained attention as vital solutions for achieving carbon neutrality in civil engineering [1]. A key approach to reducing $CO_2$ emissions in cement production involves minimizing its use. Blended cements, incorporating local supplementary cementitious materials (SCMs), offer a globally accepted solution [2]. Nevertheless, a common obstacle faced by numerous blends with low clinker dosage is the issue of low early strength, which adversely affects their competitiveness. This challenge is also evident in one of the most promising contemporary solutions: $LC^3$ cements, consisting of 50% of clinker, 30% calcined clay, 15% limestone, and 5% gypsum [3].

To address this, a deeper understanding of the hydration mechanisms of ordinary Portland cement is crucial. While tricalcium silicate ($C_3S$) has received significant attention [4, 5], the most reactive clinker mineral, $C_3A$, and its role, including sulfate balance, have been comparatively understudied. This knowledge gap is significant as the evolution of new cementitious materials leads to an increased prevalence of SCMs and elevated aluminum content in clinker [3, 6].

Tricalcium aluminate ($C_3A$) constitutes a significant portion of ordinary Portland cement (OPC), accounting for up to 15 wt.% of its composition [7]. Its uncontrolled rapid reaction with water induces a swift setting process, commonly known as a flash set, characterized by the initial precipitation of solid hydration products, specifically calcium hydroaluminate (AFm) phases. During this early stage, katoite can also form, leading to a loss of fluidity and workability in fresh concrete and, consequently, irreversible poor strength development [8]. To mitigate these rapid reactions, a small quantity of solid calcium sulfate (typically 2–5 wt.%) is introduced to the clinker during cement production [9]. Intriguingly, when $Ca(OH)_2$ and calcium sulfate coexist, the hydration of $C_3A$ experiences more significant retardation compared to the use of calcium sulfate alone, as further discussed in studies such as [10].

Similar to clinker production, the synthesis of $C_3A$ involves elevated temperatures. Achieving the desired composition in the $CaO–Al_2O_3$ system, as shown in Figure 1, requires an understanding of relevant high-temperature phase equilibria [8]. Pure $C_3A$ consists of 62.3% CaO and 37.7% $Al_2O_3$ [2]. Figure 1 indicates that $C_3A$ formation can occur within the temperature range of 1000 °C to 1400 °C, with the appearance of a liquid phase beyond 1400 °C.

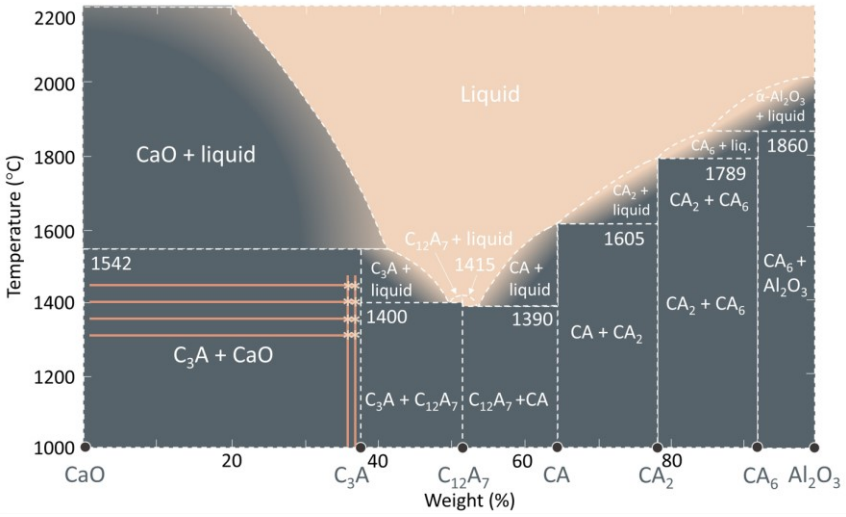



Figure 1. The simplified system $CaO–Al_2O_3$. Based on [8].

Furthermore, a deviation to the left from the line defining pure $C_3A$ (increasing CaO in the mix) results in residual lime (CaO) as a byproduct. Conversely, a higher proportion of $Al_2O_3$ in the mix, in addition to $C_3A$, leads to the generation of mayenite $C_{12}A_7$. The presence of mayenite is undesirable because, according to Quennoz [11], it significantly influences the kinetics of the $C_3A$-gypsum reaction, causing a flash set. Consequently, it is crucial to avoid mayenite formation to maintain the intended mechanisms of the studied processes when introducing synthesized pure $C_3A$ into more complex systems. It is preferable to lean towards the formation of $C_3A$ with a minimal percentage of residual CaO.

This study seeks to contribute to this understanding by designing and producing $C_3A$ on a kilogram scale under laboratory conditions. The research assesses the impact of calcination temperature and time on the final composition and morphology of the synthesized $C_3A$. The synthesized $C_3A$ will then be incorporated into blended cements, and its influence on early strength will be thoroughly evaluated. The outcomes of this investigation hold the potential to inform the design and production of greener cements, with an emphasis on superior early strength performance.

## STUDIED MATERIALS AND METHODS

In the synthesis of $C_3A$, calcium carbonate precipitated A.G., aluminum oxide A.G. (both from Penta Chemicals Unlimited), and distilled water were employed for sample preparation. The particle size distribution of the powders was determined with the help of Bettersize S3 Plus device (Bettersize Instruments), which has the ability to measure the size and shape of particles from 0.01 μm to 3500 μm. The used $Al_2O_3$ exhibited D90 of 14.37 mm, D50 of 1.13 mm, and D10 of 0.31 mm, and comparable results were observed in the case of $CaCO_3$, more specifically 17.12, 5.80, and 0.95 mm, respectively.

The mix design, outlined in Table 1 and based on prior studies [11, 12], was selected. After multiple sample preparation trials, the quantity of distilled water was adjusted to attain a consistency resembling yogurt. The specified amounts of powders and water were combined in a 3 L ball milling jar and homogenized using a rolling machine for a duration of 24 hours.

Table 1. Composition of the synthesized $C_3A$ [grams].

| | **$CaO:Al_2O_3$ ratio** | **$CaCO_3$** | **$Al_2O_3$** | **Distilled water** | **Ref.** |
|---|---|---|---|---|---|
| A | 62.5:37.5 | 713.92 | 240.00 | 900.00 | [12] |
| B | 62.8:37.2 | 713.92 | 236.94 | 900.00 | [11] |

Subsequently, the liquid mixture underwent filtration using a salad spinner to separate the material from the milling balls. The resulting samples were then poured into cylinder molds, maintaining approximately equal quantities for each cylinder. These molded samples were placed in an oven and dried for 48 hours at 60 °C. Subsequently, the temperature was raised to 105 °C until a constant weight was attained.

Following the drying process, the samples were transferred into platinum crucibles and individually heated (one sample per firing) from room temperature up to either 1300 °C or 1450 °C, employing a heating rate of 7 °C/min. The maximum temperature was sustained for 0.5, 1, and 2 hours, respectively. Upon completion, each sample was meticulously removed from the oven and promptly quenched using forced air.

The cooling process was completed in approximately 15–20 minutes. Each sample yielded 180–200 g of C3A, indicating the potential to produce one kilogram of $C_3A$ per week with this setup. Given the highly reactive nature of $C_3A$, the synthesized samples were stored in vacuum-tight plastic bags until they were crushed in an agate bowl, reaching a size of approximately 5–10 mm per piece. Subsequently, the samples underwent milling into a fine powder using a disc mill, with each milling session lasting 30 seconds and repeated twice. The resulting fine powder was then stored in a vacuum-tight plastic bag for subsequent analyses. Each sample was labeled based on the design, maximal temperature, and the duration for which the temperature was maintained.

The mineral composition of the synthesized $C_3A$ was analyzed using X-ray powder diffraction (PANalytical Aeris). The diffractometer was equipped with a CoKα source operating at 7.5 mA and 40 kV. The incident beam path included an iron beta filter, Soller slits of 0.04 rad, and a divergence slit of 1/2°. The diffracted beam path featured a 9 mm anti-scatter slit and Soller slits of 0.04 rad. A PIXcel1D-Medipix3 detector with an active length of 5.542° was employed, and the collected patterns were assessed using Profex software [13]. For the study of microstructural morphology, scanning electron microscopy (SEM) was employed. A Phenom XL electron microscope with a $CeB_6$ source operating at 5 kV and a BSE detector was used for observations, and the samples were coated before examination.

## RESULTS AND DISCUSSION

The mineral composition of all synthesized $C_3A$ was quantified, and the estimated results are displayed in Figure 2. Figure 2a distinctly illustrates that the purity of cubic $C_3A$ is significantly affected by the chosen temperature, irrespective of the selected design.

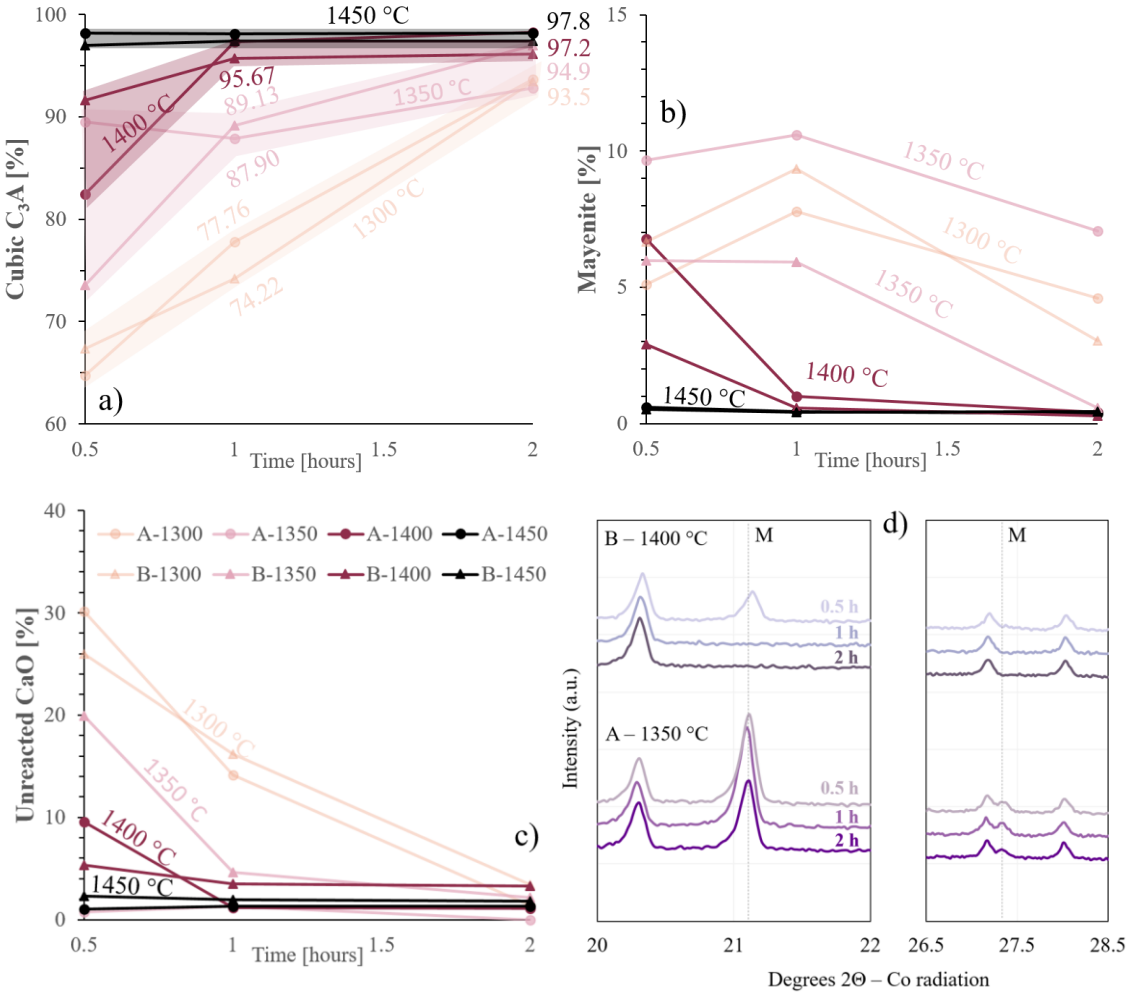



Figure 2. The mineral composition of the studied samples: a) cubic $C_3A$, b) mayenite, c) unreacted CaO, d) detail of XRD patterns displaying mayenite (M) in the structure.

At the lowest temperature of 1300 °C applied for 0.5 hours, the yield of $C_3A$ was less than 70 wt.%, while extending the calcination time to 2 hours increased this amount to approximately 93.5 wt.% for both mixtures. It can be reasonably inferred that a longer duration would likely result in a product with over 97 wt.% of $C_3A$. Moreover, with an increase in temperature, there was a corresponding increase in the amount of $C_3A$ produced. The highest amount of pure $C_3A$ above 95 wt.% was observed in samples prepared at 1400 °C (1 and 2 h, Figure 2d) and 1450 °C.

Figures 2b and 2c illustrate the levels of mayenite and unreacted CaO, respectively. These findings complement the data on $C_3A$ content, revealing a noticeable reduction in the presence of these undesirable byproducts with increasing temperature and duration. While the diminishing trend of unreacted CaO (Figure 2c) is evident in relation to temperature and time, there is an unexpected shift in the case of mayenite. Surprisingly, at synthesis temperatures of 1300 and 1350 °C, the mayenite content increases at 1 hour compared to 0.5 hours, contrary to expectations. This anomaly might be attributed to the short synthesis time and lower temperatures, resulting in highly reactive materials with a more intricate composition. Determining this composition through XRD analysis proves challenging due to its complexity.

With an increase in temperature, samples prepared at 1400 °C (1 and 2 hours) and all samples at 1450 °C exhibited a negligible amount of mayenite (under one wt.%). When the employed temperature and time parameters were compared to the literature, it became evident that the sintering process utilized in this study could potentially facilitate a faster production of $C_3A$ on a kilogram scale within a workweek. For instance, Quennoz [11] sintered her samples for nearly 24 hours, and Zunino and Scrivener [12] for 4 hours and 30 minutes. However, the exact number and quantity of samples used in these comparisons remain unclear.

Figure 3 depicts the morphology of the $C_3A$ samples, contrasting those prepared at 1300 °C (on the left) and 1450 °C (on the right), each subjected to a 2-hour duration.

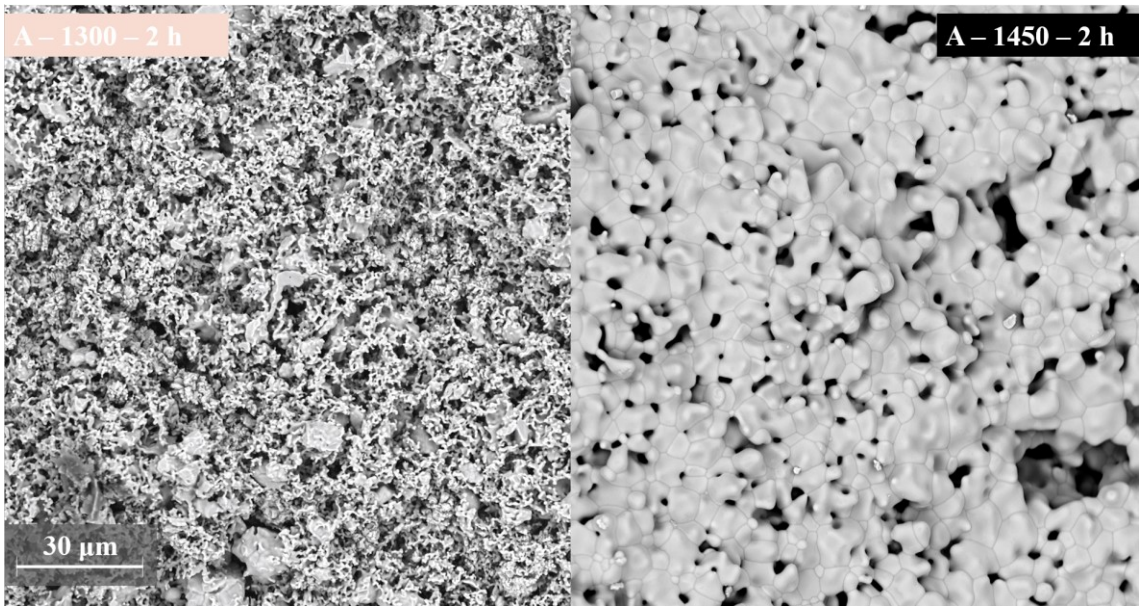



Figure 3. The morphology of the $C_3A$ samples prepared at 1300 °C (left) and 1450 °C (right) for the duration of 2 hours.

Notable features in the microstructure, such as differences in compactness, are evident and provide insight into the impact of varying temperatures on the $C_3A$ morphology. When the point analysis and mapping were done (not shown here), it was confirmed that the distribution of Ca and Al elements was homogeneous. The observations done on the powdered samples also showed that the samples prepared at lower temperatures for a shorter time had generally smaller grains when compared to the samples sintered at higher temperatures, crushed, and milled in the same way. It was expected since the higher temperature led to the formation of a more solid, better-connected microstructure.

It is worth mentioning that while the higher temperature of 1450 °C generally led to a higher yield of $C_3A$ and lower contents of unreacted CaO and mayenite were formed, the resulting larger pieces of $C_3A$, which were challenging to break due to a very condensed structure of these samples, could negatively influence the reactivity of synthesized $C_3A$ due to the lower surface area if these are not removed from the powder, e.g., by sieving, as reported in our previous study [14].

## CONCLUSION

This paper delves into the preliminary outcomes of synthesizing $C_3A$ on a kilogram scale under laboratory conditions. Two distinct $C_3A$ mix designs were formulated for comparison. The study evaluated four different temperatures – 1300, 1350, 1400, and 1450 °C – paired with time durations of 0.5, 1, and 2 hours. The primary objective was to identify a readily reproducible setup capable of yielding approximately 97 wt% of the pure cubic mineral. The key findings can be summarized as follows:

- The impact of various mix designs on the final composition of the $C_3A$ remains unclear. Nevertheless, the most significant differences were noted in samples subjected to lower temperatures and shorter calcination durations. On the other hand, for samples prepared at the highest temperatures of 1400 and 1450 °C, the variations in composition among $C_3A$, mayenite, and unreacted CaO were limited to 2 wt.% and below.
- Elevating the calcination temperature and extending the duration lead to enhancing the quality of the $C_3A$ products. Notably, as the temperature increased, samples produced at 1400 °C (for 1 and 2 hours) and all samples at 1450 °C showed a negligible presence of mayenite, measuring under 1 wt.%.
- The resulting microstructure of $C_3A$, irrespective of the temperature and duration, exhibited a compact yet porous nature. Samples prepared at lower temperatures were characterized by smaller interconnected grains. In contrast, $C_3A$ synthesized at 1450 °C displayed a seemingly more solid structure, featuring larger coral-like formations.
- Milling the sintered pieces with a disc mill revealed a challenge in breaking the larger $C_3A$ pieces prepared at 1400 and 1450 °C, which was attributed to their highly condensed structure. The presence of these larger grains could potentially hinder the reactivity of the synthesized $C_3A$, given their reduced surface area – an aspect that will be further examined in subsequent analysis.

Each factor, such as mixture design, maximum temperature, and duration, shapes the final composition and morphology of $C_3A$. Duration, notably, is crucial in influencing the presence of mayenite in the $C_3A$ structure. Compared to existing literature, our proposed sintering process could expedite kilogram-scale $C_3A$ production within a

workweek. The impact of mix design on resulting $C_3A$ will be further investigated. Future research will involve blending synthesized $C_3A$ and assessing its effect on early strength, providing insights into environmentally friendly cement design with a focus on superior early strength performance.

## ACKNOWLEDGEMENTS

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# THE INFLUENCE OF POLYPROPYLEN FIBRES OF CONCRETE PROPERTIES

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## ABSTRACT

With the development of concrete technology, the polypropylene fibers have been extensively used in concrete structures for property and durability enhancement. The polypropylene fibers have significantly low cost compared to steel, the use of macro polypropylene fiber could achieve a similar level of reinforcement in concrete at a half price of using steel. The article focuses on the influence of polypropylene fibres of concrete properities. The aim is to compare the basic material properties of concretes with the same composition but with the addition of polypropylene fibres of different lengths and in different proportions. Polypropylene fibres of 54 mm, 38 mm and 19 mm length were used as dispersed reinforcement.

**Keywords:** concrete, fibres, compressive strength, splitting tensile strength

## INTRODUCTION

The construction industry is continuously evolving and is searching for new materials and technologies which would lead to sustainable construction. A fibre reinforced concrete (FRC) is an innovative material that could meet sustainability expectations. Generally, the FRC consists of five component materials, and these are fibre, cement, aggregate, water, and additive. Each of these materials can be selected and used according to engineering demands. The quality of the FRC is determined by the material properties of fibres. The cement bonds fibres, aggregate and other materials together. The characteristic examples of natural aggregates are sand or gravel. To maintain the quality of the concrete, the fine aggregates need to be carefully employed, together with coarse aggregates. Instances of additives might be antifreeze or accelerator. These additives must be applied carefully because they may negatively influence the quality of the FRC. [1,2]
FRC which is a cementitious composite material with a dispersed reinforcement in a form of fibres, e.g., steel, polymer, polypropylene, glass, carbon, and others. The FRC is a popular material for its extremely high strength, high crack control, and high fracture toughness, and it also improves the ductility, flexural strength, and shear strength of

cementitious materials. The steel fibre reinforced concrete (SFRC) also reduces shrinkage, creep and permeability of concrete improve the fatigue, impact and explosion resistance of concrete Furthermore, the advantage of the SFRC is also its good cost-effectiveness. [1,3] The increased use of SFRC brings with its defects and failures, as with any technology. Steel fibre reinforced concrete is subject to research and development, especially in the field of shear failure [4,5], fatigue failure [6] or computational models for failure analysis. Failures, such as crack development, are contributed to, among other things, inappropriate consistency of fresh SFRC, which is a kind of technological instability.

Polypropylene fibres (PPF) are polymer fibres, they differ in the length and in the function that they perform in the concrete. Macrofibres are also called structural fibres because they are able to replace the traditional reinforcement in the form of steel bars and transfer loads acting on the structure. Their main role of microfibers is to overcome plastic shrinkage and limit the formation of cracks in the concrete. [7,8]

Usually, fibres are applied to make industrial floors as well as roads or, for instance, airport runways. This material is also used to make machine foundations and other elements exposed to dynamic loads. It is also frequently used as the shotcrete technology, for example as casing of underground structures or at renovation-repair activities. Nowadays, it is often used as material for structural elements. [9]

## MATERIALS AND METHODS

The samples were made in 3 alternatives. Alternatives were with differences in distribution of fibers in the concrete matrix. For the preparation of the samples, Forta Ferro fibres were used [10]. These are high performance fibres produced by combining polypropylene monofilament non-fibrillated fibre and mesh fibrillated fibre. They are used to reduce shrinkage of concrete in the plastic and hardened state, improve impact resistance, increase fatigue resistance and toughness of concrete. Added fibre content in a combination of lengths of 54 mm, 38 mm and 19 mm in quantities of 2.0/0.5/0.5 kilogram (C1-2.0/0.5/0.5), 4.0/1.0/1.0 kilogram (C2-4.0/1.0/1.0) and 6.0/1.5/1.5 kilogram (C3-6.0/1.5/1.5) per 1 $m^3$ of concrete. The first number indicates the amount of fibre length of 54 mm added, the second number indicates the amount of fibre length of 38 mm and the last number is the amount of fibre length of 19 mm. The proportions materials mixes are presented in table 1.

**Table 1** Composition of concrete mixtures

| | | **Quantity per $m^3$** | | |
|---|---|---|---|---|
| | | **2.0/0.5/0.5** | **4.0/1.0/1.0** | **6.0/1.5/1.5** |
| Cement CEM I 42,5R | kg | 340 | 340 | 340 |
| Fly Ash | kg | 80 | 80 | 80 |
| Fine Aggregate 0/4 | kg | 784 | 784 | 784 |
| Corse Aggregate 4/8 | kg | 920 | 920 | 920 |
| Superplastificator | kg | 5.77 | 5.77 | 5.77 |
| Water | l | 162.40 | 166.08 | 165.38 |

### Compressive strength

The compressive strength of samples was determined according to the technical standard EN 12390-3 [11]. The measurements were carried out on cube-shaped test samples with an edge length of 150 mm. The measured values of compressive strength are given in table 2 and in figure 1.

**Table 2** Compressive strength

| | Compressive strength [MPa] | Standard deviation [MPa] | Coefficient of variation [%] |
|---|---|---|---|
| C1 – 2.0/0.5/0.5 | 83.0 | 0.66 | 0.79 |
| C2 – 4.0/1.0/1.0 | 70.5 | 1.13 | 1.60 |
| C3 – 6.0/1.5/1.5 | 74.0 | 3.22 | 4.36 |

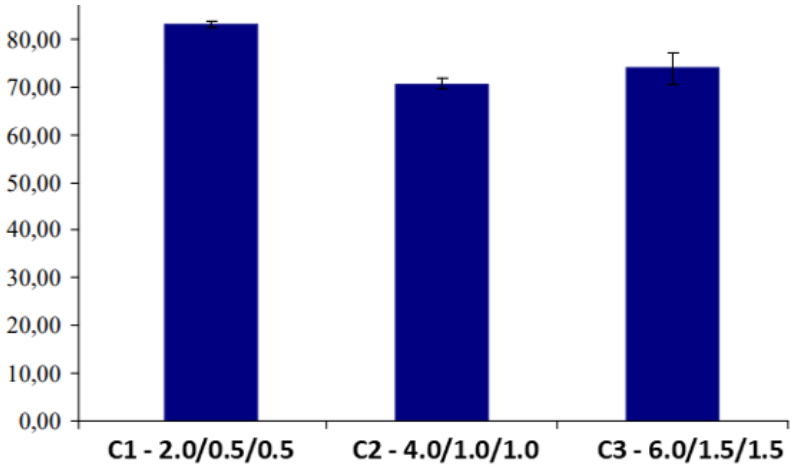



**Figure 1** Compressive strength

From the above test results it can be stated that the highest increase in the compressive strength of concrete occurred in the concrete mix with the amount of fibres in the ratio of 2.0/0.5/0.5 kilogram. The highest compressive strength was measured on concrete C1-2.0/0.5/0.5, the lowest value was measured on concrete C3-6.0/1.5/1.5 which was 12.2% lower and the lowest value was measured on concrete C2-4.0/1.0/1.0 which differed from the highest strength by 17.7%.

### Splitting tensile strength

Testing of the splitting tensile strength of the concrete was carried out in accordance with EN 12390 – 6 [12]. The cube samples with an edge length of 150 mm were tested. Three samples for each mixture were tested. Loading was carried out continuously at a rate of 0.05 MPa/s through the press until failure of the specimen. The transverse tensile strength is calculated by the maximum force, contact area and transverse dimension of the specimen.

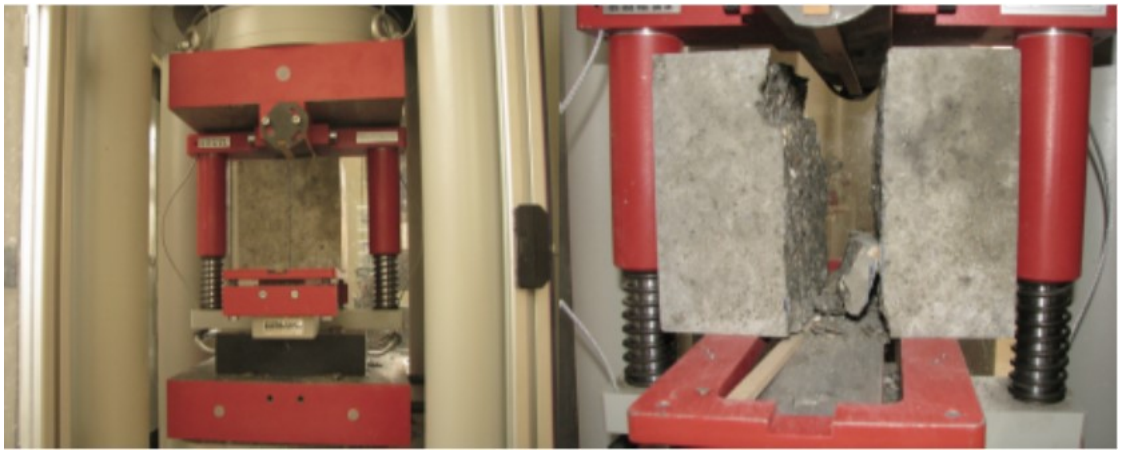


**Figure 2** Splitting tensile strength

The sample in the test machine and the sample after the test is shown in Figure 2. The measured values of splitting tensile strength are given in table 3 and in figure 3.

**Table 3** Splitting tensile strength

| | **Splitting tensile strength [MPa]** | **Standard deviation [MPa]** | **Coefficient of variation [%]** |
|---|---|---|---|
| C1 - 2.0/0.5/0.5 | 4.75 | 0.14 | 3.03 |
| C2 - 4.0/1.0/1.0 | 4.65 | 0.11 | 2.29 |
| C3 - 6.0/1.5/1.5 | 4.50 | 0.29 | 6.55 |

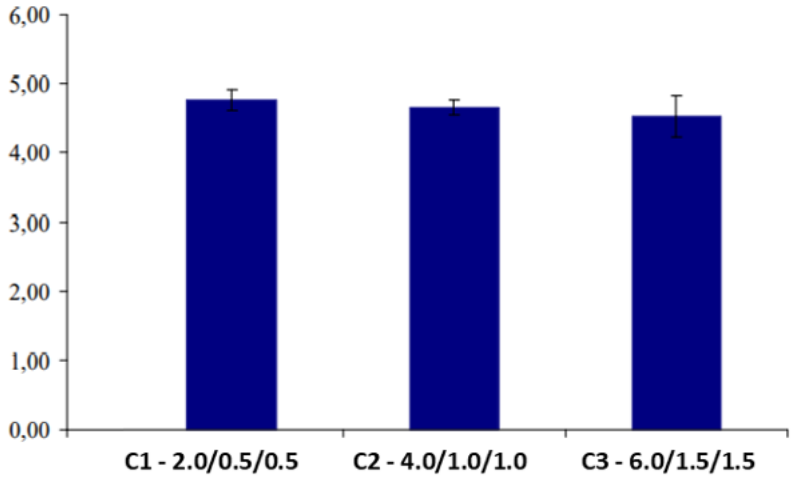



**Figure 3** Splitting tensile strength

The results of the samples are balanced, only there was a slight decrease in tensile strength as a function of the amount of fibre. Specifically, there is a decrease in strength

of 2.2% for the concrete mix with a fibre content of 4.0/1.0/1.0 kilogram and by 5.6% for concrete with a fibre content of 6.0/1.5/1.5 kilogram in relation to concrete with a fibre content of 2.0/0.5/0.5 kilogram.

## CONCLUSION

Fibres play an important role in reaching a definite load bearing capacity after the matrix fracture, depending on allocation, orientation, and embedded length. All these features are influenced by concrete composition (aggregate size, concrete content, w/c-ratio, admixtures), fibre type and others. The aim of the paper was to compare the basic material properties of concretes with the same composition but with the addition of polypropylene fibres of different lengths and in different proportions. Polypropylene fibres of 54 mm, 38 mm and 19 mm length were used as dispersion reinforcement. The results show that the best results in the compressive strength were achieved in the concrete mix with the amount of fibres in the ratio of 2.0/0.5/0.5 kilogram. The results of the samples in tensile strength were balanced, only there was a slight decrease as a function of the amount of fibre. The best results were achieved by the sample C1-2.0/0.5/0.5.

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# SECTION GREEN DESIGN

# AND SUSTAINABLE ARCHITECTURE

# BLUE-GREEN INFRASTRUCTURE AS A TOOL FOR CONNECTING AGRICULTURAL AND URBAN LAND

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## ABSTRACT

Urbanization and expansion of urban territory is a global trend that affects all major cities in the world. For this reason, the quality of the urban environment is increasingly important for human health and well-being. One increasingly popular element to improve and address the challenges associated with a warming urbanized landscape is blue-green infrastructure (BGI). Blue-green infrastructure is mainly associated with the urbanized landscape and its integration into the urban environment. In our post, we will focus on the agricultural landscape, which is in close proximity to the city of Trnava, Slovakia. The administrative territory of the city of Trnava is characterized by a low degree of ecological stability with a lack of natural elements of the landscape structure. Current vegetation in the territory of the city of Trnava (7,153 ha) is divided and represented into landscape - outside the built-up area (2.18%), urban - in the built-up area (35.40%) (Department of Spatial Development and Concepts of the City of Trnava). The aim of the contribution is to present solutions in the "Strkovisko" district, where we proposed a network of natural and near-natural measures to mitigate climate impacts related to climate change for the construction of BGI. The proposed location was designated as arable land with an area of 11.91 ha, which will be transformed into a plot of land with purpose-built agricultural protection and ecological greenery against erosive measures and measures to ensure the ecological stability of the territory. The article introduces the reader to the benefits of BGI and describes the proposed elements to mitigate climate change with an emphasis on landscape activities.

**Keywords:** Blue-green infrastructure, urbanized landscape, ecological stability of the territory, climate change,

## INTRODUCTION

Blue-green infrastructure (BGI) is the term most often used to integrate blue and green measures to mitigate urbanization and adapt to climate change by providing multiple benefits to urban areas affected by rainwater and high temperatures [1]. Blue-green infrastructure can be defined as a package of measures to support ecosystem functions with the aim of providing multiple benefits associated not only with water, but also with urban aesthetics, biodiversity, and microclimate. One of its main goals is to adapt urban areas to climate change [2]. Green infrastructure refers to nature-based systems that mimic natural hydrology and regulate surface energy processes and positively affect air movement and heat exchange. Blue infrastructure refers to natural or man-made forms of water implemented in a city that slow runoff, effectively absorb shortwave radiation and release it through evaporation [1]. Key elements of blue-green infrastructure are trees, water retention areas and other vegetation. The rainwater runoff from the

surrounding paved area is diverted into the terrain depression and infiltrates into the underground trench through the soil filter. The soil filter is used to treat rainwater to prevent clogging of the underground trench and to protect the quality of underground and surface water [3]. This multifunctional planning approach addresses different problems and objectives depending on whether the focus is on blue (water) or green (vegetation) elements. Green-motivated projects aim to thicken urban vegetation and include the growing sector of building greening. A good climatic effect of the vegetation can be achieved by sufficient irrigation [4]. With increasing urbanization, blue and green areas in cities have significantly decreased. Uncontrolled urbanization is primarily responsible for urban flooding as well as the increased problem of water scarcity worldwide [5]. Cities depend on their upper watersheds for the storage and gradual release of water into river systems [6]. Urban areas, and cities in particular, are areas of high demand for flood protection services due to the high concentration of people and infrastructure and their tendency to be located in low-lying areas. However, floods affecting urban areas can occur either locally or elsewhere in the basin; it is therefore important to consider the role of ecosystems not only within the urban areas themselves, but also across the basin landscape [5]. Climate change in a given region or locality can be considered a natural resource that shapes not only the world's ecosystems, but also the socioeconomic conditions of mankind [7]. Global climate change will have a major impact on the ecological, social, economic, and political aspects of human society in the coming decades. Extreme temperature events have increased significantly and affected different areas of the globe [8]. The overall structure of the landscape and climate change have a major impact on ecosystem services and consequently on the sustainability of regions and the entire earth system [9]. Currently, climate change also has a major impact on the agricultural landscape, which creates a mosaic of agricultural fields, human infrastructures, semi-natural habitats and scattered natural habitats. Biodiversity has different roles in agro-ecosystems and thus in the agricultural landscape [10]. From an agricultural point of view, in most European countries there is a medium rate of erosion of agricultural land, which is greater than that of forests. Eroded soil usually ends up in rivers and streams and is then carried to reservoirs where it settles. The solution can be incorporated into a combination of organizational, agro-engineering, biological and technical measures. Organizational measures include land delimitation, crop scattering to combat erosion, and proper determination of land size, shape, and layout [11]. In recent years, ecological restoration measures have been widely implemented around the world to combat land degradation and achieve the sustainable development goals. In practice, the ecological stability of a landscape can affects the ability of ecosystems to provide ecosystem services such as clean water, crops, climate regulation and biodiversity. Ecological stability and climate change are interconnected because climate change can significantly affect ecosystems and their stability. Climate change includes long-term changes in average weather conditions such as temperature, precipitation, and other climatic factors. These changes can have implications for biodiversity, ecological patterns, and the overall ecological stability of the landscape [7, 8].

## MATERIALS AND METHODS

The area of interest addressed in our publication is located near and falls under the administration of the city of Trnava. Trnava is a regional capital in western Slovakia, and due to its size and population, it ranks among the largest cities in Slovakia (the

seventh-largest city in the country). The administrative territory of the city of Trnava is characterized by a low level of ecological stability with a lack of natural elements of landscape structure. The current vegetation in the territory of Trnava (7,153 hectares) is divided and represented as landscape vegetation - outside built-up areas (2.18%) and urban vegetation - in built-up areas (35.40%). The city of Trnava has set a goal to increase the share of greenery to the optimal levels by 2035. The optimal share of greenery for landscape vegetation outside built-up areas is calculated at 14.65% (with the optimal share being 15%), and for urban vegetation in built-up areas, it is set at 42.09% (with the optimal share being 60%) (Department of Spatial Development and Concepts of the city of Trnava). Through collaboration with the city of Trnava, we selected a location north of the city in an area called "Štrkovisko." This location was chosen because it has undergone the reclamation of an old, abandoned gravel pit, turning it into a recreational area connected to other recreational zones. These recreational zones will form the basic framework of the green-blue infrastructure for the entire urbanized area of the city of Trnava.

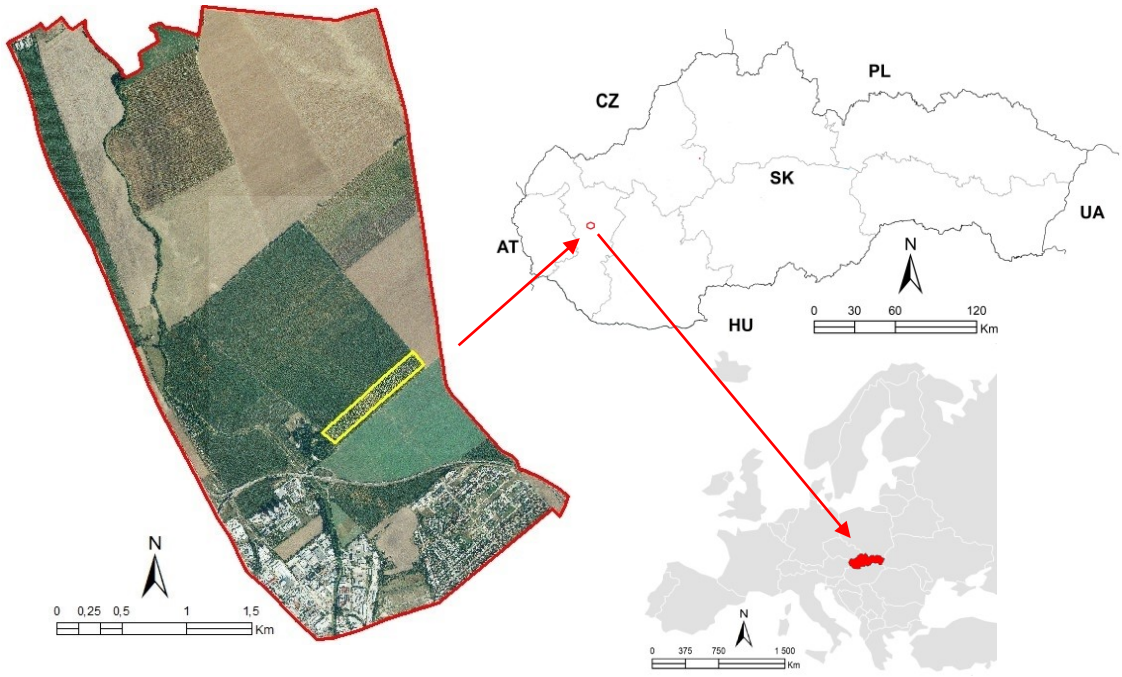



Figure. 1 Localization of the solved area

We have called the proposed location "Štrky 2", which is defined on the western side by the water course of the river Trnávka, on the southern, eastern and northern sides by a dirt road and agriculturally used arable land. The area of interest "Strky 2" includes an area of arable land in the area of 11.91 ha, including the access road. According to the valid territorial plan of the city of Trnava, the territory is intended for the development of recreation and sports when the land is transformed into a purpose-built area with agricultural protection and ecological greenery against erosive measures and measures to ensure the ecological stability of the territory. When proposing BGI natural measures to mitigate climatic impacts related to climate change, the primary goal is to ensure its long-term perspective by implementing protective and development measures. For the analysis of the area under consideration, we developed the following map materials: a map of the current state of the area, a map of hydrological conditions, a pedological map, a map of the groundwater level, a map of the concentration of the area and a map

of the ecological stability of the area. The data for determining the map of ecological stability and the associated calculation of the ecological stability coefficient were derived from the positional map VKM (vector cadastral map), the base map 1:10000, georeferenced orthophoto maps, the geological map of Slovakia 1:50000, updated BPEJ map, and the water management map 1:50000. For the calculation of the ecological stability coefficient, we utilized the methodological procedure of [12], which is based on the map of the current landscape structure (the current state of the area) and the current vegetation. The calculation takes into account the areas of individual elements of the current landscape structure and their degree of ecological stability.

$$KES = \sum_{1}^{n} \frac{p_i . S_i}{p}$$

Where: KES – Ecological Stability Coefficient of the area of interest, pi – Total area of types of landscape structure elements (in hectares), Si – Degree of ecological stability, p – Total area of the area of interest (in hectares), n – Number of elements of landscape structure in the area of interest.

## RESULTS AND DISCUSSION

The goal of implementing adaptation measures in the area was the restoration of the territory while preserving and supporting indigenous species of trees and plants. This also involved increasing landscape biodiversity, supporting biotechnical work in line with climate change adaptation measures. The fulfillment of the project's objective lay in promoting the creation of green-blue infrastructure, restoring ecological stability of the area, and reviving the area of interest to support natural fauna and flora with natural water retention in the landscape, transforming it into soil water. This was achieved by creating elements of blue-green infrastructure at the local level, connecting to the urbanized part of the city of Trnava, with the possibility of further expansion and connection of additional BGI to support biodiversity and mitigate climate impacts on agricultural landscapes.

The main goal of the project implementation was divided into stages for the time demands of the project and adherence to the vegetation period for planting and terrain adjustments. The designated area was divided into 6 zones, marked as A, B, C, D, E, and F, respectively (Fig. 2).

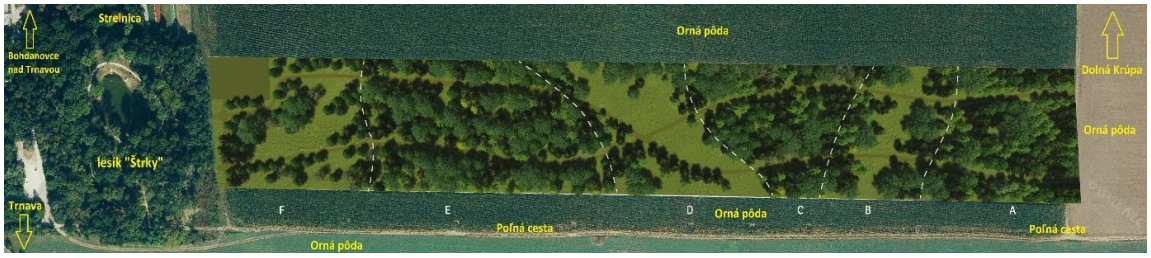



Figure. 2 The visualization of the addressed location

In the first stage, the transformation of arable land into an area with the protective function of agricultural soil and ecological greenery was necessary to implement erosion control measures and ensure the ecological stability of the area. This stage included the proposal of adaptation measures to mitigate the climatic impacts on the agricultural landscape. The second stage focused on protecting the agricultural area from weed growth before the implementation project of Blue-Green Infrastructure (BGI) for the Štrkovisko 2 location was developed. The third stage of implementation was focused on the restoration of fauna and flora to enhance the ecological stability of the area and restore the natural characteristics through the renewal of blue and green infrastructure. Another phase involved the planting of solitary trees and accompanying non-forest tree vegetation, visually supported in sections B, D, and F by a flowering meadow of grass and herbaceous indigenous plant mixture. The revitalized space, through this design, offered the surrounding fauna and flora a return to the original landscape. In addition to the ecological impact on the landscape, the project also supports regional beekeeping. The created green belt simultaneously facilitates the supportive infiltration of excess rainfall from the surrounding landscape, thereby connecting blue-green infrastructure into one cohesive element. The final stage (sixth stage) of the project involved designing a system for the restoration of the area, aiming to complete the green infrastructure in the cadastral territory of Trnava. The implementation of the project aims to coordinate the adaptation process to be comprehensive and enable local governance to respond purposefully to expected climate changes and risks. This includes addressing more frequent heatwaves, rising average temperatures, increased occurrences of drought, more frequent extreme precipitation events, and a higher frequency of strong winds and storms. The structure of systemic measures to mitigate climate change within the proposal for the Blue-Green Infrastructure project to improve the ecological stability of the Štrkovisko 2 area was assessed and evaluated based on data and analyses of possible solutions, in accordance with existing legislation for the restoration of blue and green infrastructure. The adaptation measures in this project were tailored to the specific impacts of climate change in the area, with some designed to address multiple impacts simultaneously. These measures were also formulated to withstand more frequent and intense heatwaves by creating islands of non-forest tree vegetation and providing shelters for surrounding wildlife communities. The area was also designed to withstand and alleviate the repetition of intense precipitation and the consequences of heavy rainfall. Adaptation measures include capturing rainwater and directing drainage through small hydro-technical measures, increasing the retention capacity of the area through hydro-technical measures to retain water in created depressional spaces, and establishing vegetated areas to slow surface runoff.

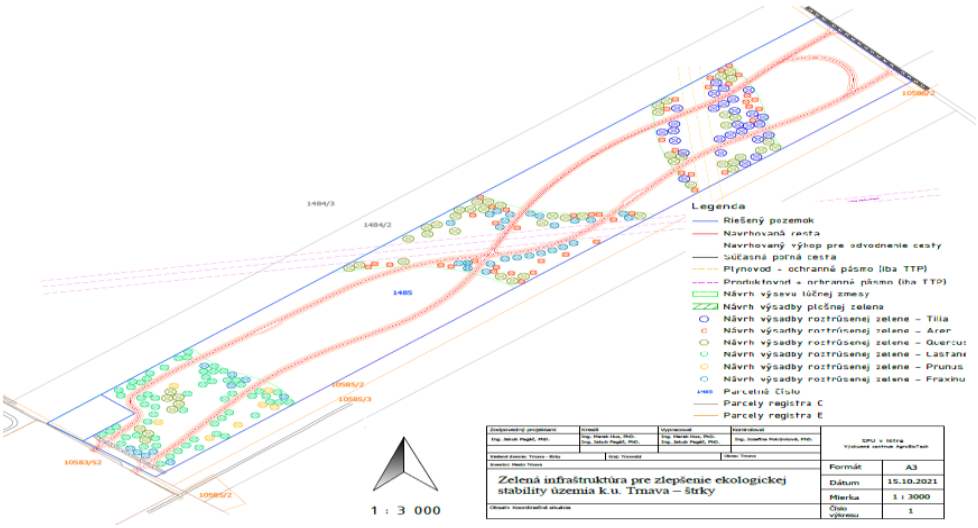



Figure. 3 Technical and implementation proposal for the addressed location

Implementation process of the project:

1. Stakeout of overall boundaries and roads – surveying work,
2. Stakeout of the course of engineering networks and edges of protective zones – surveying work,
3. Site preparation - soil processing – incorporation of current one-year vegetation cover, surface leveling,
4. Compaction of roads,
5. Establishment of a meadow community – soil preparation before establishment, supply of seeds and additional material, work,
6. Planting linear vegetation - supply of plant material, supply of other auxiliary material, work,
7. Planting solitary trees - supply of plant material, supply of other auxiliary material, work,
8. Soil preparation in areas before the establishment of integrated tree stands, temporary crops with phytosanitary effects,
9. Establishment of integrated tree stands,
10. Post-implementation surveying of the actual execution – surveying work after the completion of the components.

To support the claim that the implementation of adaptation measures in the blue-green infrastructure has improved biodiversity, we calculated the Ecological Stability Coefficient for the specified location using the provided formula before and after the implementation of planting. The representation of land types (current landscape structure of the area) in the location before the implementation of adaptation measures was as follows: Arable land 80.2%, Garden 1.4%, Orchards 0.1%, Grassland 1.2%, Forest land 1.3%, Water surface 1.1%, Built-up area 13.9%, and other area 1.8%. Applying the land type distribution to the formula for calculating the Ecological Stability Coefficient, we obtained a KES value of 1.96 for the addressed area and its catchment area. This value indicates a landscape with low ecological stability, emphasizing the need for implementing new eco-stabilizing elements and eco-stabilizing management measures [13]. After the implementation of adaptation measures in the specified location (Fig. 4), a change occurred in the representation of arable land in the addressed area, decreasing by one percentage point to a level of

79.2%. Simultaneously, there was an increase in forest land to a value of 2.3% of the total area of the area of interest. This change was reflected in the calculation of the new Ecological Stability Coefficient, showing an increase of 0.4 points to a final value of 2.



Figure. 4 The implementation of planting in the addressed location

## CONCLUSION

Until now, the prevailing opinion has been that Blue-Green Infrastructure (BGI) is associated with and built in urban and rural areas. Its contribution lies in an integrated approach to water management, utilizing natural and ecological processes to achieve a more sustainable and resilient infrastructure, improving public spaces, regulating temperature, and supporting biodiversity in inhabited areas. In this study, we wanted to emphasize that the approach to blue-green infrastructure is not limited to urban areas but can be adapted and implemented in rural areas, considering the needs of agriculture. A positive contribution of BGI is its support for sustainable development in agricultural landscapes, manifested through the preservation and protection of water resources, the promotion of biodiversity in agricultural areas, or increasing landscape resilience to extreme conditions. We presented this argument in a selected location, Štrkovisko 2, where we implemented the construction of BGI as adaptive measures to climate change in agricultural landscapes. In implementing this measure, arable land was transformed into an area with protective functions for agricultural land and ecological greenery against erosion measures, with the design of adaptive measures to mitigate the climatic impacts on agricultural landscapes covering an area of 11.91 hectares. On this plot, tree and shrub planting occurred, accompanied by the establishment of meadow communities. Terrain adjustments were made, creating purposeful depressions for capturing rainwater and its subsequent transformation and distribution to green elements during dry periods. The construction of BGI on arable land also contributed to the ecological stability of the area by 0.4% of the ecological stability coefficient. Based on the implementation and analyses conducted, we can assert that building BGI is one of the tools for connecting agricultural and urbanized landscapes to develop adaptive measures to mitigate the adverse effects of climate change.

## ACKNOWLEDGEMENTS

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# CASE STUDY: MONITORING AND ANALYSIS OF TRAFFIC BEHAVIOR IN A SMALL-SCALE CITY

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## ABSTRACT

The contribution unveils the outcomes of a case study conducted in Chotebor, Czech Republic, highlighting the pivotal role of long-term traffic analysis in fostering sustainable urban development. It exemplifies how cutting-edge technology and data can be instrumental in formulating more streamlined and eco-conscious urban blueprints. Within the framework of this initiative, surveillance cameras and traffic analysis software were deployed, yielding profound insights into the city's present dynamics and prospective demands. The findings of this case study signify that well-conceived urban infrastructure encompassing bypass routes and traffic optimization can notably amplify urban efficiency and sustainability. The study underlines the necessity for adaptability and flexibility in urban planning to effectively address unforeseen challenges. In its entirety, this research stands as a beacon of inspiration for municipalities aspiring to harness data and contemporary technology to steer towards sustainable and future-ready urban development.

**Keywords:** traffic behavior, camera system, transit rare, data visualization, traffic intensity prediction

## INTRODUCTION

The dynamics of transportation and the continuous flux of urban populations in modern cities constitute an ever-evolving landscape. This shifting terrain has profound implications for various facets, notably encompassing the environment, infrastructure, and economic progression. The quest for sustainable urban development and the integration of green architectural principles are fundamental imperatives for the future of cities, particularly in the context of global challenges such as climate change and population growth [1-3].

Key themes of this evolving urban landscape are sustainability, safety, and the holistic well-being of urban communities. Achieving sustainable development has become a lynchpin of urban planning, aiming to strike an equilibrium between economic growth and ecological preservation while ensuring that cities remain secure and thriving environments for their inhabitants. Integral to this pursuit is the vigilance and comprehensive understanding of transportation systems, which serve as the lifeblood of cities. Traffic analysis and its continuous monitoring play an indispensable role in the realization of these objectives [4, 5]

Such analysis is instrumental in crafting transportation solutions that mitigate environmental impact, enhance safety, and nurture sustainable economic development. As cities navigate their path towards becoming more environmentally friendly and secure habitats, the judicious scrutiny of transportation systems is central to achieving these collective aspirations.

## MATERIALS AND METHODS

Based on discussions with representatives of the small-scale city (Chotebor, Czechia), a pilot project for long-term traffic monitoring was implemented. For the purpose of traffic analysis in the town of Chotebor, camera systems with central management were installed at a total of seven locations in the town (monitoring point, MP). The cameras were placed at preselected locations (see Figure 1) covering the entrances to the town. The acquired data and their analysis are intended to help determine the necessity of a bypass road for the town of Chotebor in accordance with the approved urban development plan and sustainable development of the city.




**Figure 1:** Map of camera locations for monitoring traffic behavior.

The cameras operate within a closed system with communication via mobile operators, secured through a VPN with data encryption. This scheme was chosen to ensure the handling of sensitive data, even though it does not involve direct monitoring and recording of license plates or the creation of photographic documentation of passing vehicles. For the traffic analysis itself, HIKVISION cameras with license plate recognition capabilities were selected. These cameras record the edges of the roadway as well as the center lane and distinguish between the directions of passing vehicles. The cameras were mounted on public lighting poles and supplemented with communication interfaces based on MIKROTIK routers with integrated LTE modems (see Figure 2).

Each of the transit points is monitored, and it is possible to determine the intensity of passing vehicles based on the specified time or time range. For points that allowed it, a

distinction was made between the direction "towards the city" and the direction "away from the city." For points that were not differentiated into multiple lanes, only the traffic intensity is provided. The use of transit points is presented as a histogram of vehicle passages for each point, identified by the camera's name. The analysis includes the time of the analysis and all necessary information. The analysis was conducted for individual periods at each of the transit points.

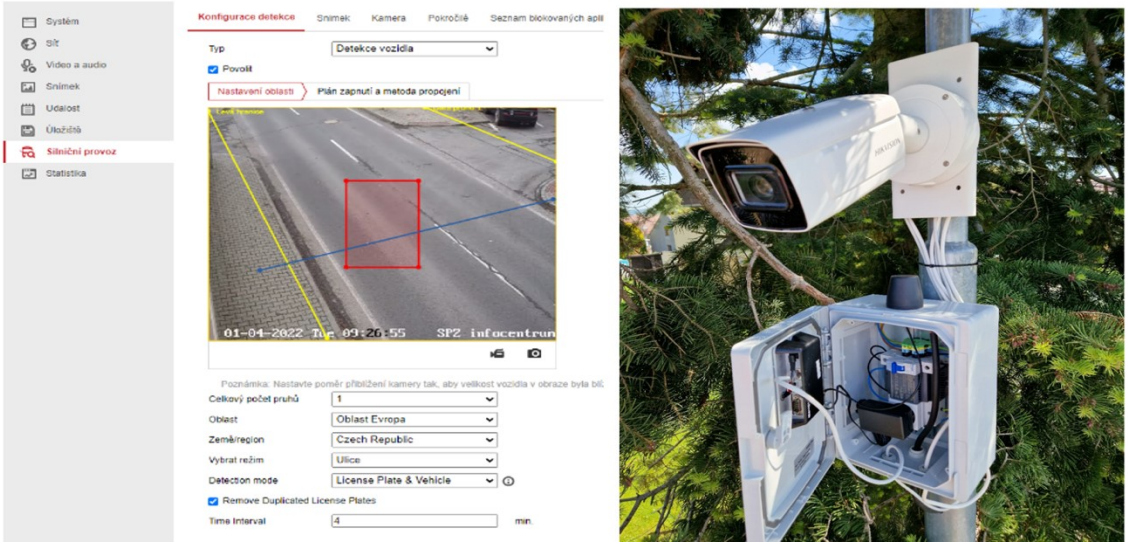



**Figure 2:** Camera system setup and camera installation demonstration.

The trends in passages are especially significant. From the graphs, it is possible to deduce the daily traffic loads at individual transit points and the overall average congestion during the given period. The horizontal axis of the graph represents the time of day, while the vertical axis represents the frequency of passages. Directions of passages from the camera's perspective are distinguished by color.

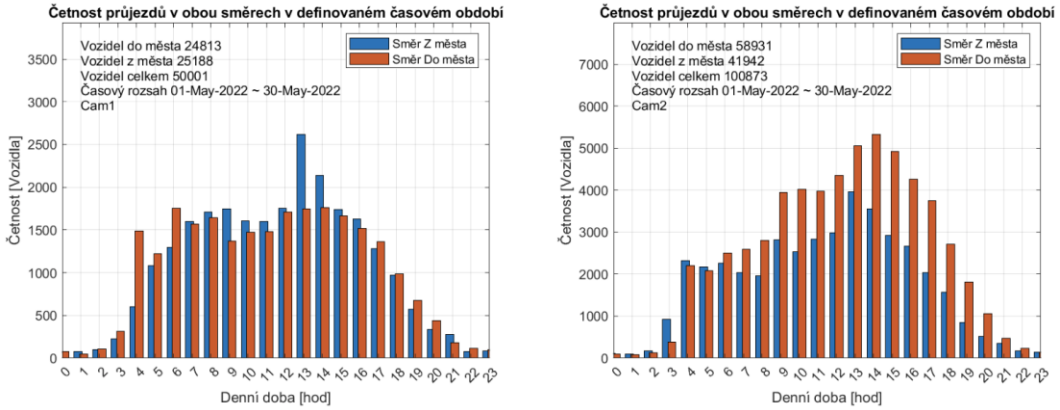



**Figure 3:** A sample histogram of passages at monitored points in May 2022 (camera 1 on the right, camera 2 on the left, blue direction out of town, orange direction into town).

One of the essential pieces of information from the ongoing analysis is transit passages through the city. The analysis has been and continues to be conducted in an offline mode based on the examination of stored data. This analysis is highly computationally intensive as it involves comparing certain vehicle UID (Unique Identifier) with the entire database

and comparing detection times and travel directions. Transit is defined as the detection of the same UID on two different transit points, in a time immediately following the originally detected entry into the city. Transit passages must be related to the absolute frequency of passages at those points to determine how many vehicles are in transit and how many remain within the city.

## RESULTS

For clear presentation and visualization of traffic behavior, a reference online mapping application has been developed and is freely accessible through a web interface at https://amg.vstecb.cz/doprava-chotebor/. This mapping application serves as an analytical tool for visualizing a camera surveillance system on a map, tracking traffic patterns, assessing the load on individual transit points over time, and providing an overall traffic analysis, among other functions. The extent of visualization and data processing is determined by the structure of the installed camera monitoring system.

The AMG analytical module is a map-centric client-server application that allows graphical data visualization based on user-defined filters, along with statistical analysis and historical data support. This application represents an effective and modern tool for presenting data suitable for management, public reporting, and control purposes. In cases where spatial information is lacking, it can be supplemented from other sources.

The analytical tool provides seamless data processing, user-friendly and intuitive operation. The application enables graphical data visualization based on user-defined filters and their statistical analysis. The specific mapping application allows for the analysis of passages and transits within a defined transit interval (from 5 minutes to 10 hours). Recorded passage data can be further filtered by time of day, days of the week, or passages based on national origin. Transit data can also be filtered based on the entry and exit points of the city, time of day, day of the week, or transit time intervals.

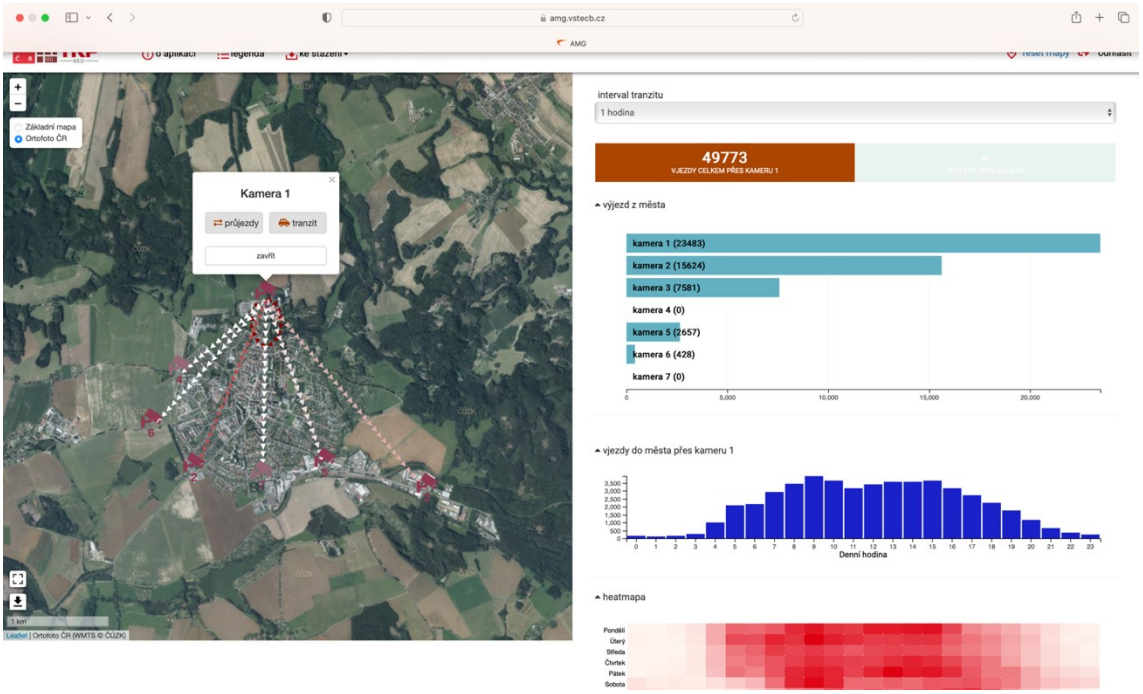



**Figure 4:** Example of data visualization of city passages in the mapping application for monitoring traffic behavior in the city of Chotebor.

The AMG client is a web application developed in JavaScript and built on our AMG platform, which also incorporates several open-source libraries such as Leaflet + OSM, DC.js, Crossfilter, and others. It allows for data input and editing through an editing module with the option to utilize the AMG backend. The client is fully responsive and prepared for mobile devices, enabling the visualization of offline data without the need for data processing on the server side using the backend.

The AMG backend is implemented in the Node.js environment and includes user management interfaces and permission allocation dialogues for individual compositions, communication with databases, and more. Additionally, it can publish APIs for communication with third-party systems or serve as an Open API utilizing Swagger UI.

The AMG analytical module is designed to meet the specific requirements of local governments, municipal and city authorities, and their departments (e.g., building, property, investment, environmental, urban planning, etc.). Apart from traffic analysis, the module can be employed for data presentation and analysis in various fields such as demographics, crime, animal capture, civic amenities, and numerous other areas.

## DISCUSSION

In accordance with data from traffic cameras installed at the main road entrances and exits to and from the city of Chotebor, an analysis of traffic flow was conducted, considering current traffic intensities and the level of vehicle transit captured between individual monitoring points. The presence of license plate agreement at two specific locations and a transit time within 10 minutes signified a transiting vehicle. In this manner, current two-way traffic intensities on individual monitored road sections were determined, as well as the daily traffic intensity variations over time. Subsequently, from these intensities, an estimation of the average daily traffic intensities for the year 2022 (RPDI) was calculated, based on the methodology outlined in technical regulation No. 189 (TP 189). Furthermore, based on the RPDI (annual average daily intensity) estimate, a projection of traffic intensities for the year 2030 was also carried out using TP 225 [6].

**Table 1:** Two-way traffic relationships between individual monitoring points for total vehicles [6].

| Total Vehicles/day | | To monitoring point | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **From monitoring point** | **1** | - | 687 | 374 | 37 | 66 | 18 | 196 |
| | **2** | - | - | 213 | 9 | 24 | 1 | 121 |
| | **3** | - | - | - | 18 | 12 | 24 | 15 |
| | **4** | - | - | - | - | 17 | 10 | 8 |
| | **5** | - | - | - | - | - | 10 | 39 |
| | **6** | - | - | - | - | - | - | 11 |
| | **7** | - | - | - | - | - | - | - |

The data analysis primarily revealed variations in transit rates, particularly between passenger vehicles and heavy-duty trucks. Heavy-duty trucks represent a significantly larger proportion of the overall transit traffic, accounting for up to 33.5%. The main transit routes occur between monitoring points 1, 2, and 3, which correspond to the north-southwest-southeast directions. Subsequently, a projection of future traffic intensities was established for each monitoring point for the year 2030. In the event that a city bypass road (Road II/345) is operational by that time, it could result in a decrease of projected

intensities by as much as 28.5% in overall traffic intensity, with a potential decrease of up to 37% in freight transportation [6].

The expected growth in traffic volume on the monitored road sections is approximately around 7% (compared to the projected values for the year 2030). However, this growth estimate is contingent on the assumption that there will be no substantial changes to the road network within the city and its immediate vicinity until that year. Significant factors include unforeseen developments in the construction of commercial and industrial facilities within the city, as well as residential units, that deviate from expected trends. Unanticipated events also play a role [6].

**Table 2:** Estimation of forward-looking values of annual average daily intensities (for 2030) without changes to the road network [6].

| Monitoring point (MP) | RPDI (2022) | RPDI (2030) | Increase/Decrease |
|---|---|---|---|
| MP 1 (Riegerova Street) | 5 411 veh./day | **5 786 veh./day** | + 6.9% |
| MP 2 (Havlickova Street) | 4 280 veh./day | **4 579 veh./day** | + 7.0% |
| MP 3 (Sokolohradska Street) | 2 436 veh./day | **2 614 veh./day** | + 7.3% |
| MP 4 (Hromadky from Jistebnice Street) | 1 088 veh./day | **1 172 veh./day** | + 7.7% |
| MP 5 (5th kvetna Street) | 1 710 veh./day | **1 834 veh./day** | + 7.3% |
| MP 6 (Zapadni Street) | 1 265 veh./day | **1 355 veh./day** | +7.1% |
| MP 7 (Zizkova Street) | 1 866 veh./day | **1 989 veh./day** | + 6.6% |

If we consider the future city bypass road planned for Chotebor (Road II/345) in its entirety, with the planned connections to the city's road network, we can determine expert estimates of changes in two-way projected traffic intensities for the year 2030 at individual monitoring points as follows [6].

**Table 3:** Estimate of RPDI at individual stations without and with the construction of a road bypass for the year 2030 [6].

| Monitoring point (MP) | RPDI (2030) | RPDI (2030) – city bypass | Increase/Decrease |
|---|---|---|---|
| MP 1 (Riegerova Street) | 5786 veh./day | **4137 veh./day** | -28.5% |
| MP 2 (Havlickova Street) | 4579 veh./day | **3379 veh./day** | -26.2% |
| MP 3 (Sokolohradska Street) | 2614 veh./day | **1845 veh./day** | -29.4% |
| MP 4 (Hromadky from Jistebnice Street) | 1172 veh./day | **1089 veh./day** | -7.1% |
| MP 5 (5th kvetna Street) | 1834 veh./day | **1742 veh./day** | -5.0% |
| MP 6 (Zapadni Street) | 1355 veh./day | **1285 veh./day** | -5.2% |
| MP 7 (Zizkova Street) | 1989 veh./day | **1655 veh./day** | -16.8% |

The provided data, as well as the results of the analysis, can be used to create a traffic intensity prediction model for the road network within the city and its immediate vicinity, taking into account the construction of the city bypass road. The authors recommend this approach, especially given the fact that the city bypass road will reduce travel times between significant regional centers and induce additional road traffic as a result [6].

## CONCLUSION

The conclusion of this case-study in the small town of Chotebor provides key insights into the importance of long-term traffic analysis and its impact on the sustainable development of cities. It also demonstrates how modern technology and data can play a pivotal role in the creation of smarter and more environmentally friendly urban plans.

The project in Chotebor vividly illustrates how cameras and modern software can be used to monitor traffic, enhancing our understanding of the current situation and the future needs of the city. This project extends beyond mere traffic analysis, showing us a path to utilize data for more comprehensive urban planning. The findings from the Chotebor project suggest that well-planned urban infrastructure, including the construction of bypass roads and traffic optimization, can help cities function more efficiently and sustainably. Nevertheless, it is important to bear in mind that urban development is a complex process influenced by numerous factors, including economic and social variables. Plans must be flexible and adaptable to changes, and data can aid in responding to new challenges in real time.

Overall, this study can be regarded as an inspiration for other cities that wish to harness modern technology and data to become more sustainable and future ready. By combining data and urban planning, we can create cities that are prepared to face the challenges of the 21$^{st}$ century.

## ACKNOWLEDGEMENTS

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# COMMUNICATING SCIENCE IN A BIOLOGICAL NATURAL PARK TO ENHANCE CITIZENS' PRO-ENVIRONMENTAL BEHAVIOURS

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## ABSTRACT

The consequences of human actions on Earth are becoming increasingly evident, leading us to consider that the "climate collapse has begun", as the General Secretary of the United Nations (UN) warned at the beginning of September 2023. At less than a decade from 2030, the goals and its targets for sustainable development (SD) in the UN 2030 Agenda are far from being achieved, requiring urgent actions. Their fulfilment is at risk, particularly considering recent events such as the war(s) and the pandemic. Developing individual and collective awareness for action is essential. To this end, it is necessary to develop pro-environmental knowledge and attitudes in all citizens, not just the scholar public, which can translate into behaviours favouring the environment and SD. Based on this framework, quantitative research was developed into the scope of communication workshops in a natural biological park in Portugal. This study aimed to evaluate visitors' knowledge about SD and pro-environmental behaviours (PEB) after participating in two science communication workshops: *Night of the Fireflies* and *Recreation of the Wool Cycle*. In both workshops, the activities highlighted sustainable development themes in the scope of species extinction and biodiversity. A questionnaire was applied to 47 visitors (n=47) at the end of the activities. The results indicated that all visitors became familiar with SD, although they evaluated their knowledge as just sufficient. Participants also indicated that reducing waste and using plastic are PEBs that most contribute to SD. Finally, visitors considered that the PEB they would be willing to adopt due to their participation were the preservation of cultural and natural heritage and the non-disruption of fauna and flora during a visit to a park or natural area. Developing workshops enhanced citizens' awareness and reflection on aspects of nature protection and SD.

**Keywords:** natural park; communication; pro-environmental behaviours; sustainable development.

## INTRODUCTION

Humanity faces multiple sustainability challenges due to the complex interactions between humanity and the Earth system. The consequences of human actions on Earth are becoming increasingly evident, leading us to consider that the "climate collapse has begun", as the General Secretary of the United Nations (UN) – António Guterres – warned at the beginning of September 2023. Given the complexity of these interactions, often non-linear, their understanding is sometimes incomplete and insufficient [1]. Our individual and collective choices can exacerbate environmental, economic, and social problems [1], and their understanding and mitigation require interdisciplinary approaches [2]. With less than a decade remaining until 2030, the sustainable development goals (SDGs) and the targets outlined in the UN 2030 Agenda still need to be met, demanding immediate and decisive actions. Therefore, citizens need to be educated about sustainable development (SD) issues and improve individual and collective awareness for action. To this end, it is necessary to develop pro-environmental knowledge, attitudes and behaviours in entirely citizens (and not just the scholar public under the formal education settings), which can translate into real and tangible actions favouring the environment and SD.

Environmental and sustainability education and communication have the common objective of teaching and raising citizens' literacy about the consequences of human behaviours on the planet to support the development of knowledge, favourable positions and actions with the protection of the environment, consistent with SD – named pro-environmental knowledge, attitudes and behaviours [2,3,4]. However, to raise awareness among a broad spectrum of society, it is necessary to develop non-formal and science communication activities that address these issues. A recent publication from The National Academies of Sciences, Engineering, and Medicine [5] delineated five overarching objectives for science communication, including impacting individuals' opinions and behaviours towards socio-scientific issues.

It is essential to clarify the difference between pro-environmental attitudes and behaviours, meaning that attitudes correspond to a set of individual beliefs, values, and intentions regarding environmental issues, which may or may not translate into favourable actions concerning the environment, that is, into pro-environmental behaviours (PEB) [6,7,8]. However, developing PEB is essential for minimizing anthropic impacts on the planet [7]. The relationship between pro-environmental knowledge, attitudes and behaviours has been intensely investigated, and the adoption of PEB is difficult, involving multiple factors, both internal and external [3,4,6,9]. Over the last few decades, the determinants or predictors of PEB have been investigated. The relationship between factors such as knowledge, values and attitudes and the development of PEB is complex [7]. One of the first predictors investigated for the development of PEB was knowledge [7,10,11,12]. However, its impact is less significant than other factors, such as values and beliefs [7]. In the case of values and beliefs, research indicates that, given their stability over time, they strongly condition the development of PEB [7], with pro-environmental attitudes having an effect similar [11,13].

Scientific communication actions in natural parks are of robust importance for SD. By providing accessible and engaging platforms, these activities familiarise visitors with biodiversity, ecosystems, and the significance of responsible consumption. They raise

awareness of SDGs related to environmental conservation and cultural heritage preservation, inspiring sustainable behaviours. Natural parks serve as living laboratories, offering hands-on experiences that deepen understanding and foster a connection to nature.

## METHODOLOGY

Based on this framework, quantitative preliminary research was developed into the scope of two science communication workshops in a natural biological park in Portugal. This study aimed to evaluate visitors' knowledge about SD and PEB after participating in two science communication workshops in the scope of a PhD research in science education and communication.

### Sample

A sample of this study consists of 47 visitors (n=47) from a biological natural park in the northern region of Portugal. Most participants were female (n=27; 57.4%) and had a higher education degree (n=32; 68.1%), namely a first degree (n=23; 48.9%). Participants average age was 38.4 years old (SD=11.83).

### Instrument

A questionnaire was developed and applied at the end of the activities. This data collection instrument was comprised of 12 questions: 11 closed questions (including multiple choice and Likert scale items) and one open question. The questionnaire aimed to evaluate visitors' knowledge regarding SD and the conceptual and behavioural impact resulting from the two science communication workshops developed. Conducting this questionnaire took an average of 10 minutes, and the data was collected anonymously and treated confidentially.

### Communication workshops

Within this study, two science communication workshops were crafted: *Night of the Fireflies* and *Recreation of the Wool Cycle*. Both workshops focused on SD topics related to species extinction and biodiversity. Through these activities, awareness is sparked, interest is nurtured, and visitors' informed opinions and understanding are reinforced. This enables them to gain the knowledge and skills required to advocate for SD.

The standout attraction in the natural park is the *Night of the Fireflies* communication workshop. Like ladybugs, beetles, or weevils, fireflies exhibit a distinctive feature wherein both larvae and adults of most species emit light, making them visible during dusk and early evening (bioluminescent). Consequently, every June, the park opens its doors at night to observe fireflies and other nocturnal creatures. This communication activity aims to foster the knowledge and skills needed to support SDG4 (target 7) and SDG12 (target 8). It promotes sustainable agricultural practices, yielding environmental advantages such as habitat improvement and the proliferation of native fauna and soil biota populations (SDG 15, targets 5 and 7). Additionally, it encourages sustainable management and efficient use of natural resources (SDG 12, target 2), raises awareness

of SD and lifestyles in harmony with nature (SDG 12), and safeguards cultural and natural heritage (SDG 11, targets 4 and 7) (Figure 2).



**Figure 1**. The science communication workshops: *Night of the Fireflies* (photos from the left) and *Recreation of the Wool Cycle* (photos from the right).

The workshop on communication on the *Recreation of the Wool Cycle* commences with the traditional shearing of the Serra da Estrela sheep breed. This process highlights the Serra da Estrela sheep breed, which originated from ancient roots and evolved from wild forms. Emphasizing sustainable wool production practices, this activity contributes to environmental benefits such as the augmentation of native fauna populations, enhancement of soil biota, and the promotion of greater diversity in pastures, aligning with SDGs 15 (targets 5 and bg 7). It also promotes sustainable production and consumption, fostering awareness of sustainable development and lifestyles in harmony with nature (SDG 12, target 2) while safeguarding cultural and natural heritage (SDG 11, targets 4 and 7) (**Figure 2**).




**Figure 2**. SDGs addressed in the two-science communication workshops developed (retrived from United Nations [14], p.40).

## RESULTS AND DISCUSSION

The findings revealed that all participants (n=47; 100.0%) were aware of SD. When asked where they learned about SD, the two options most selected by respondents were through their formal education (n=26; 55.3%) and the media (n=25; 53.2%). Although the previous results, respondents evaluated their knowledge after participating only as moderately adequate (Mean = 3.4 [in a 5-point Likert scale]; SD = 0.85) (**Chart 1**).

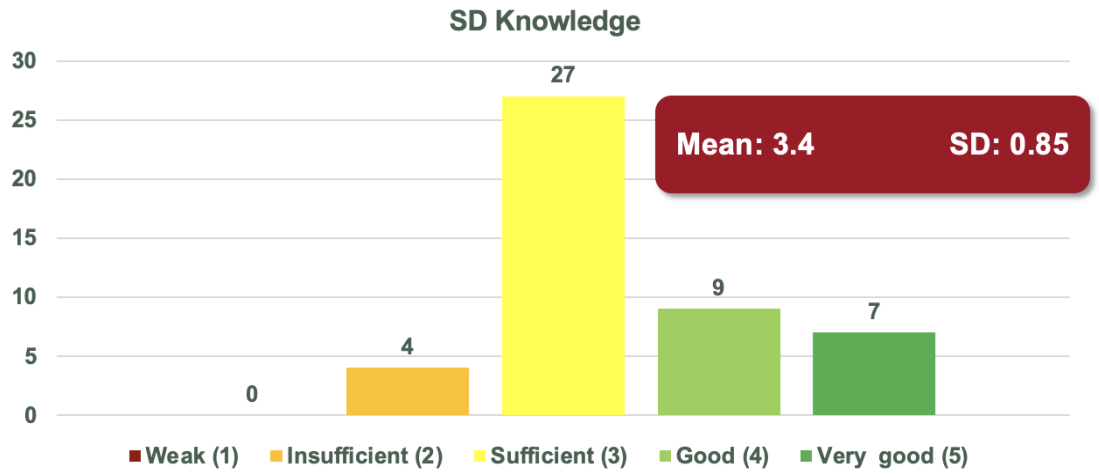



**Chart 1**. Visitors evaluate their knowledge about SD.

The results of the study indicate a high level of awareness of SD among the participants. Despite this awareness, participants assessed their knowledge after the activity as sufficient. These indicators denote the need to promote more science communication initiatives to improve knowledge about SD among citizens [15].

Regarding the SDGs covered in the activity they had participated in, respondents indicated (in descending order):

- SDG15 – Life on land (n=47; 100.0%).
- SDG12 – Responsible consumption and production (n=34; 72.3%).
- SDG4 – Quality education (n=26; 55.3%).
- SDG14 – Life bellow water (n=25; 53.2%).

Visitors reported that the three most common environmental problems discussed in the workshops were species extinction (n=34; 72.3%), biodiversity (n=32; 68.1%), and water and pollution (n=8; 17.0%). Furthermore, in the visitors' opinion on which behaviours would most contribute to SD, respondents indicated that reducing waste (n=32; 68.1%), energy consumption (n=20; 42.6%) and plastic use (n=16; 34.0%), and thinking responsibly (n=22; 46.8%) are PEB that have the most significant impact on SD.

Lastly, visitors expressed, based on their participation, a willingness to embrace PEB related to (in descending order):

- Preserving cultural and natural heritage (n=30; 63.8%).
- Avoid disturbance to fauna and flora during visits to parks or natural areas (n=25; 53.2%).
- Encouraging others to protect the environment (n=20; 42.6%).
- Choose regional flora (n=16; 34.0%).

These findings highlight both the positive impact of science communication and outdoor learning activitivies in raising awareness and the potential for encouraging sustainable behaviours among citizens, corroborating the importance of developing this kind of workshop to promote a more sustainable future [15].

## CONCLUSION

The study's results highlighted the participants' high awareness of SD issues, indicating familiarity with the concept. However, it is noteworthy that despite this awareness, visitors evaluated their knowledge after the activity as only moderately adequate. This discrepancy signals a potential gap between awareness and perceived understanding, indicating the importance of continuous efforts to enhance knowledge about SD.

One notable aspect of the study is the prioritisation of specific SDGs by participants following their involvement in the activity. This reflects the participants' engagement with topics related to nature preservation, responsible consumption, and life protection, emphasising the influence of education (SDG4) on identifying diverse concerns within the broader scope of sustainable development. Environmental problems discussed during the workshops further underscore the participants' keen awareness of species extinction, biodiversity, and pollution. This indicates an understanding of environmental challenges and a recognition of the multifaceted nature of sustainability.

The participants' insights into PEB that contribute significantly to SD reveal a collective consciousness toward practical solutions. Notably, reducing waste, managing energy consumption and minimising plastic use are measures with the most significant impact. This awareness suggests a readiness among participants to translate knowledge into behaviours for a more sustainable lifestyle. Finally, the expressed willingness of participants to adopt PEB underscores the potential for positive behavioural change. These findings highlight the efficacy of workshops in raising awareness and inspiring a genuine commitment to SD.

The study highlights the positive impact of science communications workshops developed in fostering awareness and encouraging sustainable behaviours among citizens. It emphasises the necessity of ongoing science communication initiatives to bridge the gap between awareness and perceived knowledge, ultimately contributing to the collective efforts for a more sustainable and resilient tomorrow. The findings reinforce the importance of workshops as valuable tools to promote environmental consciousness and sustainable actions, paving the way for a more green society.

## ACKNOWLEDGEMENTS

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# GEOETHICS AND SUSTAINABLE SOIL USE: FROM THE CURRICULUM TO THE BRAZILIAN CLASSROOMS IN ELEMENTARY EDUCATION

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## ABSTRACT

In 2015, the Food and Agriculture Organization of the United Nations (FAO-UN) declared soil a finite natural resource in the face of anthropogenic use, occupation, and exploitation. The importance of soil for maintaining life and other natural resources must be adequately addressed in the curricula for natural sciences and geography teaching. The National Common Curricular Base – BNCC, defined for elementary education in Brazil, and the Paulista Curriculum, specific for the state of São Paulo, imply that full training related to sustainability requires the student to have competencies intrinsic to the socio-environmental values development and decision. Resorting to field and laboratory activities on the soil in the classroom, it was possible to approach geoethics when discussing the valorisation of georesources. The educational actions involving the inseparability between human activity and the impacts on abiotic cycles were simultaneously addressed in line with the curricular recommendations (BNCC and Paulista Curriculum). Thus, using the natural resource soil, students participated in activities involving environmental awareness, water consumption/preservation, air pollution, waste reduction, and sustainable consumption, among others. This work addressed the teaching of the soil as an irreplaceable resource for protecting life and environmental balance in elementary school, resorting to geoethics as an approach that allowed the development of non-cognitive competencies related to the environment, such as reflection, awareness, and argumentation. The meaningful impact on students made it worth highlighting this geoethics approach to increase knowledge and develop competencies.

**Keywords:** environmental education; geoethics; geosciences; soil rational use.

## INTRODUCTION

Soil constitutes an intricate system through the atmosphere, cryosphere, geosphere, hydrosphere, and biosphere crossroads. This natural resource is vital for supporting societal and ecosystem services such as food production and ecosystem services

maintenance, and ultimately supporting life on Earth, being a keystone for sustainability [1,2]. Soil health is the ongoing capacity to operate as a crucial living ecosystem, supporting the well-being of biodiversity, including humans. Soils are essential in providing diverse ecosystem services (**Figure. 1**).

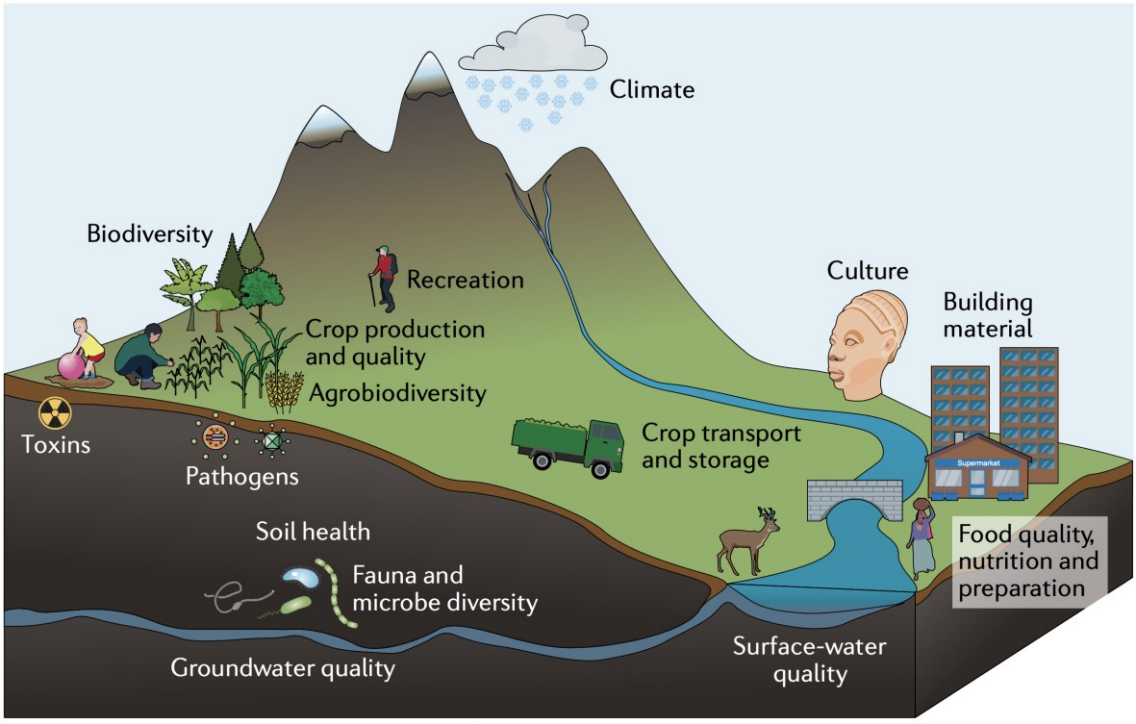



**Figure 1.** Soil-health and ecosystem services (Retrieved from Lehmann et al. [2], p.4).

Soil health and ecosystem services link agricultural and soil science, policymaking, stakeholder requirements, and sustainable supply chain management [2]. Consequently, effective soil-health management to support sustainability needs to address three critical considerations according to Lehmann et al. [2]: i) the recognition that enhancing multiple soil-ecosystem services requires a multifunctional approach; ii) the understanding that efforts to improve one soil service may lead to positive or adverse effects on another service; iii) the imperative that soil-health management should aim to sustain soil services over the long term. In this context, we highlight four primary soil-ecosystem services: sustainable plant production, water-quality control, human health advancement, and climate change mitigation.

In 1972, the Gaia hypothesis brought forward the perspective of Earth as a self-regulating system on a global scale, explicitly incorporating soil-ecosystem concepts and extending beyond soil services exclusively for human benefit [2,3]. Enhanced understanding of soil bio and geological processes has been made possible mainly due to substantial progress in analytical capabilities since the 1980s. Introduced in 2012, soil security addresses the necessity for equitable access to soil ecosystem services, treating it as a fundamental human right.

Establishing the United Nations' Sustainable Development Goals (SDGs) of the 2030 Agenda in 2015 underscored the necessity to harmonize soil functions with sustainability, making soil health an appropriate foundation [2]. In 2015, the FAO (UN

Food and Agriculture Foundation) declared that this natural resource is finite and conscientious care for this resource is needed [4]. Society, governments, and other bodies must discuss this natural resource. Since its degradation accelerates yearly, we witness environmental impacts and scarcity compromising its use for ecosystem services. Approximately 23% of the Earth's surface is already experiencing degradation, and the current estimated rate of arable land loss due to soil degradation is 30–35 times higher than the historical rate [5]. Consequently, soil security is frequently employed in policy discussions, considering human culture, capital, and legal dimensions of soil management. Significantly, soil security facilitates meaningful discussions regarding soil as a shared resource akin to water and [2]. The discussion on effective soil management can be enriched and deepened by integrating geoethics. This recent interdisciplinary scientific area enables a more profound reflection, harmonizing the consequences of human actions concerning the Earth system [6] and enabling soil degradation mitigation measures.

The geoethical and sustainable understanding of soil is a cornerstone for maintaining Earth's holistic and dynamic balance and securing the well-being of present and future generations. Geoethics emphasizes the importance of responsible stewardship of natural resources, such as soil [6]. Education can be a powerful tool for shaping a conscientious and informed society on this topic. Geosciences education could catalyze geoethical and sustainable soil use by fostering awareness and promoting responsible practices. Geosciences teaching, grounded on an ethical and sustainable approach, can empower individuals to actively preserve and enhance this critical natural resource. This awareness can begin in schools and extend to general society, encompassing several scientific areas such as geosciences, biology, and human health [7]. Also, sustainable soil governance is also critical to advancing several SDGs [2,7].

Promoting a solid and geoethical soil education at formal and non-formal levels holds significant potential to enhance the opportunity for an integrated perspective on environmental issues. A crucial objective in achieving high-quality education through a soil-focused approach is to improve citizens' awareness to recognize and value the correct use of this finite natural resource [7]. Thus, learning about soils can foster an increased sense of responsibility and appreciation of this natural resource as an integral component of our delicate Earth system.

From an educational standpoint, the present work intended to foster learning activities that address human actions and soil valorization from a geoethical view, promoting socio-environmental competencies linked to the soil and present in the National Common Curricular Base and Paulista Curriculum from Brazil [8,9].

## METHODOLOY

A soil competencies development program, based on geoethics and sustainability, was developed based on the action-research methodology. Tests and discussion/debate sessions were conducted to evaluate participants' knowledge development.

The activities developed were conducted at a public school in São Paulo, Brazil, with 2$^{nd}$ to 9$^{th}$-grade elementary and 1$^{st}$-grade high school students. From 2021 to the present day, the proposed class program promoted inseparable, complex, holistic learning of soil and human interactions, focusing on geoethics and sustainability, thought

laboratory, field, and theoretical activities. The soil sustainability education program, focusing on geoethics, included a school community with more than 2500 active students, the largest state school in São Paulo. The work sought to develop the following objectives: i) recognize the importance of soil for life, environmental balance, and maintenance of georesources based on socio-environmental competencies based on geoethics; ii) introduce issues regarding local pollution and develop competencies related to soil value; iii) act in the culture of local soil pollution and contaminations with problem-solving proposals in which students playing an active role.

To achieve the mentioned objectives, several initiatives were carried out:

- Theoretical classes: to promote student learning on soil recognition beyond the "land or floor" concepts (**Figure 2**).



**Figure 2**. Theoretical classes on soil: origin, resource, and impacts.

- Field classes: to recognize the soil types in the school surroundings and study the human impacts, such as pollution.
- Laboratory classes: to recognize the origin of the soil, texture, color, types, and functions, among other characteristics (**Figure 3**).



**Figure 3**. Laboratory classes about the soil.

- Multiplier students: After completing the learning program on the soil, the 6th to 9th-grade elementary school students and 1st-grade high school students assisted students from the 2nd to 5th grade of elementary school (**Figure 4**).



**Figure 4**. Multiplier students assisting younger learners.

- Final debate session: supported by the research teacher and after learning to recognize the soil value, a debating circle was held to discuss soil pollution at school. Actions and projects were created for the preservation, pollution reduction, and discussion on global warming, among other issues.

It is worth highlighting that with the support of a PhD research on the Earth Sciences Program at the University of Campinas (Brazil) and aims to develop geoethical and environmental awareness in which “humanity is nature with nature”.

## RESULTS AND DISCUSSION

The educational activities described in this study have demonstrated significant success across multiple dimensions, such as:

i) Environmental awareness development:

The educational activities have effectively contributed to developing environmental awareness, particularly concerning soil ecosystems. Participants showed an increased understanding of the importance of soil health, biodiversity, and the broader environmental implications of human actions [10].

ii) Hands-on field and laboratory activities:

Incorporating field and laboratory activities proved essential in providing participants with practical environmental insights into soil science [11]. Field activities allowed for direct observation and interaction with soil ecosystems, while laboratory work enhanced analytical and investigative competencies and more profound knowledge of soil properties [12].

iii) Active involvement of multiplier students:

The engagement of multiplier students played a crucial role in the success of the educational activities developed. These students, acting as catalysts, actively shared knowledge and skills achieved from their participation in this PhD program with a broader audience, involving the surrounding community [13]. Their involvement effectively translated theoretical and geoethical knowledge into practical solutions, raising public awareness about the local problems concerning soil health and how to improve human-soil-related actions.

iv) Problem-solving competencies enhancement:

The educational activities were structured to promote critical and systemic thinking and problem-solving competencies. Participants, including multiplier students, demonstrated an improved ability to address challenges related to soil management and environmental sustainability. This enhanced capability indicates the PhD program's success in fostering practical and problem-solving skills among participants [14].

v) Transformation of idle spaces and waste deposits:

The overarching goal of this PhD research was to repurpose idle spaces and waste deposits into authentic educational environments (**Figure 5**).




**Figure 5**. Students transforming idle spaces into immersive educational spaces.

This research showcases the potential for transforming neglected areas into hubs for learning and developing ethical and sustainable thinking. This not only addresses issues related to soil/environmental degradation but also creates valuable spaces for educational engagement [15].

## CONCLUSION

Converting idle spaces into educational environments aligns seamlessly, intending to foster a geoethical and sustainable awareness among this study's participants. The ambition to repurpose neglected areas represents a significant stride towards creating immersive educational spaces that deepen individuals' connection with the natural world and instill a commitment to sustainable and geoethical behaviors. The geoethical reflections on soil governance tailored an imperative and comprehensive sustainability education program to this crucial natural resource. While the scope of this research is currently limited to a single school in São Paulo, Brazil, this study reimagines

educational strategies, mainly through innovative mind and hands-on laboratory and field classes that emphasize soil and socio-environmental competencies, such as environmental insight competency. The presented approach aims to nurture a high standard of environmental education, setting the stage for broader educational and research applications in the future.

The educational activities implemented in this research are noteworthy as key achievements that include the development of heightened environmental awareness stemming from soil-centric engaging activities. The active involvement of students has proven influential in solving environmental problems, developing practical skills, and communicating knowledge effectively based on geoethical and sustainable soil use. This ambitious goal addresses the critical issue of environmental degradation and embodies a commitment to reshaping educational landscapes. In essence, this research indicates the transformative power of education in cultivating environmental awareness and envisioning and actively working towards a more sustainable and prosperous future. As the effects of this research extend beyond the confines of a single school, its potential impact on shaping the future of elementary sustainability education is promising.

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# IDENTIFICATION OF URBAN LANDSCAPE UNITS BASED ON SENTINEL-2A IMAGERY AND ILLEGAL LANDFILLS: CASE STUDY FROM VELINGRAD CITY (BULGARIA)

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## ABSTRACT

The variety of urban landscapes, especially of urban green spaces provide a wide range of human well-being opportunities, physical recreation, sport activities, and urban heat mitigation. Therefore, there are many studies applying remote sensing and GIS methods, which were related to the sustainable city development focused on the "green network", "green spaces" and land use. Conversely, illegal landfills in and around settlements and rivers have a negative anthropogenic impact on the environment. Therefore, their study is crucial for sustainable planning and landscaping. In this work, the study on urban landscape units and illegal landfills in the Velingrad City, Bulgaria is presented.

The following main urban landscape units (ULU) were identified using remote sensing and GIS techniques for calculation of several vegetation and built-up indices from Sentinel 2A satellite imagery (Date: 2023-02-14) and Mean Shift segmentation: 1. Green areas; 2. Residential area/ Impervious surface; 3. Industrial area; 4. Stadium; 5. Broad-leaved forest; 7. Coniferous forest; 7. Mixed forest; 8. Grass/ Pasture/ Shrub; 9. Arable land; 10. Complex cultivation patterns/ significant areas of natural vegetation; 11. Water body/ River Bank Trees; 12. Lake, and 13. Bare Earth/ Paths.

The illegal dumps have been investigated in the summer of 2023. The results show that the main types of unregulated landfills in the city of Velingrad are household, construction/ industrial and plastic waste. They are distributed in the river and the riverside, near construction areas, hotels and restaurants, as well as in Lake Kleptuza, and most of them can be cleaned, re-cultivated and landscaped.

The methodological approach and obtained results in this work will be further used to elaborate models of anthropogenic impact at river basin scale.

**Keywords:** urban landscape units, illegal landfills, Sentinel 2A, remote sensing, Geographic Information Systems

## INTRODUCTION

The variety of urban landscapes provide a wide range of human well-being, sport and recreation opportunities. Therefore, the study of the urban land green spaces and land cover is crucial for sustainable landscaping and urban planning, as well as for the human quality of life in the cities. In the last decades there are many studies based partially on use of remote sensing and GIS techniques and methods, which are related to the sustainable development of the urban areas such as “green network”, “green ways”, “green cities” [1-3] and “green spaces” [4-5]. Other works are focused on identifying the most appropriate assessment methods used in researching ecosystem service regulation in the context of urban planning and on empirical analysis of how green, resilient, and inclusive cities are in a climate change conditions, e.g. [6-7]. Remote sensing data is being widely used for mapping and land use/ land cover classification also in the urban areas based mostly on vegetation and built-up indices, e.g., [4, 8-9], and others.

Unlike urban green spaces, which benefit people's well-being and provide opportunities for sports and recreation, illegal landfills in and around settlements and rivers have a negative anthropogenic impact on the people’s living environment not only in Europe, incl. Bulgaria, but also in the others regions and countries around the world, e.g. Asia and Pacific, North and Latin America, Caribbean, and Africa [10]. The problem is global and is an object of many studies, applying a set of indicators, remote sensing and GIS, as well as methodologies for sustainable reclamation of landfills [11-13].

The leading objective of this study is to identify the main urban landscape units (ULU) in the Velingrad city, Bulgaria, as well as the land use/ land cover in the surrounding area in order to find the mostly appropriate methodological approach that best represents the current land surface and help the categorization of the urban green architecture. The obtained results and the unauthorized landfills are analysed in an integral way to provide information about areas that are subjected to anthropogenic pressure and in the long term could be re-cultivated and transformed into green spaces.

## MATERIALS AND METHODS

### STUDY AREA

The city of Velingrad (N 42.027772, E 23.995328) with is surrounding area is located in the Chepinska Valley (Western Rhodopes) and belongs to the Pazardzhik District – Velingrad Municipality. It covers an area of 32.0 $km^2$ with an average altitude of 833.1 m. The Chepinska River flows through the city with its tributaries Matnica, Elenka, Lukovitsa, and Katarov dol (Fig. 1).

The study area is a part of the Moravian-Rhodope zone and more specifically in the Rila-Rhodope tectonic unit (parts of the Western Rhodopes Zone) [14] and characterized by various geological settings with Precambrian high-grade metamorphic rocks, Paleozoic medium-grained biotite granites, Late Cretaceous aplitoid-pegmatoid granites, Neogene sediments and Quaternary alluvial, deluvial, proluvial deposits – boulders, gravels, sands, and clays. The specific intertwining of metamorphic rocks and limestones has influenced the formation of a characteristic karst complex with a karst field around the city of Velingrad and numerous karst springs, e.g. Kleptuza (580 − 1160 l/s) [15]. The investigated area is characterized by four hydrothermal deposits –

Chepino, Ladzhene, Kamenitsa and Draginovo with total flow rate of the mineral water amounts to about 175 l/sec with an average temperature of about 60°C [15]. Together with the transitional continental climate [16] they have contributed to the development of the economic activities, SPA and balneotherapy and tourism in the city.

The architectural structure of the city is characterized by significant fragmentation and spatial diversification of various land use types. It comprises mainly of individual houses, small and medium-sized buildings, industrial/ commercial areas, as well as of green spaces e.g., parks, small house and kinder gardens, sport grounds, and small and medium-size forest areas.

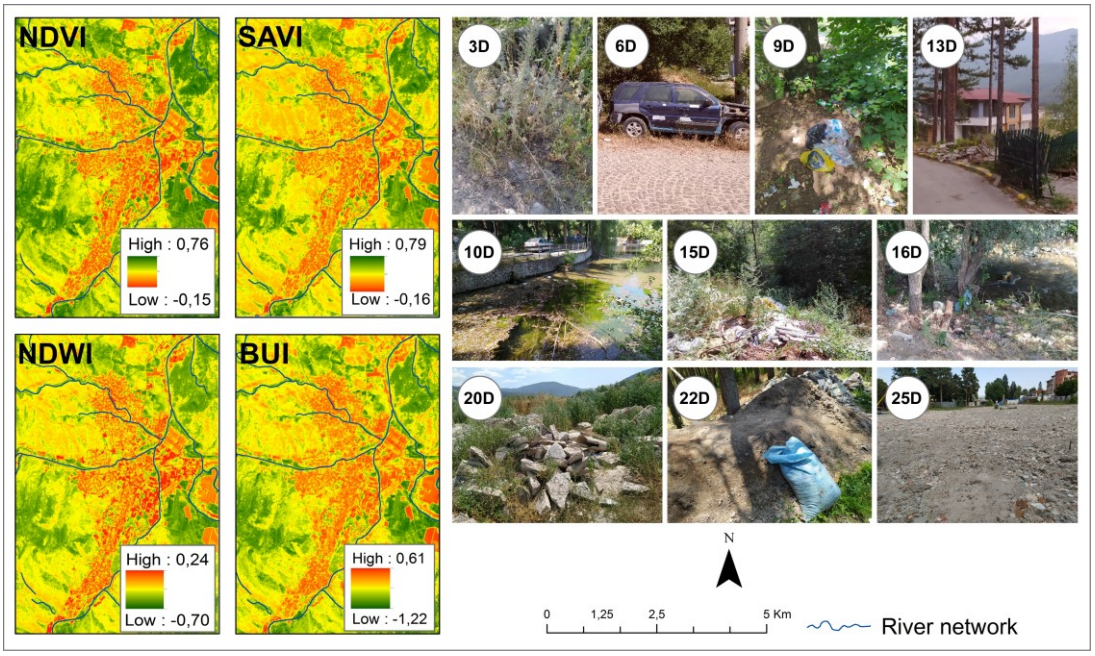



Fig. 1. The calculated vegetation, water, and built-up indexes NDVI, SAVI, NDWI, and BUI and some illegal dumps in the city of Velingrad

*Legend – landfill types: 3D – tire; 6D – abandoned car; 9D, 15D and 16D – household waste; 10D – plastic waste; 13D, 20D, 22D, and 25D – construction waste*

The solid waste in the city of Velingrad has the following origin: households, construction, industrial, and tourist sites, sanatoriums, hotels, administrative and public units. They are deposited at the regional depot in the village of Aleko Konstantinovo outside the city. It is planned a construction of pre-treatment facility for mixed household waste and a plant for composting green waste for the municipalities of Velingrad and Rakitovo, which will be for a great ecological importance [15].

## DATA

For the purposes of the present work, the following satellite data was used: standard Sentinel-2 Level 2A (Atmospherically corrected Surface Reflectance in cartographic geometry) optical satellite imagery with at high resolution level 10 m was used in this study. This product is available in 100 x 100 $m^2$ tiles in UTM/WGS84 projection and was obtained from Copernicus Open Access Hub (https://scihub.copernicus.eu/dhus/#/home). It contains spectral bands, as well as

Bottom-Of-Atmosphere (BOA) reflectance in cartographic geometry, an Aerosol Optical Thickness (AOT), a Water Vapour (WV) map, and a Scene Classification Map (SCM) together with Quality Indicators (QI) for cloud and snow probabilities at 60 m resolution. The Sentinel-2A image used in this work has the following main characteristics: date – 2023-02-14, MultiSpectral Instrument (MSI) sensor, cloud cover percentage 1.18, and is in format SAFE (Sentinel online: Accessed at 1st Aug. 2023: https://sentinels.copernicus.eu/web/sentinel/user-guides/sentinel-2-msi/processing-levels/level-2).

The unauthorized landfills have been investigated on the basis on data obtained from own mapping conducted in the summer of 2023 (Fig. 1 and 2).

## METHODS

The pre-processing and resampling to 10 m was done with SNAP software v. 9.0 (ESA: Accessed at 1st Aug. 2023: https://step.esa.int/main/download/snap-download/).

The described by [8], [17], and [18] vegetation, water and built-up indices were performed from the Sentinel 2A data using QGIS Software: Normalized Difference Vegetation Index (NDVI), Soil-adjusted Vegetation Index (SAVI), Normalized Difference Water Index (NDWI), and Built-up Index (BUI) (Fig. 1).

In a next step the Mean Shift Segmentation was performed [19]. The obtained result was vectorized to perform an object-based identification, as well as an expert knowledge-based classification based on field work, ESRI, Maxar, and Google Earth Engine data.

The investigated unauthorized landfills were converted as point layer in ESRI Shape format and classified according to their generation sources and constituent components as household, construction/ industrial, and plastic dumps, tires and abandoned (dumped) cars (Fig. 1 and 2).

## RESULTS

The typical waste deposited in the dumping sites in the city of Velingrad includes textiles, plastics, paper, construction materials, tires, and abandoned cars. The most of them are located in the residental area and have been investigated on the basis on data obtained from own mapping conducted in July 2023. Some examples are illustrated in Figures 1 and 2.

The calculated indices NDVI, SAVI, NDWI, and BUI are presented in Figure 1. The image correlation matrix between them is shows that NDVI and BUI have the best correlation which other, followed by NDVI – NDWI, and SAVI – NDWI (Table 1). Although these indices represent variations of the basic land cover units in the study area compared to the original land surface from large-scale Google Earth data, these results show that it is difficult to automatically extract built-up, arable land and bare-earth areas separately.

As shown in Figure 2 (A) the Mean Shift Segmentation (MSS) with spectral detail 20 delivers better results. It has 191538 segments, which were grouped into 13 land use/ land cover classes through knowledge- and object-based identification (Figure 2, B, C,

and D). The result shows that the main green spaces in the residential and impervious areas of the Velingrad City are small-size coniferous, broad-leaved and mixed forest, parks, house gardens, and stadium.

Table 1. Correlation matrix between the NDVI, SAVI, NDWI, and BUI indices

| | NDVI | SAVI | NDWI | BUI |
|---|---|---|---|---|
| **NDVI** | 1,0000 | | | |
| **SAVI** | 0,9834 | 1,0000 | | |
| **NDWI** | **0,9815** | **0,9758** | 1,0000 | |
| **BUI** | **0,9844** | 0,9743 | 0,9588 | 1,0000 |

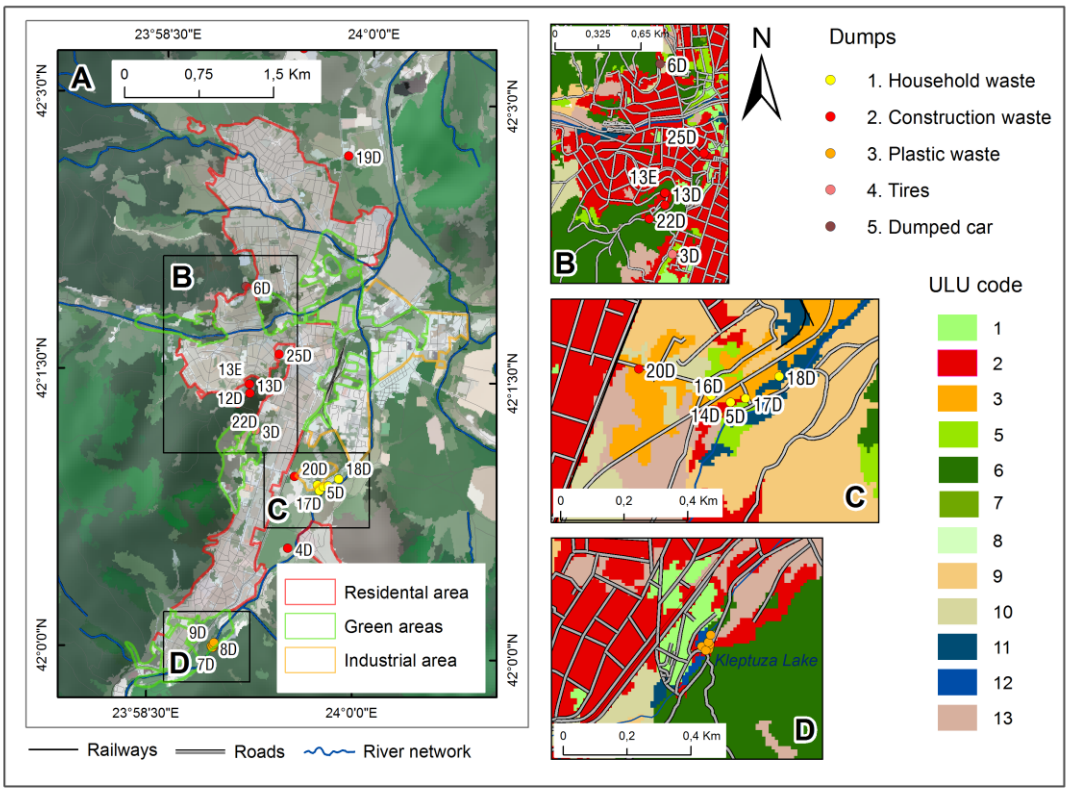



Fig. 2. Urban landscape units: **A.** ULU extracted using Mean Shift Segmentation (MSS); **B-C-D.** ULU identified on the basis on MSS and knowledge based approach

*Legend – ULU code in this figure: 1. Green areas; 2. Residential area/ Impervious surface; 3. Industrial area; 5. Broad-leaved forest; 7. Coniferous forest; 7. Mixed forest; 8. Grass/ Pasture/ Scrub; 9. Arable land; 10. Complex cultivation patterns/ significant areas of natural vegetation; 11. Water body/ River Bank Trees; 12. Lake, and 13. Bare-Earth/ Paths.*

An integral analysis of the ULU-land use/ land cover map and the unauthorized dumps shows that the household waste is deposed mostly in the river bank areas, where the

construction and plastic dumps were generated mostly near the newer buildings and commercial units like hotel Maxi and residential buildings, the construction company "Metalica" Ltd., as well as touristic sites, e.g. the Kleptuza Lake and hotels "Maxi Park Hotel and SPA" and "Panorama" (Fig. 2).

Their constituent components are mostly residential and construction materials, plastic, paper, metals, and wood and therefore could be cleaned and the dumping sites – re-cultivated and landscaped.

## CONCLUSION

Although the calculated vegetation, water and built-up indices – NDVI, SAVI, NDWI, and BUI indicate the main thematic classes built-up, non-tree covered, and forest areas, they don't represent all the classes identified in this study.

Unlike them, the object-based and expert knowledge-based methods using as basis the performed Mean Shift Segmentation of the of the S2A band set B04-B03-B02 – Natural Color deliver the best classification method for recognizing and identification of ULU and land use/ land cover in the city of Velingrad. The following main ULU and land use/land cover type in the city's surrounding area were identified using Sentinel 2A satellite imagery (Date: 2023-02-14) and GIS techniques: 1. Green areas; 2. Residential area/ Impervious surface; 3. Industrial area; 4. Stadium; 5. Broad-leaved forest; 7. Coniferous forest; 7. Mixed forest; 8. Grass/ Pasture/ Shrub; 9. Arable land; 10. Complex cultivation patterns/ significant areas of natural vegetation; 11. Water body/ River Bank Trees; 12. Lake, and 13 Bare-Earth/ Paths.

The green spaces in the residential part of the city of Velingrad are parks, small house gardens, sport grounds, incl. stadium, and small- and medium sized mostly coniferous forest areas.

In this work the investigated unauthorized landfills lack the waste pre-treatment in the study area and according their generation sources were grouped as household, construction/ industrial and plastic waste, tires, and abandoned cars. They were mostly distributed in the river and the riverside, near construction areas, hotels and restaurants, administration and public authorities, as well as in the area of the lake "Kleptuza". According to their origin and constituent components most of them can be cleaned, re-cultivated and landscaped.

The methodological approach and the obtained results in this work will be further used to elaborate models of anthropogenic impact at river basin scale on example of the Chepinska and Chepelarska River Basins (Rhodopes).

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# LAND MANAGEMENT FOR INCREASING OPERATIONAL EFFICIENCY OF DISTRIBUTION ELECTRIC NETWORK COMPANY 'SADALES TĪKLS' IN LATVIA

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## ABSTRACT

In order to reduce the costs of AS ´Sadales tīkls', the largest distribution electric network company in Latvia, within the framework of the work efficiency improvement program, the facilities of the support bases where production resources were concentrated - people, equipment and materials, and the geographical location of real estate - were analyzed. A real estate survey of the company's support bases was conducted and the efficiency of use was assessed. The geospatial analysis included the distance and time on the way from the support base to the repair facilities, the risk of damage to the distribution network. As a result, the optimal geographical location of support base objects in Latvia was determined and the number of real estate properties was reduced as a result of the study. At the same time, the company's performance in preventing damage in the event of a power failure was maintained.

**Keywords:** land management, efficiency, distribution electric networks.

## INTRODUCTION

As a natural monopoly, distribution electric network companies have an obligation to the state and society to increase their operational efficiency in order to ensure competitive economic development and citizens to receive high-quality electricity supply services at the lowest possible price. The studies so far have looked at the operational efficiency of distribution network companies, focusing mainly on the modernization of technological processes. Due to the impact of climate change, attention is paid to access to distribution networks and connection fees, taking into account the rapid development of electricity generation with renewable energy sources (Wainer & Petrovics N.van der Grijp, 2022)[17]. A weatherproof network is considered by converting overhead lines to underground cable lines (Saastamoinen & Kuosmanen, 2014)[16]. An independent area of research is investment planning, the impact of electricity production with renewable energy resources on the operating costs of the distribution network (Noltinga *et al*., 2019; Vesterberg, Zhou, Lungren, 2021)[14,18]. Over time, the analysis of total operating costs and the possibilities of creating new network connections have not lost their relevance (Filipini, Hrovatin, Zoriča 2004; Wainer, Petroics, Van der Grijp., 2022)[8,17]. However, even in countries with developed economies, such as Australia, studies show that 70% of distribution network companies have reserves for increasing efficiency (Boon et. al.,

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2021)[4]. In connection with the use of land in energy, the relevance is the interconnection of electrical networks, the selection of sites for power plants that produce electricity with renewable energy resources (Guillot *et al*., 2023; Linberg, Birging, Widen, 2021)[9,12], the planning of the expansion of the distribution network (Bosio *et al*., 2021)[6]. The authors of the article have not been able to find research on the real estate management of distribution network companies, which is not the company's core business, but is nevertheless a reserve for increasing efficiency. The purpose of the article is to present a study on the implementation of the project of reducing the number of support objects in Latvia, which was one of the sub-tasks of the company's efficiency improvement program. The task of the article is to show the effect of cross-sectoral cooperation in cases where the company's activities are related to land and real estate. Distribution electricity networks in Latvia are maintained and developed by the state company AS ´Sadales tīkls´, hereinafter referred to as the Company, providing service to 790,000 customers (Sadales tīkls, 2022)[20]. The company's operations cover 99% of Latvia's national territory in an area of 63,943 $km^2$. Land management as a comprehensive concept in the article is related to the evaluation of the efficiency of the company's real estate use and the subsequent measures taken to reduce operating costs. Land management in Latvia is seen as the rational placement of infrastructure (Auziņš, 2016)[2], but there are no studies on the effective use of real estate in electricity supply companies in Latvia. The importance of the research is indicated by the fact that the population in Latvia has decreased by 21.9% over the last 25 years (Official statistic of Latvia, population)[19], so each user must bear a greater amount of the comparative costs of infrastructure maintenance. Before the implementation of the project, the company managed 5262 land units and 5713 buildings and structures, which can be divided into 2 groups differing in meaning: Support base facilities - units of land for placement of personnel, equipment and materials. Technical objects – land units for the placement of transformer substation structures. It is emphasized that due to the natural nature of monopoly, competition is impossible. The interests of society to receive services at affordable prices and the development of the distribution network are contradictory (Ajodhia & Hacwort, 2005)[1]. When looking at the literature on distribution network maintenance issues in Latvia, there is no lack of information that determines the safe operation of the network (Budahs & Zviedrītis, 2012)[7], but there is a lack of information on solving land management issues. In energy, land units, buildings and structures are one of the 3 main groups of fixed assets. The company's total number of support bases was 73. Historically, they were created with the following structure: Regional base 14% of the total, department base 46% of the total, Precinct base 40% of the total. The classic steps of the land management process (Larsson, 1997; Auziņš, 2008)[11,3] were applied to the evaluation of the usable efficiency of the distribution network Support objects as follows: The need to reduce the company's operating costs. The overall goal was to reduce the number and cost of real estate. The task was to analyze the number and geographical location of real estate, justify and implement a liquidation of the Support bases. In order to get an overall view of the use of objects, a 100% survey of all objects was carried out. The results of the survey indicated that only 22% of the number of support bases are used effectively, unused premises were in 29% of objects, unused territories were in 23% of objects, and in 18% of objects both premises and territory were unused. This would be feasible if the fault repair time of power grids would not be increased and the performance coefficients – System Average Interruption Duration Index (SAIDI) and System Average Interruption Frequency Index (SAIFI) of the distribution grid would not deteriorate. The coefficient

SAIFI stands for the index of the average frequency of electricity supply interruptions. In the SAIFI coefficient is determined by dividing the total number of power supply interruptions of customers by the total number of customers. On the other hand, the SAIDI coefficient determines the duration of the average outage of the company's customer, which shows the technical quality of the network. The main factor for the location of the support bases is the distance to the network objects, in order to ensure the fastest possible repair of the damage. In land management in Latvia, the location of objects has so far been viewed more in relation to the location of land areas in relation to production centers (Locmers, 1974)[13] and the effect of distance on economic activity (Boruks, 2001)[5]. In order to get a visual impression of the coverage of the territories of the Support Base crews in the territory of Latvia, the operational territories of the bases within a radius of 30 km were marked on the cartographic material (Figure 1).




**Figure 1.** Areas of operation of support objects without Riga city bases, radius R = 30 km. Note: Blue color - the base of the department. Green – the base of the precinct.

In order to solve the task, a geospatial survey of the Support Bases had to be carried out in 2 steps. First, the development of a pilot project, simulating the scenarios of the liquidation of individual support bases, and then, in the 2nd step, looking at the entire territory of Latvia.

**Materials and Methods**

The study used descriptive, pilot and optimization research methods. The following data were used to solve the task.

Data of the company ’Sadales tīkls’:

- Addresses of support bases;
- Spatial data of the location of technical objects (TO);
- Spatial data of customer locations;
- Spatial data of substation locations;

In the analysis of geospatial data ´Mappost´, Ltd. information on road network coverage data of Latvia was used, as well as experience in optimal route planning. The company

has geospatial information about the road network, which is classified by road importance, maintenance class, surface, permitted speed. Based on long-term data accumulation and experience, possible movement speeds (km $h^{-1}$) in 3 variations: Tractor in winter and summer (v= 20 to 28 km $h^{-1}$), light car summer (v= 20 to 85 km $h^{-1}$), light car in winter (v=13 to 85 km $h^{-1}$).

Conditions for evaluation of pilot project support bases:

- Number of bases from 3 to 7 pcs.;
- Number of Network technical objects (TO) 7099 pcs;
- The research area is 'isolated' from neighboring areas;
- Resources are unlimited;
- 3 inviolable bases – current department centers (Jēkabpils, Preiļi, Aizkraukle); Untouchable objects: <60 min away from the base (for the tractor); 30 min away from the base (car);
- Distance time from base to the fastest TO reached (minutes).

Proposed indicators:

- Proportion of distant TO in risk sum (%): shows the distribution of total risk in time zones;
- Average distance (minutes): average time spent on the road to the number of objects closest to each base. The drawing shows the influence of the river as an obstacle on the travel time (Figure 2);
- Proportion of distant high-risk TO in the amount of time (%): shows how much burden can be caused by TOs where the probability of work is higher;
- A very high value of the indicator shows a potentially high time consumption on the way to the actual execution of the work.
- A very low value of the indicator shows an oversaturation of the number of bases.

Based on the damage analysis of the electrical network, it was assumed that the risk of damage to overhead line objects is 4.5 times higher than the risk of damage to other objects. The risk of damage to the rest of the network was determined by the number 1.

$$\boldsymbol{R = \frac{A \cdot 4{,}5 + B}{A + B}} \qquad (1)$$

R – weighted average damage risk;
A – number of objects of repair overhead lines, pcs;
B – number of other types repair objects, pcs.

In the territory of the pilot project, GVL TO was 3286 units, and other TO 3813 units. The total amount of risk for 7099 objects of the pilot project was 18600, but the weighted average damage risk R=2.5458 for each object, taking into account the risk weight of the overhead line object and the rest of the object.

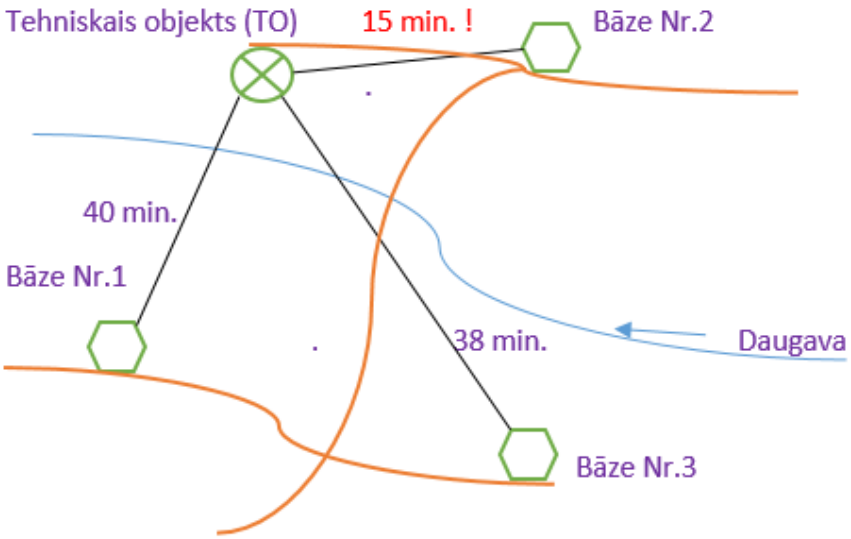



**Figure 2.** Time in minutes from the base to the technical object (TO). The blue line is the Daugava. The brown line is the road.

**Results and Discussion**

An analysis of 16 different base structure scenarios was performed, with the tractor as the slowest but most significant vehicle travel time to the point of failure.

All 16 scenarios were analyzed, but scenario S-1 (Figure 3) with all the support bases of the pilot project and scenario S-16 (Figure 4), in which only Aizkraukles, Jēkabpils and Preiļu divisions remain, were taken for comparison. It can be seen that in the case of S-16, relatively many distant high-risk objects remain in the Southern part of the territory. This means that in the case of scenario S-1, the Aknīste station must be left in operation in order to reduce the travel time to network objects. The distance to the objects is increased by the factor of the river Daugava, which can be crossed only in two places near Aizkraukle and Krustpils.

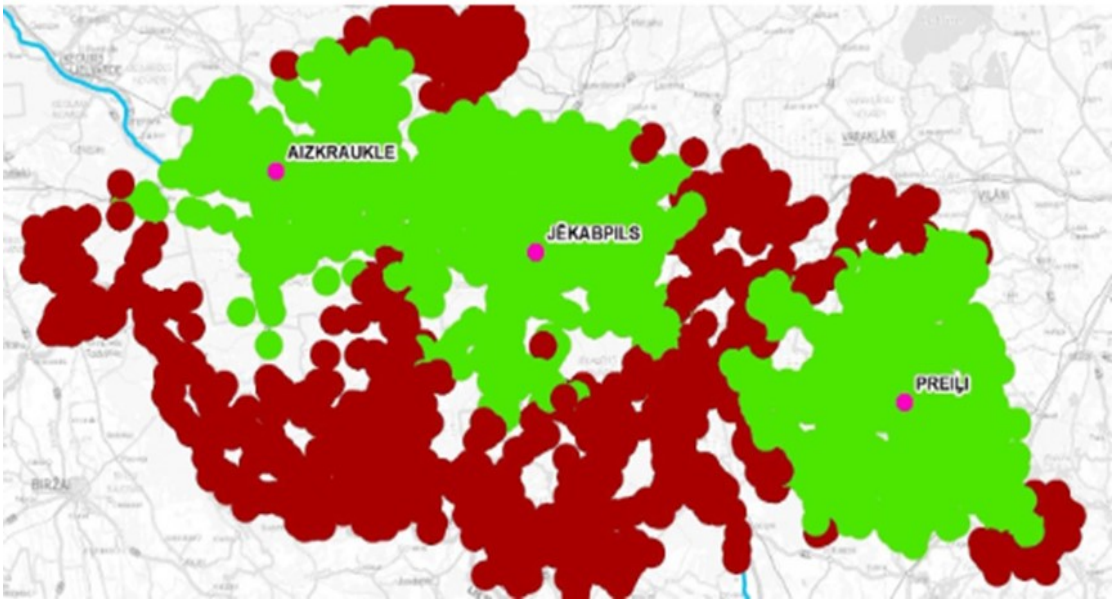



**Figure 3.** Scenario S-1. Aizkraukles, Jēkabpils, Preiļu, Aknīste, Līvāni, Pļaviņas. Note: Brown objects where the travel time to the place of damage t > 60 min.

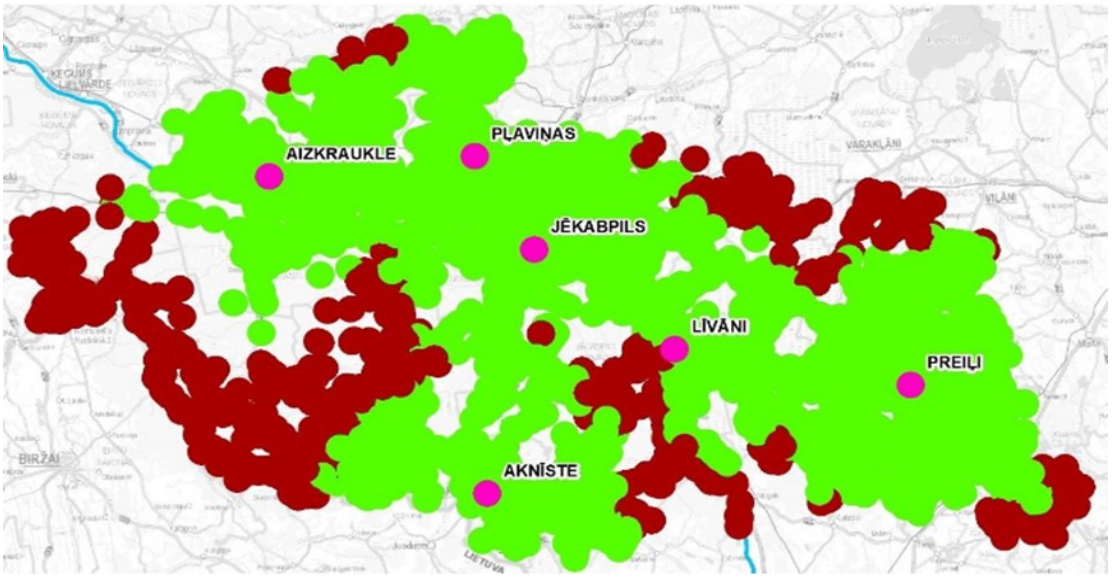



**Figure 4**. Scenario S-16 Aizkraukles, Jēkabpils, Preiļu divisions. Note: Brown objects where the travel time to the place of damage t > 60 min.

Table 1 shows the indicators to be analyzed for scenarios S–1 and S–16 of the location of support bases and their deviations, which allow determining the influence of the Daugava river on the possible damage risk and time on the way to the damage site. It can be seen that the influence of the location of overhead lines as distant high-risk objects will not be confirmed. The results indicate that the objects of overhead lines are not located further than the other objects of the electrical network and the value of the indicator even decreases by 4.1% in case of scenario S-16.

**Table 1.** Changes in the evaluation indicators of the optimal placement of support bases

| Number of support bases scenario | The proportion of distant objects in the risk sum, t > 60 min, % | Average time to get to object t, min | The number of distant high-risk objects in the sum of time, % |
|---|---|---|---|
| S – 1 | 21.0 | 38,0 | 36.0 |
| S – 16 | 39.0 | 50.5 | 31.9 |
| Δ S16 - S1 | + 18.0 | + 12.5 | - 4.1 |

In the course of the pilot project, after evaluating the scenarios of the placement of various support bases, the following optimal values for the placement of the support bases of distribution electric networks were proposed:

- Proportion of distant TO in the risk amount: 18–33%;
- Average time to the object: 35–45 min;
- Proportion of high-risk TO in the amount of time: 15–30 %.

The conclusions about the indicator values are as follows. The optimal values were deliberately chosen in a wide interval, since the indicators were based only on calculations for this particular pilot project model. In practice, the situation of each support base should be looked at individually. An indicator interval that is too narrow would allow more support bases to be closed, but would increase the time to repair a fault, which is a measure of the efficiency of electrical grids. Further research would be necessary so that

the indicator values could be considered as valid for the analysis of the geographical location of the support bases of other distribution network companies. Looking at the studies of the geospatial analysis of distribution network objects, the authors of the article did not find a similar approach, because currently in the geospatial analysis of electrical networks, the choice of possible locations for renewable energy power plants is relevant (Lindberg *et al*, 2021; Patanhar *et al*., 2023)[12,15]. In the case of Latvia, our research also had its own advantage, because its territory is served by one main operator of the distribution system and the economic effect of the implementation of the cost reduction project is felt by the entire population of the country. In countries with a larger territory, for example, in Sweden, 175 distribution system operators work (Johansson, Vendel, Nuur, 2021)[10], which would fragment the economic effect of a similar study. According to the developed methodology, an evaluation of all support bases in the territory of Latvia was carried out, but with one difference. As an additional layer, the places of high electricity consumption were added, which are highlighted in green in Figure 5. As a result, the optimal solution in the territory of Latvia would be 29 pcs. support bases. Of them, 27 pcs. would be located outside the capital Riga and 2 pcs. the city of Riga, one of which would be the company's management base and the other the central warehouse base.. In Figure 5, the white areas mean that there is a small number of users and the density of networks, so the company will not have problems in case of network damage, even though the average time spent on the road to the place of damage will be higher than indicated by the indicators.

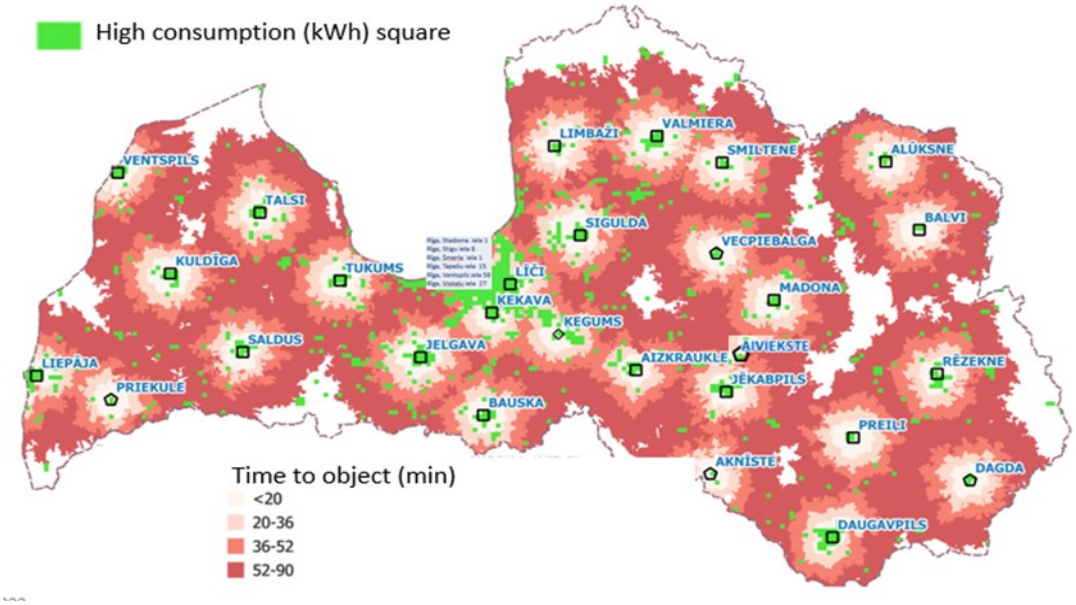



**Figure 5**. Scheme of optimal placement of support bases in Latvia.

Values of indicators of optimal location of support bases in the territory of Latvia:

- Proportion of distant TO in the risk amount: 35%;
- Average time to the object: 49 min;
- Proportion of high-risk TO in the amount of time: 36 %.

As a result of the simulation, a decision was made that the company can reduce the number of Support bases by 46%, remaining in 34 geographical locations, while the repair construction personnel remain in 29 locations. Geospatial analysis showed that a new

base location in Ķegums is needed. The base's proximity to high consumption areas was assessed as an additional important factor. In order to improve the company's performance in damage prevention, the possibility of separating 2000-5000 $m^2$ of areas for the placement of damage prevention materials from the territories of the liberated bases was considered during the design. By implementing the analysis of land management and geospatial location of objects, the company reduced the number of land units required for the number of support bases by 50.5% (from 101 to 50 land units), and the number of buildings by 35.2% (from 389 to 252 buildings), thus significantly reducing real estate maintenance cost. During the implementation of the project, the safety of electricity supply to customers was preserved, because the average electricity supply interruption duration index for one customer in 2021 was 208 minutes (SAIDI index) and the average number of electricity supply interruptions per year for one customer (SAIFI index) coefficient 2.3 times, have continued to decrease since 2017 by 53 minutes and 0.5 times, respectively. Land management processes contribute to increasing the overall efficiency of the company's economic activity, and as a result, the company was able to reduce the cost of its services by an average of 5.5%.

## CONCLUSIONS

1. By evaluating the land management and geospatial analysis of the support bases of the distribution electric network company, it was possible to reduce their number by 50%.
2. The reduction in the number of support bases did not affect the SAIDI and SAIFI indices characterizing the efficiency of the company's economic activity.

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# LOCATION INTELLIGENCE APPLYING ODDS FOR SHAPING SUSTAINABLE SMART CITIES

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## ABSTRACT

By steady scientific research carried out in the last decades was possible to register wanted technological advance oriented also to succeed in shaping sustainable smart cities. Nowadays several cities on European level and not only are starting the implementation of smart city design strategies, which are foremostly developed by access to needed databases as well as by specific approaches related to location intelligence supporting by this shaping appropriate policies on local level. Smart cities imply effective integration of physical, digital, and human systems to ensure a wanted sustainable, and prosperous future for their citizens. Location intelligence represents the ability to visualize spatial data to identify and analyze relationships and trends on local level, answering questions regarding available infrastructure, as well as roads with highest priority for maintenance by minimizing potential traffic disruptions. The more location intelligence offers diverse odds for an efficient urban planning by considering precise historical and present traffic patterns with the goal of designing short- and long-term urban development strategies for the benefit of citizens. The five Green Initiatives for Location Intelligence will be presented as well as a case study as an application example of applying location intelligence in a Romanian city with the goal of assuring best strategies for shaping sustainable smart cities.

**Keywords:** location intelligence, sustainable cities, infrastructure, traffic, electric vehicles

## INTRODUCTION

It is an uncontroversial fact that applying current and historical data on location movements allows town planners to understand past events as well as present challenges. A direct consequence of this action is the trying of city planners to design flexible and efficient cities by respecting sustainability requests, in other words to try shaping currently most debated sustainable smart cities [8, 12].

After the release of the Brundtland Report in 1987 defining for the first time the concept of sustainable development [5], sustained debates followed 1992 during the Conference for Environment and Development in Rio de Janeiro, that ended with adopting the much discussed document called „Agenda 21" [3, 6]. As a direct result of these events, it became more than clear that there is a need to simultaneously consider several aspects for successfully succeeding to operationalize the concept of sustainable development on

different levels, as on global, national, regional as well as on local level [6, 11]. The attempt is to work out best strategies to assure local sustainability by considering several aspects related to technological, economic, and social activities on a regional level [10, 13].

## MATERIAL AND METHODS

In the worldwide debates regarding currently existing possibilities for assuring the sustainability of our human society it has been several times emphasized the main role played by shaping on a local level cost and energy friendly housing alternatives. At the same time there is a need to assure high living standards for inhabitants, including not only technical and economic aspects, but also environmental, social, and cultural ones [1, 9]. All these requirements are directed to the vision of shaping in the future all over the world the needed technical infrastructure and in the same time the appropriate mentality for living in the much desired *sustainable city* [1, 12].

Worldwide began in the last time sustained debates on scientific, political, and social levels to find best possible strategies for shaping *sustainable smart cities*, as presented in Figure 1. Such strategies should be applicable to several countries all over the world, anyway by considering diverse odds of *location intelligence* on local level [3, 7].

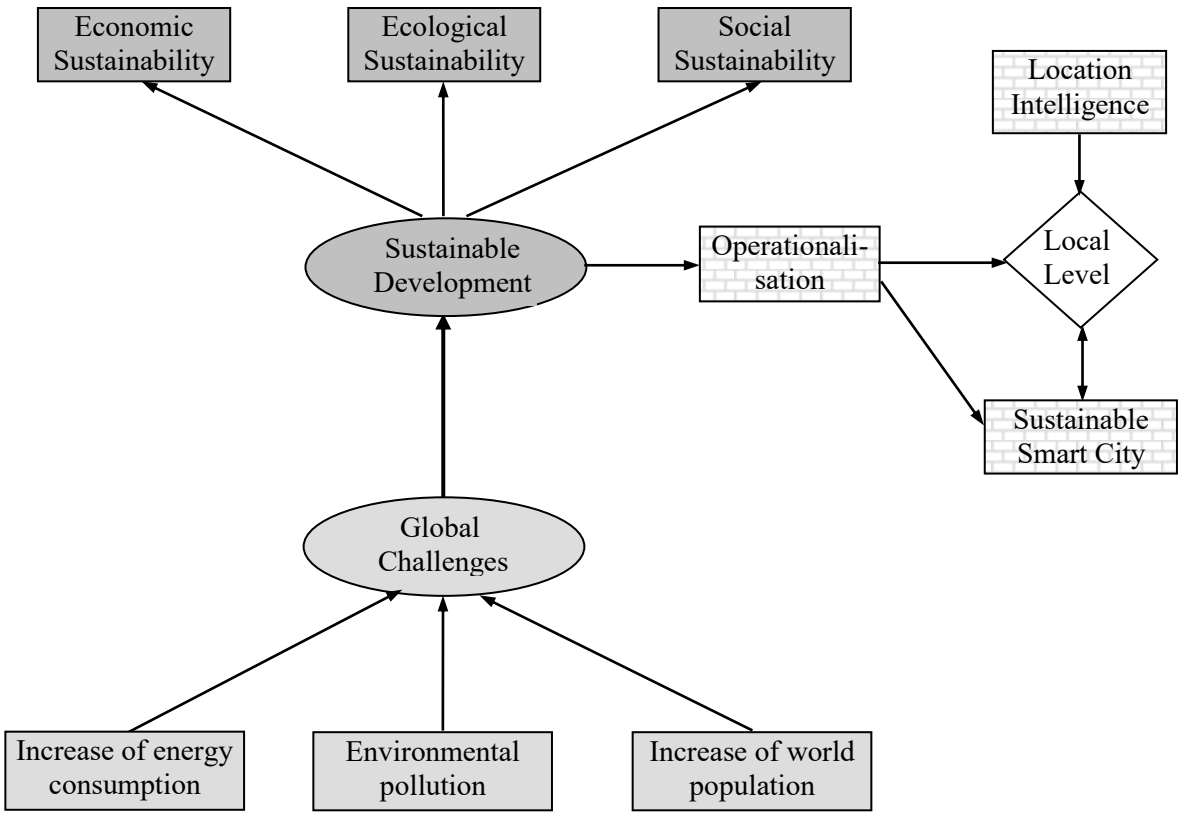



**Figure 1:** Global challenges, the concept of sustainable development and the role of location intelligence for its operationalization on local level.

## ABOUT LOCATION INTELLIGENCE

Location intelligence represents the resulted comprehension after monitoring and analyzing specific geospatial data. Presenting on a smart map some specific data, having lot of relevance regarding the evolution of diverse aspects on considered level, such as related to demographic aspects or to traffic related ones as well as to economic situation in considered area and to environmental quality or to specific weather conditions assures insights about the analyzed area, for instance a certain region or a city. Location intelligence can also be applied for better understanding certain processes taking place in certain places. A challenging issue regarding shaping sustainable smart cities is the request of respecting specific regional mentalities especially by considering cultural differences [1, 6, 13]. On the other side newer applying odds when designing scenarios for potential future developments on local level to support decision making are given by using location intelligence for optimal planning of next activities with the goal of shaping sustainable smart cities. As it is known, location intelligence represents the intuitive methodology, which supports providing interested people with insights about spatial phenomena by using geographic and geospatial data, that include [7]: Geocoding data, Points-of-Interest data, Physical addresses, Latitude and longitude, Postal Code, and Aerial maps.

In the last time applying location intelligence has been understood as being a necessity in our real complex life especially in technological and economic field but not only in these fields. This insight results because it supports developing best strategies and taking decisions upon analysing in details consumer and competitive landscapes. It is a recognized fact that main advantages of adopting location intelligence in several regional economic activities are represented by diverse fields, especially the ones of competitive intelligence, commercialization, and location selection, which proven during the time their relevance for the success of a certain activity [4, 7].

## SHAPING SUSTAINABLE SMART CITIES BY LOCATION INTELLIGENCE

Recent events on a worldwide level brought the recognition of the fact that the impact of huge industrialization and urbanization is severe on our planet Earth [10, 11]. As a result of these developments the world currently faces several challenges regarding climate changes, unanticipated natural disasters, and other potential events. However, it should be recognised that technological advance on a global level is rapidly contributing to succeed making the world a better place to live [6]. Therefore, important steps are currently made especially by government organizations, environmental institutions and even by companies for applying location intelligence to try reducing registered negative impacts on the population and on Earth. In this regard there are presently in sustained debates five so-called *Green Initiatives*, which base on *Location Intelligence* by using geospatial data, as presented in the following [7].

### 1. Sustainable Agriculture

Agriculture related activities demonstrate a main contribution in registered environmental changes such as climate change, water scarcity, deforestation, and land debasement. In this context sustainable agriculture assures performing mindful cultivation that means fulfilling population needs by saving resources for next generations. Using location intelligence, agricultural land is analyzed based on its site

location and surrounding areas, as well as sustainable farming methodologies are adopted for better assets. Another relevant usage case of location intelligence in sustainable agriculture is represented by naturally powered energy farms. It is a main task to find the ideal location to build sun-powered and wind-energy farms. Location intelligence supports the process of finding the right location of generators, substations, warehouses, and other related objects.

**2. Electric vehicle charging stations over harmful emissions.**

It is to be mentioned that in the last decade the global electric vehicle market has significantly improved. The sales of electric vehicles grew by 43% in the first half of 2020 compared to 2019, although pandemic time. In 2021 it came back stronger, breaking the sales record for electric vehicles by doubling compared to the previous year. The electric vehicle, EV, industry is trying to achieve its ambitious goal of *zero emissions target by 2050*. In this regard by using location intelligence, it is easier to recognize the right places to set up the EV stations. It supports cities with maps to emphasize population density, closeness to focal points, and segment data to ensure that the EV stations are on the right places.

**3. Sustainable smart cities**

The real estate market is currently booming, and location data is supporting estate agents in building sustainable smart cities for people to live in [2]. Real estate is defined as the land and any permanent structures, like a home, or improvements attached to the land, whether natural or man-made. Real estate is real property that includes land, and anything permanently attached to it or built on it, whether natural or man-made. Generally, it is accepted that there are five main categories of real estate, which include residential, commercial, industrial, raw land, and those with special use [2]. Location intelligence assists city designers in building greener spaces around the city. Through geospatial data, network planning models and infrastructure investments are made accurately, which is driven to make a sustainable smart city. Using location intelligence, road and traffic management can be monitored to reduce harmful emissions through vehicles; and energy plants can be positioned upon gaining better insights for shaping sustainable smart cities.

**4. Faster healthcare during natural disasters**

Although Covid pandemic has strongly impacted the world by severely impacting business life of the common man, location data supported governments and other organizations in many ways to combat this zoonotic disease. Location data can provide insights into the number of cases in a particular location, and faster healthcare to the localities. Location intelligence assists governments in catastrophic disasters to control and provide a safer environment for the public. On the other side this technology supports understanding air quality in a specified location, and polluting aspects, so that it supports choosing the right place before development and planning accurate strategies after development [7].

**5. Sustainable transportation routes**

In this context it is known that location intelligence supports governments and administrators to study, analyse, and proceed with project plans on how to dispose traffic infrastructure, where to foster bike rides, and specific racing events. This means that location intelligence supports mitigating the air pollution in the main hub of the

location and reducing traffic congestion. Spatial examination can indicate in the best way the place where each individual is located and where bike offers must be situated for better audience engagement. This procedure includes ingesting geospatial information and working with other data sources to try designing *greener* strategies for shaping sustainable transportation routes [8, 10, 13].

## CASE STUDY

Considering made presentation a case study will be debated as direct application realised in Open Street Map for the city of Alba Iulia [14]. The very good known historical area in the city called "Alba Carolina Fortress" has been considered. In the studied area were identified main following tourist attractions: "Apor" Palace, "1 Decembrie 1918" University of Alba Iulia, National Unity Museum, Princely Palace, and "Saint Michael" Cathedral, Reunification Cathedral, as to be recognized in Fig. 2. As it is to be recognised in this figure, Location Intelligence allows the visualization of spatial data for identifying and analysing relationships on local level, giving information about available infrastructure, and roads. In addition by location intelligence diverse odds for an efficient urban planning can be gained by considering precise historical and traffic patterns with the goal of designing short- and long-term urban development strategies on a local level.

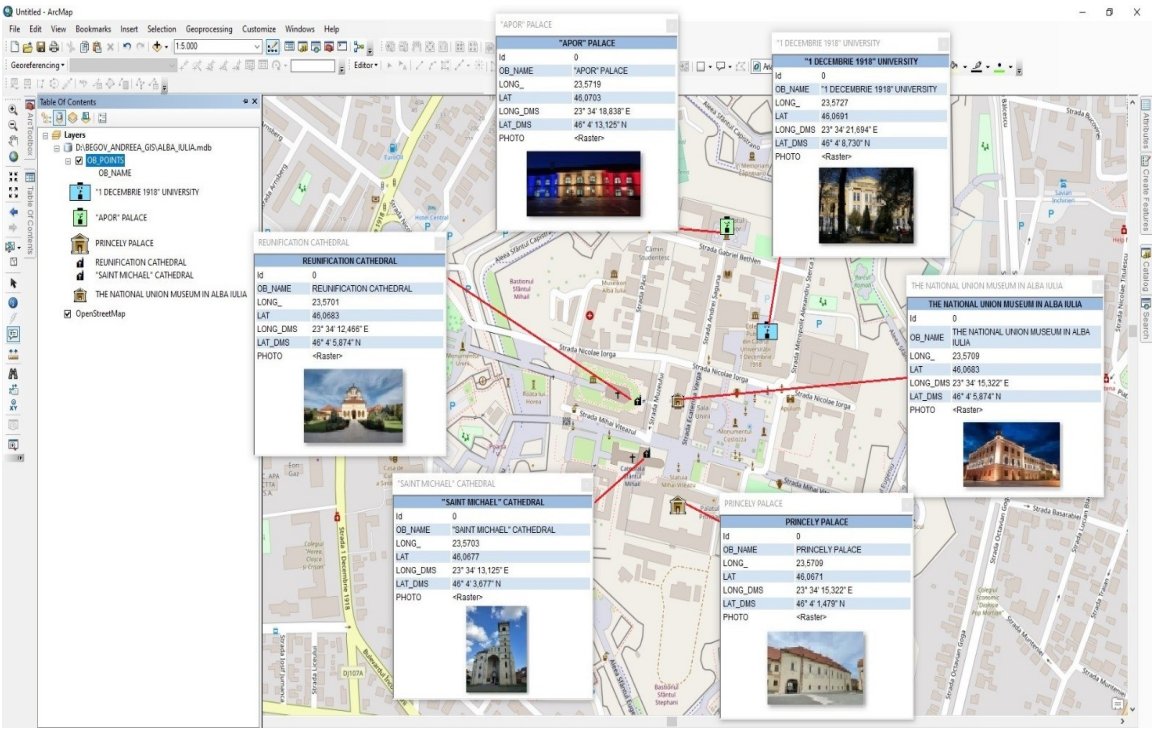



**Figure 2:** Presentation of the "Alba Carolina Fortress" in Open Street Map for the city of Alba Iulia.

Considering presented example of applying Location Intelligence for the city of Alba Iulia in Romania to each objective has been associated its characteristic photo and its coordinates have been calculated in the Stereographic 1970 national projection

system but also in the WGS system. As it is emphasized in Figure 3 in the example of “Apor” Palace, if somebody is on the application and the application identifies the position of the person, it will automatically show the picture of the identified objective nearby, its name and coordinates [14].

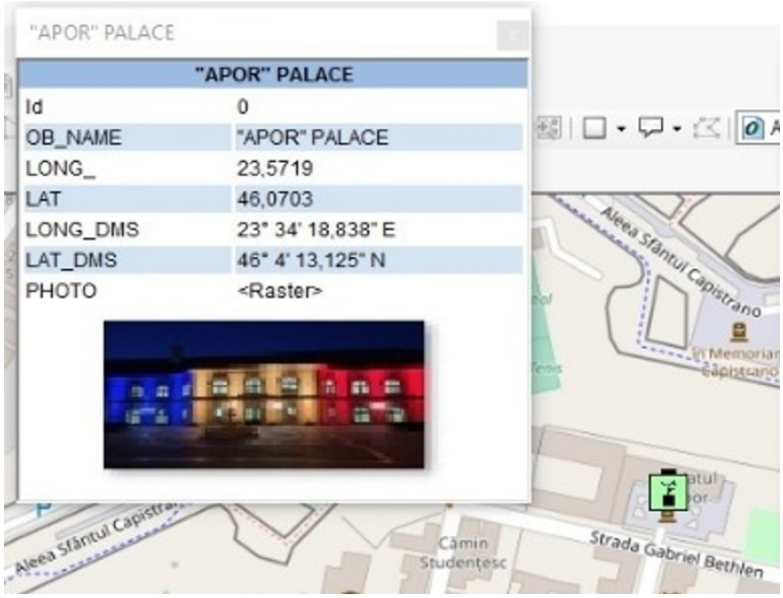



**Figure 3:** Characteristic photo and specific coordinates of the objective called “Apor Palace” in the Alba Carolina Fortress of the city of Alba Iulia.

## RESULT AND DISCUSSION

Regarding the role of location intelligence for shaping sustainable cities it is of foremost relevance and importance [2, 4, 7]. On the other side shaping a green real estate is requested as a base for bringing clarity in organizing manner and in management of built infrastructure in cities, what brings advance in the field of location intelligence for shaping sustainable cities.

Location intelligence made possible designing five *Green Initiatives* by using geospatial data: Sustainable Agriculture, Electric vehicle charging stations over harmful emissions, Sustainable Smart Cities, Faster healthcare during natural disasters, and Sustainable transportation routes. Therefore, continuous environmental concern connected to applying location intelligence for sustainable smart cities began to emerge, known as the notion of green development. Green development is represented by the real estate development concept, which considers social and environmental impacts of development, and it is defined by the following three criteria: environmental responsiveness, resource efficiency, and community and cultural sensitivity. Hence there has been an ongoing argument of whether green strategies should be *sustainable* as well as economically stimulating.

Presented case study as direct application realised in Open Street Map for the city of Alba Iulia in the historical area of the city called “Alba Carolina Fortress”

demonstrates the usefulness of Location Intelligence in finding more details about tourist attractions in considered city and in bringing the city closer to its inhabitants and tourists.

As it is to be remarked, sustainable communities are starting to emerge all over the world and sustainable cities are rising too by applying location intelligence. In this context solutions to some of the greatest issues facing humanity such as poverty, climate change, healthcare and education will be found in new forms of leading our day-to-day lives. The foremost goal of such strategies is the decarbonization of local economies for enabling new standards of living by location intelligence. It is to be emphasized that in recent time the vision of Sustainable Living by appropriate Location Intelligence. This is relevant especially because currently the goal is the clean energy transition and the use of Location Intelligence for succeeding in shaping sustainable cities.

## CONCLUSION

A multitude of single fields on a local level have to be considered at the same time, as technical field, economic and socio-political one, as well as environmental field for succeeding in shaping sustainable cities. Independently of which field is more or less considered in a certain situation with regard to assuring sustainability on local level, location intelligence plays a main role in this context. In this regard *Green Initiatives* by using geospatial data connected to Location Intelligence are of foremost importance, as Sustainable Agriculture, Electric vehicle charging stations over harmful emissions, Sustainable Smart Cities, Faster healthcare during natural disasters, and Sustainable transportation routes. In this regard there is a need of changes in human behavior as well as mindset changes. The local level has to be at first considered for finding concrete strategies for urban sustainability starting with Location Intelligence. The vision of shaping sustainable cities, by taking into account regional differences, is currently worldwide a pretty discussed topic, where Location Intelligence receives a main role in this regard. To Location Intelligence is linked the concept of green development, which represents the corresponding concept considering social and environmental impacts of this development. The application shown for the historical area in the city of Alba Iulia called “Alba Carolina Fortress” has demonstrated the power of Location Intelligence even for touristic usage. Location Intelligence for shaping sustainable smart cities must consider environmental responsiveness, resource efficiency, and community and cultural sensitivity. Debates in this regard are interdisciplinary, so that cooperation among political and public actors, civil society and researchers is foremost requested with the goal of assuring Location Intelligence for shaping sustainable cities.

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# NONVIOLENCE OR REVOLUTION. TOWARDS A HYBRID AND ANTI-SPECIESIST ARCHITECTURE

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## ABSTRACT

In 1923 Le Corbusier's *Vers une architecture* was published for the first time, maybe the most influential essay for the discipline ever. Imprinted on the title page of that first edition was the photo of the deck of the steamer Aquitaine, at the time the most up-to-date result of the industry: from there, the "machine" became the model for a radical renewal of architecture. Nowadays, in 2023, who would ever dream of presenting the latest cruise ship or a super-jet as a paradigm for a renewed architecture? In fact, in the meantime a century has passed and a turnaround has taken place. The current environmental crisis, with ever more violent and frequent extreme events on the one hand and an increasingly widespread rampant eco-anxiety on the other, is changing our sensibility. The paradigm for the architecture-to-come that is starting to glimpse in this first part of the XXI century is hybrid, "monstrous", made of vegetable and animal matter: it is more similar to a tree than to an airplane, more similar to an animal than to a ship; finally, more similar to Buddha than to Christ. This article will try to argue how and why Buddhist *nonviolence* can be assumed – in an ecological key – as a revolutionary posture to try to access in a new era, also for the discipline of architecture.

**Keywords:** architecture, nonviolence, environmental crisis, nature, city.

## 1923, OR SOME PREMISES

Exactly one hundred years ago, the most influential architectural essay of the twentieth century – and perhaps ever – saw the light for the first time: it was 1923 when the Parisian publishing house Crès published *Vers une architecture* by Le Corbusier [1], a text which was groundbreaking for its ability to have established, through words and images, the boundary conditions of possibility for a new practical language. In summary, *Vers une architecture* was the treatise which, more than any other, at least until that historical moment, had the ability to create a concrete world starting from a theoretical speculation: a book with unprecedented tangible evidence. Before this one, the only other theoretical operation of (almost) comparable power, media diffusion and international influence maybe was *I quattro libri dell'architettura* (1570) by the Italian architect Andrea Palladio [2]: however, there are some important differences between these two written works. The first lies in the fact that Palladio makes use of an iconographic apparatus that's strictly architectural (elevations, sections, axonometries, construction details, etc.); otherwise, Le Corbusier is less "rigorous", and leans on images and pictures of other provenances, which he takes mainly from the world outside that of the discipline. The second important difference is that Palladio speaks of

constructions that are individual in their "loneliness", and his influence will be such, that is, Palladio's language will have a great echo in architectural projects in their solitude, often designed for a cultured and wealthy client; on the contrary, Le Corbusier's verb will end up building the outskirts and suburbs of the cities as we know them today. The third and final main divergence lies in a more simple fact: the Italian architect published his written treatise at the age of sixty-two, during the final part of a career which had seen him as the creator and chief builder of numerous buildings; the Swiss one, however, he entered the world of architecture with this first essay at the age of thirty-six. If Palladio's one is a "wise" and retrospective collection that looks at a life prior to the book, Le Corbusier's one is a pseudo-youthful manifesto that expresses the desire to change the world through a renewed architecture. And the second, the one that originally should have been titled *Architecture or Revolution*, will overtake the first one in terms of power, propagation and social influence.

It's starting from this book that the architectural twentieth century established itself. It's because of this text that a good part of the cities in which we live were created – and are still being created nowadays. And it's only by starting from this publication that, today, we can begin to question a world, that of those who came before us. Not doing so would mean making an unfounded critique.

## AT THE BOTTOM OF A PHOTOGRAPH

So, let's start right from there, from the title page of that first edition of *Vers une architecture*. There, at the center of a light blue monochromatic background, stands a black and white photograph: the one immortalized in its cold, "rational" rigor is the deck of the Aquitaine, one of the most up-to-date advanced steamships of the time. Set afloat in 1913 and demolished in 1950, the transatlantic was owned by the Cunard Line company: it was 275 meters long and 30 meters wide, a sort of symbol of the industrial power of the early twenties, the pinnacle and final outcome of a mechanical and engineering refinement unprecedented in human history. Now, I think setting the premises for this discussion starting right from here makes sense, as the Aquitaine deck – together with the many other bombastic engineering, industrial and "machinic" references within Le Corbusier's text – is there to show us a way, that of progress in and for architecture, a progress that can only go in that precise direction. Either that or nothing.

Well, nowadays it is as if we were there, at the bottom of that perspective, near that vanishing point: the twentieth century has come to an end and we have ideally crossed that deck. In the meantime, the world has changed and the transatlantic has begun to falter under our feet: we are there at the end of that path and we turn back, sensing that perhaps the optimistic disillusionment of the Modern Movement had not taken into account many factors. We are there feeling our legs wavering and we go in search of a haven where we can put our feet back on the ground. The great promise of the "infinite well-being" of the Modern has collided with the *finitude* of our reality. Now we urgently need a new perspective to frame, through which to face and shape the near future – which otherwise will find us unprepared and will be able to overwhelm us.

## A SIXTY-EIGHT IN THE MIDDLE

We were saying, in the meantime, during this last century, the world has changed: we have proof of this profound mutation simply by the fact that, in 2023, no one would ever dream of presenting a cruise ship or the latest super-jet as a paradigm for a revolutionary architecture-to-come. The underlying reason for this review and new look at things-in-the-world can be reduced, in a nutshell, to the macro-theme of *energy*. Between the 1950s and the 1960s, the prodromes of the ecological turning point that we are experiencing with greater emphasis today were born: almost paradoxically, they have to do with a *distancing* from our earthly *òikos*. Le Corbusier had recently completed his ecclesiastical masterpiece of the Chapel of Notre-Dame-du-Haut in Ronchamp (1955) when, in 1957, the Soviets launched Sputnik into orbit. In 1961 Jurij Gagarin made about ten orbits around our planet. In 1963, a woman, Valentina Tereškova, did the same for the first time. Two years later, Aleksej Leonov floats for a few minutes in interstellar space and, as is well known, in 1969 Neil Armstrong, an American astronaut, leaves the first human footprint on the Moon. But it is above all in the previous year, in 1968, that our perspective and usual gaze on things is forever upset and overturned. It was Christmas Eve of that year, in fact, when the crew of the Apollo 8 mission took the famous *Earthrise*, the first photograph of our planet taken from our satellite. In the eyes of the whole world, Gaia now appears in its solitude, a fragile rock mass orbiting in cosmic space: but above all, our planet appears to us in its finitude, we can visually perceive its boundaries, we can test its perimeter. «The Earth, until now seen as an *unlimited* supplier of space and energy, following the new point of view imposed by space travel, appears as a system with a labile and precarious equilibrium. The *ecological awareness* is born» [3]. The paradox of the Sixty-Eight is therefore also of this type: moving away from the ground as never before, we now offer a foreign (and harbinger) look at our common shared house. Only now does it truly appear to us as it really is, or at least as we seem to have never perceived it, that is, as finite, discrete, defined. Looking at things from the outside for the first time, we understand our earthly condition a little better.

## A SILENT SPRING

From an architectural point of view, it is precisely during the same years – and also because of the aforementioned issues – that the relationship between built-matter and natural-matter, between architecture and environment, between the circumscribed of the project and what's around it and external to it (up until that historical moment) began to change. Around 1966, Archigram looked at architectures so much lighter, less sedentary and more interrelated with the surrounding. At the 1967 Expo in Montreal, Frei Otto and Richard Buckminster Fuller attempted to overwrite the traditional norms of the relationship between architecture and external landscape following two opposite ways: the former's well-known tensile structures seem to trace the sinuous forms of nature – almost always denied, during the past centuries, by a building's theory handed down by tradition –; the geodesic domes of the second, on the contrary, seek the construction of a totally new, extremely hi-tech and controlled landscape. Then, the American Lawrence Halprin, together with his wife Anna, explores «the interactions between the body and nature. [If] the reference to body art and the performances of Allan Kaprow, Yoko Ono, Yayoi Kusama [and] Bruce Naumann is evident [...] there is, with respect to these

artistic research, an original and interesting approach towards nature, as something to enter into symbiosis with» [4]. Another interesting episode of this season is the experimental community of Arcosanti (Arizona), in which members of hippie communities, alternative intellectuals and some students stayed for more or less long periods, fascinated by an innovative and totally anti-traditional idea about inhabiting our world and the urban environments. Arcosanti was conceived and designed by the Italian Paolo Soleri, an architect fascinated throughout his life «by self-sufficient communities, founded on natural, spiritual and rational principles, and so dense as to favor an intense activity of meeting and cultural exchange» [5]. To be short, it seems that 1968 took no prisoners, ushering in a new season in all disciplines, including architecture. The premises of the current political, social and obviously urban events occurred precisely during those years, although for a few decades they remained taken into very little consideration by the official and mainstream culture. The ecological "architectural spring" of the second half of the 1960s remained, for the most, a *silent spring* – the reference here is to the homonymous book by Rachel Carson (among the founding mothers of environmentalist sentiment), published for the first time in 1962 and that remained almost completely unheard until the mid-1990s [6].

Now, positioning ourselves in the wake of the interesting reading that the historian Barnabas Calder makes of the architectural Modern Movement and its derivatives which still crowd the architectural agenda of the XXI century [7], it seems correct to affirm – without too many regrets – that we can no longer afford a similar way of doing-and-thinking architecture: that which was born at the beginning of the last century, in fact, was the architecture of *low-cost energy*. By contraction, says Calder – reversing Louis Sullivan's famous mantra of *form follows function*, transforming it into a more updated (and maybe sincere and useful) *form follows fuel* –, what we have been experiencing in various manners for a century right now is the architecture of *oil*. Analyzing it with the eyes of the present, the architecture of the twentieth century can be said, in energetic terms, to be predatory, brazen and, if we want, short-sighted – as it doesn't take the slightest consideration of the finiteness of our planet's resources (during these lines I use the present tence since by "twentieth century architecture" I mean a widespread and shared *way of doing* architecture, rather than a repertoire of built projects). Oil flows in very large quantities, it is liquid, it seems like it could never end. And it took decades before we truly began to understand what it means to make running the great flame of the world on oil – and gas, or coal –, without a single interruption. What Le Corbusier's *modulor* presents to us is a Man still at the center of the world, still violent towards his surroundings, towards the environment, towards the so-called *natural*.

## A FORM OF SPECIES AUTISM

The key point of the discussion opens right here: using the words of Philipp Blom, that of the twentieth century is still a mostly and chiefly *subjugated nature* [8], often and willingly reduced to a mere handmaid of an austere and maximally rational architecture, made of lithic and non-living matter, built with the prodigious tools and incredibly energy-intensive materials of the oil – the "petrosexoracial" contemporaneity of ours. On the other hand, one of the most sincere, up-to-date and sharp definitions of city is perhaps the one that Emanuele Coccia gave some time ago. According to the

philosopher, «city is the name of the condition of possibility and the expression of *a form of species autism*» [9]. This definition is very interesting, first of all, because it starts from a finally mixed and interrelated vision of things: by speaking of our urban condition as a sort of "species autism", Coccia is implicitly endorsing the first – and perhaps most important – of the four known Dipesh Chakrabarty's thesis on climate in history, which highlights the collapse between the history of human events and that of natural events: continuing to separate them is no longer admissible, given that «anthropogenic explanations of climate change entail *the crisis of the secular distinction between natural history and human history*» [10]. Secondly, this definition is a harbinger of renewal as it is comparing our way of urban living to a pathology and, even better, to a psychic solipsism of ours, caused by a mental attitude: the *urbe* as we know it has operated violently towards other living species, eradicating them and leaving them outside its perimeter, to allow a more peaceful coexistence between beings belonging to the same species only – if not even to the same clan or social class. However, we are realizing that this form of speciesist autism cannot be the solution for the future to come. This way of thinking about the urban needs to be revisited – and it is Coccia again, a few lines later, who assures and reassures us that this pathology does not represent a destiny for *Homo sapiens*. «The question is no longer who we are, but what we will transform into» [11].

Our granted and perpetuated *subjugation* of the natural element has very ancient roots and finds its maximum expression in that Enlightenment, which in many disciplines and branches "secularized" the world, but which from the point of view of (not) questioning the centrality of Man – who is always male, white, heterosexual and who knows why he is always alone, in his muscularity, at the beginning of the history (because such is Locke's first man, as well as the atavic "Noble Savage" of Rousseau) – nothing changes, or almost-nothing, of the Christian vision which in the meantime, over the centuries, had established itself in the West. The Man of Enlightenment remains delightedly where he is, at the center of a cosmos we have learned to technically control even better. Two hundred years later, 1968, its derivatives and nowadays ecological crisis, in fact, have dealt the last fatal blow to the centrality of the Vitruvian man – which adapted is that of Christianity, that of the Enlightenment and that of Le Corbusier's *modulor*. The Copernican revolution broke the first arm of the Vitruvian man in two: we are no longer physically at the center of the universe; Darwin's evolutionary theory broke his second arm: we are no longer at the center of the divine creation; the psychoanalysis of the unconscious broke his first leg: we are no longer so sure that we are owners and masters in our own bodily home; and now the ecological-environmental crisis is breaking the last limb: we're not all that unique in terms of what we live with on this planet. The Man at the center of the "perfect circle" who no longer has any handholds, now he falls.

## TOWARDS A NONVIOLENT ARCHITECTURE

Architecture has always been part of what, according to historiography before Dipesh Chakrabarty, we can define as strictly-human occurrences. However, due to that *collapse* between the two Histories referred to previously – which is essentially an ontological and epistemological turn –, architecture is about to live and is already partly experiencing a huge transformation. Architecture too can no longer allow itself to

remain separated from the so-called natural events given that, as we have understood, the distinction has been totally lost (now we have proof that it never really existed). As a reaction to all of this, I suppose we can say that, more and more widely, *ecosophy* – as a practice of thought that subverts, in a non-dual and scientifically updated meaning, our relationship with what surrounds us – is, in practice, influencing the thoughts and "the hands" of numerous contemporary architects, entering into and defining, to different layers, the preliminary conceptual panorama of many project creators.

It was the year 1992 when Zygmunt Bauman allowed himself to write down what would end up appearing to the most as a prophecy – and not a very well-founded one – rather than an observation or a fact expressed by looking at reality: «The postmodern mind *opens up* to Buddhism with greater fullness than its predecessors» [12]. Now, in the wake of authoritative and influential voices, it can be considered valid to speak of Buddhist thought – or at least "Buddhist-like" – at the basis of contemporary ecological sentiment. Just think about the theses forwarded by authors such as Arne Næss, Gary Snyder, Donna Haraway (her profound interconnection inherent in the constituent things of the *chthulucene* reflects that typical Buddhist "feeling"), Jane Bennett or Timothy Morton (who even uses as a metaphor to talk about the ecological crisis the condition of the *bardo*, a topic and specific place of Tibetan Buddhism): it seems proven that «the interest that many exponents of contemporary ecology have shown in the vision of the Buddhist world now has its own history» [13]. In summary, trying to keep together the various considerations discussed so far, we can affirm that Bauman was right to the extent that (and thirty years later we can say that this is actually what happened), following the historical collapse of the twentieth century which caused from opening up to an even more pervasive crisis, the current climate-environmental one, *Homo sapiens* has been changing and downsizing – thanks to reasons known to all of us – his *ecological dimension*. Precisely this ecological downsizing has brought him closer to a vision of the things-in-the-world that has much in common with the Buddhist one: this because, as we have attempted to explain in the previous lines, ecology and ecosophy own speculative roots strongly soaked and imbued of Buddhist culture, metaphors and cross-references. In this very case, coming to the heart of the matter here, a direct consequence of the *interpenetration* – or also *strong interconnection* – of things, in things and between things which is found in many if not all of the fundamental texts of the Buddhist tradition, is precisely the institution of *nonviolence*. Taking this argument to its extreme consequences, when one has that «himself [is] resident in all beings, [and] all beings [are] resident in him» [14], nonviolence towards the Other-from-itself must be called into question. In that way, we are able to strictly speak of a *nonviolent ecology* and, from this, of a *nonviolent architecture* when it relies its intentions on an ecosophical matrix of thought.

## CONCLUSIONS

The current ecological crisis is forcing also architecture, together with all other scientific and non-scientific disciplines, to change. This change does not appear to be purely formal: on the contrary, it seems to be starting to affect the most intimate and speculative part of the architectural discourse. If at a first more superficial reading we can affirm that we see a world of design that is veering towards a somewhat easy "naturalization" that is increasingly pushed – even the most miserable greenwashing

falls within, as far as what it wants to appear to be, internal of this turn: one could actually say that the (too) widespread greenwashing somehow corroborates this hypothesis –, some more interesting works, attempting to place themselves on a deeper level than a simple formal or material analogy, I believe they are tracing the premises for a radical renewal within the world of architectural theory and criticism. We understand this renewal to concern the preliminary approach and posture to the contemporary project, which is transforming itself *ecosophically* and *nonviolently*. What I have attempted to explore in depth here, I would like to be taken as a long reasoning or premise, which tries to build a renewed interpretation to understand the underlying reasons that are driving more and more contemporary projects. An example of this – among the many possible ones – are the "playful" attempts to reposition the borders between human and non-human by Studio Ossidiana (Giovanni Bellotti, Alessandra Covini). They do it sometimes through an almost-lithic elements which in reality are also made of plant matter, including edible seeds for many species of birds – architectural partitions designed to fade and melt over time, which have mixed functions depending on their shape and location (The Garden of Intersections, Utrecht, 2019) –, sometimes instead with floating artificial islets designed solely and exclusively for bird species (The Bird's Palace, Amsterdam, 2020).

In 1923, Le Corbusier's famous *Vers une architecture* should have been entitled *Architecture or Revolution*. What the Swiss master feared was the ghost of the Soviet revolution, which managed – according to its author's intentions – to convince the European bourgeoisie to ensure that the task of quelling the less wealthy classes could be left to the new "architecture for all" brought forward by the Modern Movement (an economical, "efficient" and mass-produced *machine à habiter*), thus limiting their psycho-physical discomfort – which is the premise to organized riots into the streets, which are usually in turn the run-through for the revolution. A century after that title was never awarded, today the conditions have profoundly changed. The revolution (which is more of an upheaval) of an ecological-environmental nature that awaits us all – without exception – could be much deeper and more pervasive: a *hyper-revolution* of the "climate" intended as an *hyperobject* [15]. The turning point that the world of architecture seems to have undertaken – in its less superficial, more intimate and profound aspects –, of which I have tried to delve into the possible theoretical bases here, is the attempt to reformulate our approach to the project, trying to avoid stumble upon a fate we don't want. Or at least can be assumed as a way to try to get there prepared. Because history is made at the crossroads: *nonviolence or revolution*.

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# RENEWABLE ENERGY INTEGRATION, CLIMATE ANALYSIS, AND EFFICIENCY OPTIMIZATION FOR GREENER TRANSPORTATION - CASE STUDY IN DOBROGEA

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## ABSTRACT

This paper presents a model for super-rapid charging stations powered by renewable energy sources and conducts an analysis of land suitability based on climatic parameters. Initially, data on temperature, solar radiation, and specific humidity were collected for three designated areas, followed by an examination of the variations in these values.
Among the three cities, Cernavoda, Medgidia, and Ovidiu, two equal segments of approximately 30 kilometers each were established for the installation of photovoltaic panel systems on both sides of navigable canals. With each segment measuring 30 kilometers in length, a linear area of 60 kilometers was covered on both sides, resulting in approximately 767 photovoltaic panels per kilometer and an installed power of 514 kW per kilometer. The total installed power for the entire 60 kilometers is approximately 30.84 MW, necessitating the division of the network into 1028 kW segments, each with a 1250 kVA transformer with a voltage of 20/0.4 kV. Fifteen transformer substations must be installed on each side of the canal, totaling 30 substations for the entire stretch. The energy produced by this system is utilized for three electric vehicle charging stations, positioned every 15 kilometers on each side of the canal, resulting in six charging stations per segment. Excess energy is injected into the national electrical grid (SEN) via 20/110 kV substations. To achieve optimal efficiency, a 2x25 MVA substation is supplied by 15 transformer substations, one for each side of the canal.
The electric vehicle charging stations are of the super-rapid type, with a capacity of 2x175 kW. Initially, these stations exclusively rely on photovoltaic panels for approximately six hours each day, but energy storage batteries can be installed to ensure continuous operation, even during the evening hours. Comparing the analysis of the two segments, it is observed that there are slight differences. In the second segment, closer to the Black Sea with higher specific humidity, there is a slightly higher annual energy production of 390 kWh compared to the first segment, despite similar solar radiation values. Notably, this analysis reveals that specific humidity as a climatic parameter does not significantly influence the efficiency of photovoltaic panels compared to the other two parameters.
Through this project, the aim is to reduce pollutant emissions from vehicles on public roads while simultaneously decreasing carbon emissions by replacing conventional electricity generation plants.

**Keywords:** Renewable energy, photovoltaic, solar radiation, carbon emissions.

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## INTRODUCTION

Romania, a nation at the crossroads of Central and South-eastern Europe, has long been a cultural and geographical bridge between the Eastern and Western worlds. In this diverse and vibrant European landscape, one region, in particular, stands out for its unique potential in the quest for greener transportation solutions: Dobrogea. Nestled in the south-eastern corner of Romania, Dobrogea is characterized by a rich tapestry of landscapes, from the shores of the Black Sea to its fertile plains and navigable canals[1]. This region, steeped in history and teeming with natural beauty, presents an ideal backdrop for the exploration of renewable energy integration in transportation [2].

Within Dobrogea, three cities, Cernavoda, Medgidia, and Ovidiu, have captured our attention for their strategic geographical location and climatic characteristics. These cities, known for their historical significance and modern vitality, are poised to play a pivotal role in the sustainable transformation of transportation in the region. Cernavoda, with its roots stretching back to ancient Dacia, represents a link between the past and the future[3]. Medgidia, founded during the Roman Empire, reflects the region's enduring legacy of connectivity. Ovidiu, named after the renowned Roman poet, Ovid, adds a touch of cultural richness to the region. Each of these cities, with their distinct historical backgrounds, is positioned to contribute to the innovative vision of greener and more efficient transportation through renewable energy integration [4].

Furthermore, as a member of the European Union (EU), Romania operates within the framework of a continent that has set ambitious targets for reducing carbon emissions and embracing sustainable energy practices. The European commitment to combat climate change has ripple effects on the nation's policies and practices, making Romania, and regions like Dobrogea, central players in the broader transition to a low-carbon future. The exploration of renewable energy sources and their integration into transportation infrastructure aligns with the EU's sustainable development goals, and it presents a unique opportunity for Dobrogea to be at the forefront of these efforts [1].

In recent years, electric vehicles (EVs) have gained prominence as a promising alternative to internal combustion engine vehicles. These vehicles, powered by electricity, produce zero tailpipe emissions, offering a tangible solution to the environmental burdens associated with traditional vehicles. However, the effectiveness of electric vehicles in promoting environmental sustainability hinges significantly on the source of the electricity used for charging. Renewable energy, harnessed from sources such as solar and wind, has emerged as a compelling choice to power electric vehicle charging infrastructure. By using renewable energy to charge electric vehicles, we not only reduce the overall carbon footprint of the transportation sector but also contribute to the broader goals of reducing reliance on fossil fuels and combating climate change [5].

Against this backdrop, this paper delves into the potential of renewable energy integration, drawing inspiration from the picturesque Dobrogea region and its cities. The study aims to not only address the practicality of incorporating renewable energy into the transportation sector but also to highlight the role that regions like Dobrogea can play in advancing these goals. By considering the specific climatic parameters and geographical features of this area, we aim to explore the feasibility and efficiency of photovoltaic panel systems in powering electric vehicle charging stations, ultimately contributing to greener, more sustainable transportation solutions that resonate with Romania's European commitments [6].

## MATERIALS AND METHODS

With the rapid growth of industries, the transportation sector is faced with new challenges, most notably the need to reduce carbon emissions while meeting the ever-increasing demand for transportation services. Within the European Union, transportation stands as the second-largest source of $CO_2$ emissions, accounting for approximately a quarter of total emissions in 2018. This substantial contribution to emissions is primarily attributed to the fact that nearly 94% of the transportation fleet consists of internal combustion engine vehicles powered by fossil fuels. Without effective measures to curb pollution, it is projected that, by 2050, the transportation sector could become the largest contributor to atmospheric pollution. This is, in part, due to a projected increase in demand for transportation by 40% for passengers and 60% for freight between 2010 and 2050, as per EU scenarios 7].

The movement to address vehicular emissions aligns with the EU's long-term objectives to annually reduce factors contributing to global warming and to limit temperature increase to 1.5°C, as agreed upon in the 2016 Paris Agreement [2]. In 2016, the European Commission fortified its stance on the decarbonization of transportation by emphasizing the need to enhance transport system efficiency. This emphasis involves the incorporation of low-emission alternative energy solutions and a shift toward low-emission or zero-emission transport modes. In light of this, the EU has established new $CO_2$ emission standards for vehicles manufactured after 2020, including passenger cars, freight vans, and large trucks. However, despite clear political intentions and requirements, progress is hindered by uncertainties surrounding key aspects such as necessary infrastructure conditions and technology availability [2], [8].

The transition to zero-emission electric vehicles (EVs) is further complicated by challenges within the National Energy System. An assessment is needed to determine if the national system can generate sufficient electricity to charge all EVs and if there are enough charging stations available for these vehicles. Additionally, the cost of energy storage batteries in new vehicles poses a significant hurdle [3]. Recent developments suggest that the production cost of energy storage batteries can decrease more rapidly and dramatically than anticipated in 2017. The prevalence of electric vehicles on the market by 2050 can vary from insignificant to widespread adoption, with the primary determinant being the trajectory of battery costs [3, 7].

Due to the multitude of unknown and unpredictable factors, the EU has developed a wide range of future scenarios regarding the electric vehicle market. The European Climate Foundation, for example, presents hypotheses for electric vehicle sales in 2030, ranging from 15% to 85%. Recent EU policies have narrowed down the decarbonization project range for the transport sector in 2030, setting reference values for new vehicles in 2030 at 35% for passenger vehicles and 30% for trucks and freight vans to be low-emission or zero-emission, compared to 1.1% for full electric and plug-in hybrid vehicles in 2016 [9]. To mitigate carbon emissions from the transportation sector and meet the growing demand for electricity, we propose the use of photovoltaic panel systems. These systems are central to renewable energy sources and have the potential to eventually replace fossil fuels without harming the environment. Yet, photovoltaic systems are not without limitations. One major concern is their efficiency, as they convert only a small percentage of solar energy into electricity, with typical panels having an efficiency of around 15-20%. Efforts have been made to surpass this technical constraint, with some systems

achieving efficiencies exceeding 40%. However, as efficiency improves, acquisition costs rise [10].

Furthermore, a significant amount of land is required to generate substantial electric power with photovoltaic systems. The cost of acquiring this land increases as available land diminishes. To overcome space limitations and meet increasing energy requirements, we propose the installation of photovoltaic systems in densely populated urban areas. This approach, particularly on building rooftops, offers a more practical solution and is preferable to large centralized solar facilities [6]. Distributed generation using such systems is environmentally friendly but presents challenges in terms of building renovations, safety, and public perception [11]. For both the National Energy System and the general population, an optimal solution is to install photovoltaic systems along navigable canals. This approach is promising for several reasons. Firstly, it maximizes the use of public, easily accessible, and underutilized canal-side land without additional costs. Additionally, it results in high energy efficiency, given minimal obstructions such as trees or structures that could block sunlight and improved panel cooling due to wind movements over water. As a result, panel wear-and-tear expenses are significantly reduced [12].

## RESULTS

Dobrogea, a region in south-eastern Romania, boasts a unique and diverse geographical landscape. Situated between the Danube River and the Black Sea, Dobrogea is known for its rich cultural history and stunning natural beauty. The region's climate plays a significant role in shaping its environment and daily life.

The climate in Dobrogea is characterized as a continental-temperate one, with influences from both the Black Sea and the continental landmass. This results in a climate that features hot, dry summers and relatively mild winters. The temperature variations throughout the year are quite pronounced. During the summer months, which typically span from June to August, temperatures can rise significantly, often exceeding 30°C (86°F). This warm period is complemented by ample sunshine and low precipitation, making it a prime season for outdoor activities and tourism. The Black Sea's proximity also means that coastal areas enjoy a milder climate during the summer, offering relief from the inland heat [13].

Conversely, the winter season, from December to February, brings cooler temperatures to the region. Daily highs can range from 5°C to 10°C (41°F to 50°F), and night-time lows can drop below freezing. While snowfall is infrequent, it does occur, adding a touch of winter charm to the landscape. The transitional periods of spring and autumn are generally mild, with pleasant temperatures and less extreme weather conditions. These seasons are perfect for exploring the natural beauty of Dobrogea and enjoying outdoor activities without the intensity of summer heat or winter cold.




**Figure 1**. Map site of Dobrogea

We conducted an analysis of three areas along the navigable canals in Dobrogea. Starting from Cernavoda, which serves as the most distant reference point, and ending at Ovidiu, where the canal flows into the Black Sea, with Medgidia as the intermediate point between these two extremes. Our data collection focused on climatic parameters, including solar radiation, temperature, and humidity.

In the following sections, we will present the results of our measurements taken in 2021, with data broken down on a monthly basis. This data encompasses solar radiation on one hand and temperature and specific humidity on the other [14].

**Table 1.** Geographical data for the first location: Ovidiu

| | | | | |
|---|---|---|---|---|
| Location: Latitude 44.3436 Longitude 28.0258 | Ovidiu | | | |
| Elevation from MERRA-2: Average for 0.5 x 0.625-degree lat/lon region = 57.89 meters | | | | |

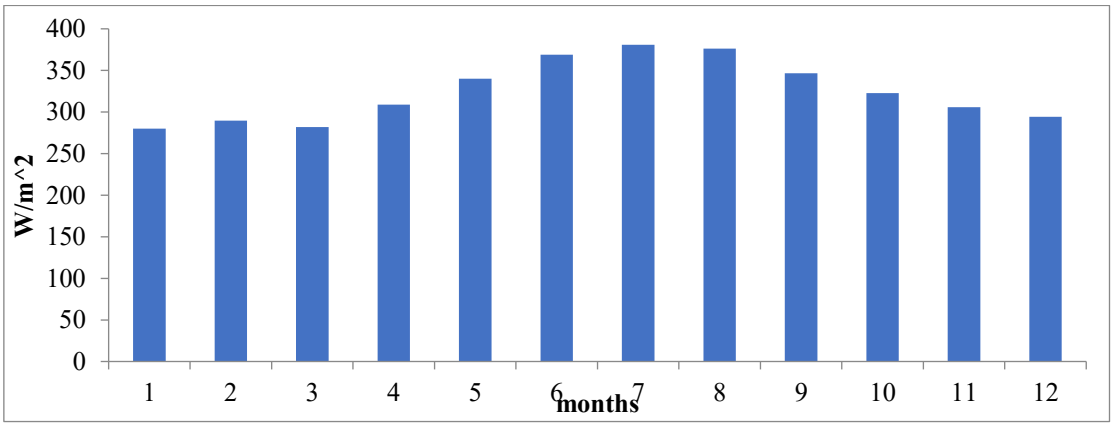



**Figure 2**. Ovidiu: Radiation 2021 - Monthly Average.

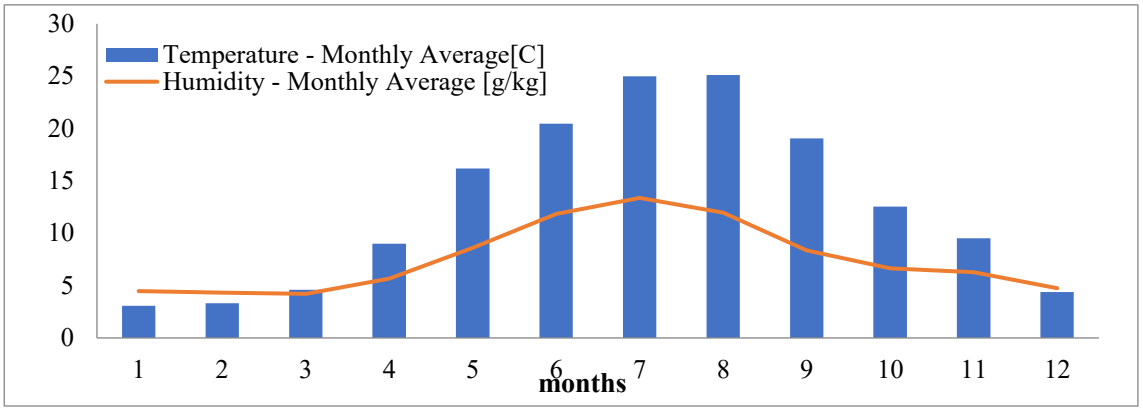



**Figure 3**. Ovidiu: Temperature and Humidity 2021 - Monthly Average.

The parameters of the last analysed zone are represented in Figures 1,2, around the city of Ovidiu, where the chosen canal flows into the Black Sea.

In terms of solar radiation, the values remain unchanged, hovering around 390 W/m$^2$,

In the second case, the annual average temperature exceeds 14°C, and the annual specific humidity reaches values above 7 g/kg.

A significant difference observed among the three zones is in terms of temperature and specific humidity. These values are higher near the Black Sea.

**Table 2.** Geographical data for the second location: Cernavoda

| | | | | |
|---|---|---|---|---|
| Location: Latitude 44.3436 Longitude 28.0258 | Cernavoda | | | |
| Elevation from MERRA-2: Average for 0.5 x 0.625-degree lat/lon region = 57.89 meters | | | | |

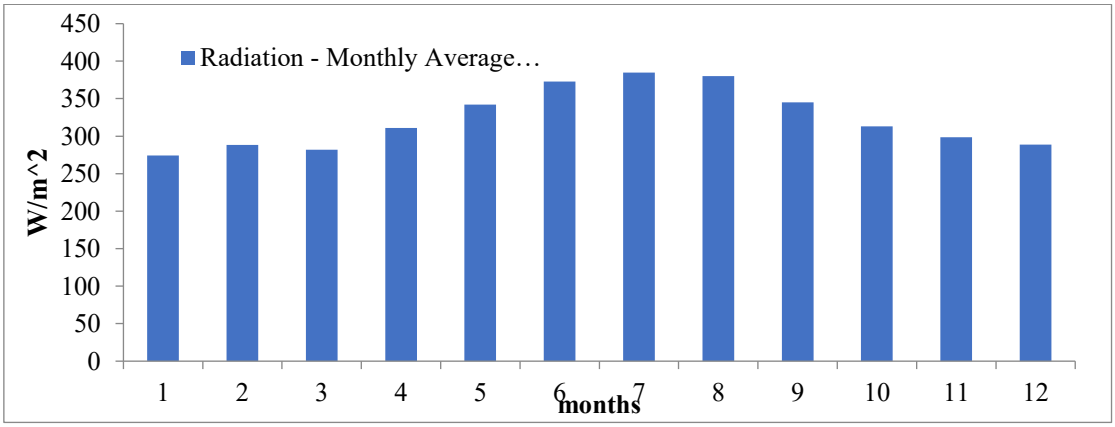



**Figure 4.** Cernavoda: Radiation 2021 - Monthly Average.

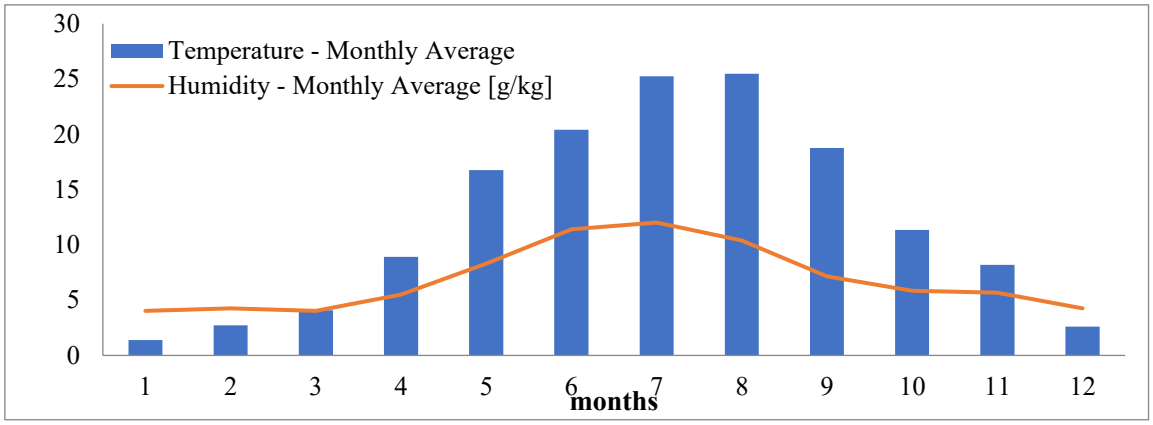



**Figure 5**. Cernavoda: Temperature and Humidity 2021 - Monthly Average.

Figure 6 and Figure 7 as previously provided, depict the climatic parameters in the zone for analysis. We selected a point on the navigable canal near the city of Cernavodă, acting as a transition point from the Muntenia region to Dobrogea.

Illustrates the variation in solar radiation around the city of Cernavodă in 2021. The X-axis represents time, indicating the months of analysis, while the Y-axis displays the monthly average values of solar radiation. It is observed that the minimum radiation value occurs in the first month of the year, January.

The second type of graph, presented in Figure 7 represents the variation in temperature and specific humidity around the city of Cernavodă in 2021. The X-axis represents time, indicating the months of analysis, and the Y-axis displays the monthly average values of both parameters [15].

In comparison with the minimum values of solar radiation, for temperature, it can be observed that the lowest monthly average value occurs either in the first month of the year or in the last month, while the maximum values oscillate between the three summer months. Similar to solar radiation, the minimum specific humidity values are in January for all years, with the maximum values present during the summer months.

**Table 3.** Geographical data for the third location: Medgidia

| | | |
|---|---|---|
| Location: Latitude 44.3436 Longitude 28.0258 | Medgidia | |
| Elevation from MERRA-2: Average for 0.5 x 0.625-degree lat/lon region = 57.89 meters | | |

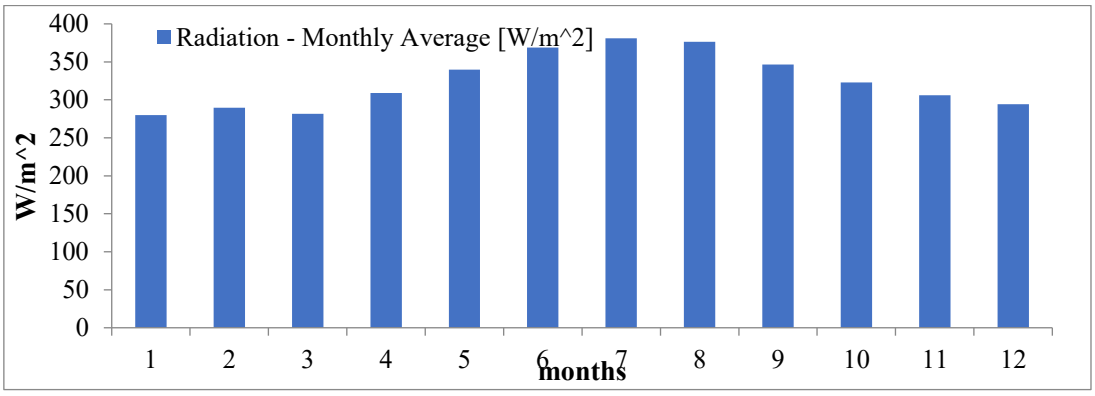



**Figure 6**. Medgidia: Radiation 2021 - Monthly Average.

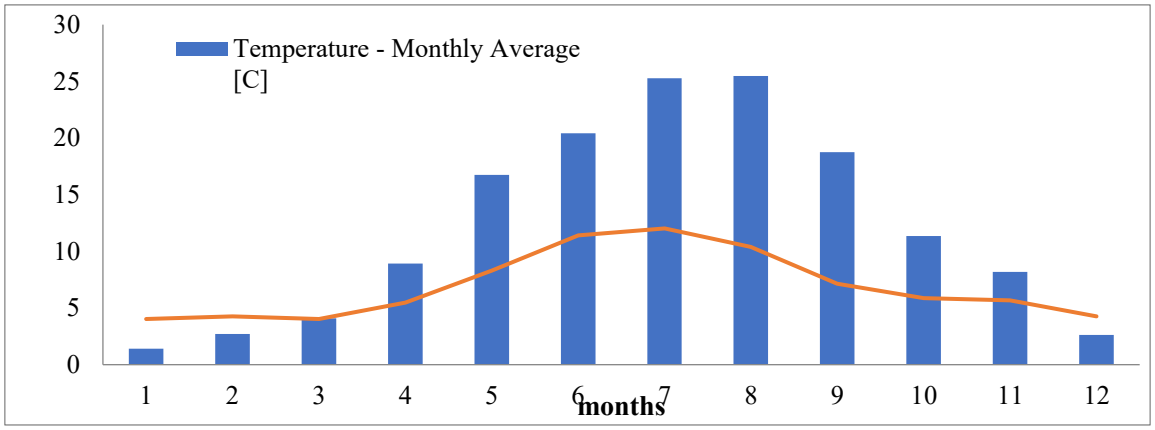



**Figure 7.** Medgidia: Temperature and Humidity 2021 - Monthly Average.

In Figures 6, 7, investigate the climatic parameter data in the second zone presented, which is in the vicinity of the city of Medgidia.

Although the second analysed zone is close to the first, we observe a different behaviour in solar radiation compared to the first case. The maximum annual value is 390.63 W/m². In terms of atmospheric temperature and specific humidity, no significant differences are observed. These two parameters are similar to those in the first zone, where the annual average temperature remains below 14°C, and the annual average specific humidity remains under 7 g/kg.
If we look at the annual average values in each individual graph, we can see that, in most cases, the ratio is 6 months out of 12 with radiation values above this average.

The Various software programs were utilized, including NASA Site, PVSyst, and PVGys. The first two programs mentioned are employed to access historical meteorological databases. NASA Power is an online platform that provides sets of solar and meteorological data from NASA research to support renewable energy, building energy efficiency, and agricultural needs. The database includes parameters for specific years categorized into annual, monthly, and hourly averages. Data on radiation, temperature, humidity, and wind intensity were extracted in 2021. The data obtained from NASA Power were analyzed and compared with those extracted from PVSyst [4]. Using the PVGys software, the total energy produced in different sections of the navigable canal with the same installed solar panel capacity was calculated. This analysis helps determine whether panel efficiency is influenced by temperature, specific humidity, and solar radiation, which vary as the canal approaches the Black Sea. Three analysis zones are taken as reference: Cernavoda, the farthest point from the sea; Medgidia, an intermediate point; and Ovidiu, where the canal flows into the Black Sea. The panels will be installed on both sides of the canal. Considering that there is an approximate distance of 25 km between Cernavoda and Medgidia, a length of 50 km (25 km on each side) is taken into account. These panels can provide electric power for electric vehicle charging stations, illuminate nearby cities, or inject electricity into the national grid. Radiation has a high value in the summer and lower values in other seasons [5].

## DISCUSSION

i) Electricity Generated on Sections

We install photovoltaic panel systems on both banks of the navigable canal at equal distances, with the same number of panels and installed power. In the end, we calculate the annual installed energy to determine if climatic parameters influenced by the Black Sea affect the amount of energy generated from the panels.

We consider the two canal sections between Cernavodă - Medgidia and Medgidia - Ovidiu as reference points. These two distances are approximately equal, with a length of about 30 km. As photovoltaic panels are mounted on both banks, we obtain a linear space of 60 km to install the equipment.

From the technical specifications of the panels, it is evident that we can install approximately 767 panels per kilometer. According to the calculations for these panels, we have a power of 514 kW (670 W/panel). For the entire 60 km stretch, we obtain a total installed power of 30.84 MW.

Using the PVGys program, we simulate the total energy over the course of a year for the two mentioned sections. The two results obtained will be compared in the end to determine if specific humidity influences the efficiency of the photovoltaic panels, even if radiation is only slightly higher, with humidity being the only parameter with significantly different values in the last zone analysed compared to the previous two.

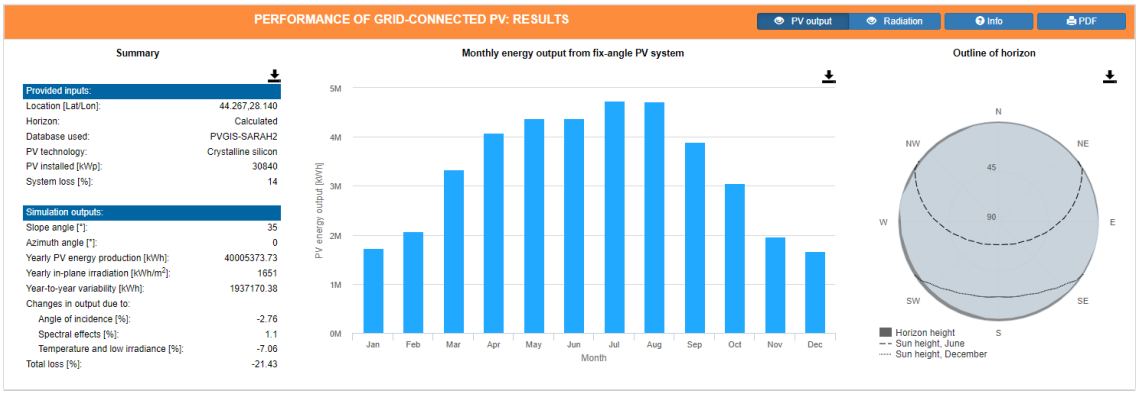



**Figure 8.** PVGys Simulation of the Cernavodă - Medgidia Section [6]

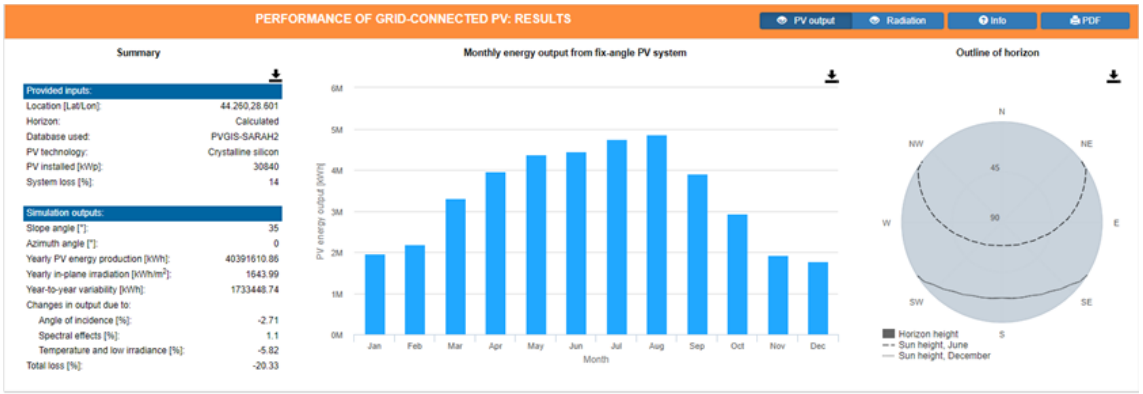



**Figure 9.** PVGys Simulation of the Medgidia - Ovidiu Section [6]

ii) Modeling Navigable Canals in Dobrogea (MATLAB Code)

$$one_km = \frac{1000}{W} \quad (1)$$

% Where one_km is the distance in one kilometer, W = 1.303 is the width of the photovoltaic panel;

$$P_i_km_1 = \frac{P_i * one_km}{1000} \quad (2)$$

% Pi_km_1 is the installed power of photovoltaic panels per one kilometer of highway [W], Pi = 670 [W] is the installed power of one photovoltaic panel;

$$P_i_km_60 = \frac{P_i_km_1 * 60}{1000} \quad (3)$$

% Pi_km_60 is the installed power of photovoltaic panels on a 60 km highway stretch

Superfast EV Charging Stations 2x175kW every 15 km in 15 km = 3 charging stations.

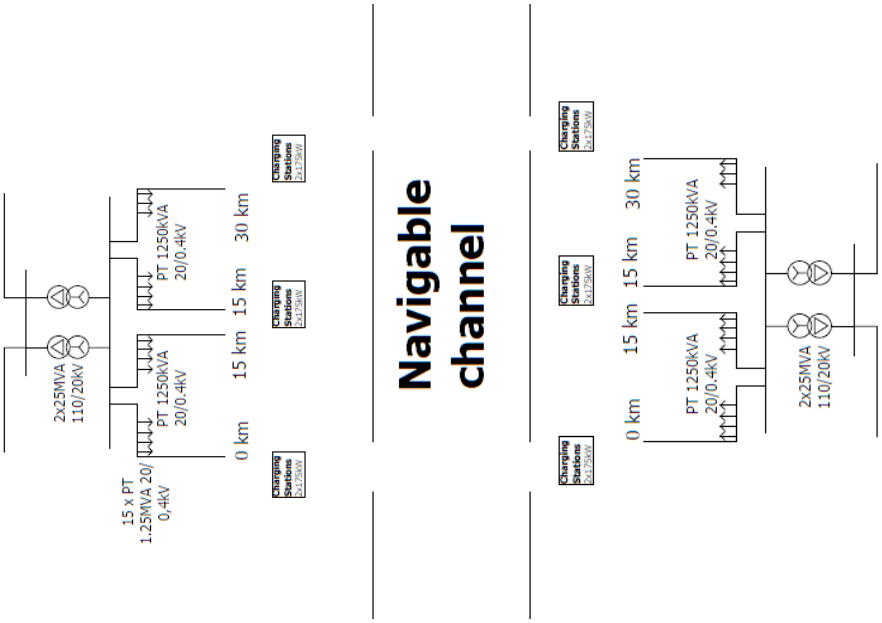



**Figure 10.** Monofilament diagram for our simulated system

iii) Simulation Results

Cernavoda - Medgidia Section
Performance of Grid-Connected PV System:
Power for each panel = 670 W
Distance of 1 km = 767 panels
Installed power for 1 km = 514 kW
Installed power for 60 km = 30.84 MW
Annual photovoltaic energy production = 40 GWh
Superfast EV Charging Stations 2x175kW every 15 km = 3 charging stations
Medgidia - Ovidiu Section
Performance of Grid-Connected PV System:
Power for each panel = 670 W
Distance of 1 km = 767 panels
Installed power for 1 km = 514 kW
Installed power for 60 km = 30.84 MW
Annual photovoltaic energy production = 40.39 GWh

Superfast EV Charging Stations 2x175kW every 15 km = 3 charging stations

Explanation of Results:

These simulation results provide valuable insights into the photovoltaic (PV) power generation potential and electric vehicle (EV) charging infrastructure for two distinct sections of the journey, namely the Cernavodă - Medgidia and Medgidia - Ovidiu sections.

**Cernavodă - Medgidia Section:**

The PV system in this section consists of panels with a power rating of 670 W each.
With 767 panels per kilometer, the installed power for 1 kilometer of this PV system is 514 kW.
Over a 60-kilometer stretch, the cumulative installed PV power reaches 30.84 MW.

The annual energy production from this photovoltaic array is estimated at 40 GWh, which represents a substantial amount of renewable energy generation.

To support electric vehicles, there are three Superfast EV Charging Stations along this route, each with a power output of 2x175 kW, positioned at 15-kilometer intervals.

**Medgidia - Ovidiu Section:**

Similar to the previous section, this part of the journey also features a PV system with panels rated at 670 W each.
The installed power for 1 kilometer remains at 514 kW.
Over the 60-kilometer span, the total installed PV power equals 30.84 MW.
The annual photovoltaic energy production for this section is slightly higher at 40.39 GWh.

Like the previous section, there are three Superfast EV Charging Stations with 2x175 kW charging capability, placed at 15-kilometer intervals.

In summary, both sections of the journey offer substantial photovoltaic power generation potential, with each having an annual energy production of around 40 GWh. Furthermore, the availability of Superfast EV Charging Stations at 15-kilometer intervals contributes to the development of an EV-friendly infrastructure, promoting sustainable and eco-friendly transportation options.

## CONCLUSION

The simulation results for the Cernavoda - Medgidia and Medgidia - Ovidiu sections offer a robust assessment of the photovoltaic (PV) power generation potential and the electric vehicle (EV) charging infrastructure along these routes in the Dobrogea region.

In both sections, the PV systems demonstrate substantial capacity, with annual energy production estimated at approximately 40 GWh. This capacity represents a significant milestone in the region's quest for renewable energy generation, a crucial step in mitigating carbon emissions and advancing sustainability efforts. By harnessing the power of the sun, Dobrogea has the potential to make a substantial contribution to cleaner and more eco-friendly energy production.

The strategic placement of Superfast EV Charging Stations at 15-kilometer intervals in both sections is a pivotal move towards encouraging widespread EV adoption. This development not only serves as a convenience for EV users but also plays a crucial role in creating a more environmentally conscious transportation network. These charging stations, with a powerful 2x175 kW output, will undoubtedly incentivize the use of electric vehicles, leading to a noticeable reduction in the overall carbon footprint of

transportation in the region. This dual approach of clean energy generation and EV infrastructure development aligns with global trends promoting sustainability and the transition to more eco-conscious modes of transportation.

In conclusion, the integration of PV power generation and EV charging infrastructure in these sections of Dobrogea represents a significant leap towards a greener, more sustainable future. These initiatives are in harmony with the worldwide shift towards renewable energy sources and the widespread adoption of electric transportation. Through these efforts, the region contributes to a cleaner environment, a reduced reliance on fossil fuels, and a brighter, more sustainable tomorrow.

## ACKNOWLEDGEMENT

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# SUSTAINABLE SOLUTIONS FOR TEMPORARY ACCOMODATION IN POST-DISASTER MODULES. SPATIAL CONNECTION IN STRUCTURES.

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## ABSTRACT

The report explores spatial connectivity options for housing modules for post-disaster and emergency shelters. Various high-density module clustering archetypes are being explored and sustainable solutions are elaborated regarding saving usable space, utilizing resource efficiency and implementing environmentally friendly materials. Usually, the people affected by disasters are accommodated in unfavorable conditions, so the study explores decisions that ensure comfort during temporary habitation along with a regard for nature. Through the synthesized principles, it is demonstrated that even in distress conditions imposing severe constraints, a quality housing can be achieved.

**Keywords:** emergency accommodation, comfort of habitation, sustainable post-disaster residential modules, residential modules clustering

## INTRODUCTION

This report forms part of a research project, *"Prefabricated Modules for Temporary Accommodation in Disaster and Crisis - Alternative Digital Conceptual Solutions"*. One of the key aims of this project is to develop a systematic database categorising the currently available modular and prefabricated temporary shelters according to their intended purposes. The analysed data will be used to develop a digital prototype of a prefabricated modular temporary shelter for disaster and crisis relief. The study will investigate techniques for integrating and assembling shelter modules into different structures. The ultimate goal is to build a prototype module.

According to data from the National Statistical Institute in Bulgaria [1], heavy rainfall has caused the major disasters in recent years, resulting in flooding and landslides in coastal and mountain regions (Fig. 1). As a consequence, a significant proportion of the housing stock is unsuitable for prolonged occupancy. The lack of prompt response from accountable entities requires alternative options for temporary lodging that meet contemporary standards of living comfort, housing durability, and timely assembly as required. In reality, currently available solutions are restricted to placing temporary containers on one level in close proximity to flood-affected areas, erecting tents for mass accommodation of displaced residents, or arranging lodging in nearby hotels (Fig. 1). However, these solutions are inadequate in their fragility and discomfort, and are prolonged due to institutional hurdles. This prompts the question of how to improve the conditions in which destitute individuals conduct their daily activities and work,

positioning them as inactive or inferior members of society. **Providing shelter alone may not suffice - what measures involving thermal, health, psychological and social comfort are necessary? How can architecture be used to facilitate this?**



*Fig. 1 - Flood damage in Bulgaria (2022, Karavelovo village, Karlovo district - left), temporary shelters for victims (right) - Source: personal records and online sources*

## MATERIALS AND METHODS

According to Quarentelly's [2] classic distinction, there are three stages in the recovery process: **temporary**, **transitional**, and **permanent housing**. The latter categorises them within the crisis-recovery timeframe. The first group provides relief: temporary accommodations during which people do not establish routines. It encompasses the first group - "sleeping spaces" in large existing buildings - and the second group - "sleeping units" categorised based on Shigeru Ban's work. The present research centres on enhancing and advancing the quality of living in the transitional shelter category, referred to by Quarentelly as temporary accommodation that facilitates the re-establishment of daily routines, designed for short-term stays. These shelters comprise housing modules and micro homes. Restoration of permanent housing is overseen by the government and lies beyond the scope of this study.

This report provides a systematic overview of various forms of temporary accommodation, such as those created for recreational and tourist purposes, mobile homes, modular solutions, and the work of Shigeru Ban in the field of post-disaster housing. **The primary aim of this study is to summarise these forms of temporary accommodation. A. The potential for clustering separate modular housing units into a unified structure, B. The strategies for improving comfort in compact dwellings, and C. The formulation of remedies and standards for constructing such comfort in an emergency shelter.**

- **Temporary accommodation for leisure and tourism purposes**

This could be exemplified through various implementations of lightweight and mobile housing units, allowing for an adaptable and innovative approach to recreational housing. The Cocoon Tree & Cocoonsplash project (Fig. 2), for instance, suggests a spherical solution consisting of "a lightweight aluminium frame and waterproof membrane that can be suspended between trees (stretched with ropes) or be on the ground on supports" [3]. The accommodation consists of two base modules, a sleeping module (D=2.40 m; Total Area ~ 4.5 $m^2$) and a sanitary module. Suspending the structure at height proves to be a desirable option in situations of subsequent intense rainfall or landslides as it can be detached from the ground. Nonetheless, the minimum capacity of the module and the separation of the sanitary unit from the actual accommodation pose disadvantages.



*Fig. 2 - Cocoon lodge, floor construction + sanitary module; Berni du Payrat ; source: https://www.cocoontree.com/gallery-cocoon-tree [24.10.2023]*

- **Mobile homes**

The mobility of Bulgarian development KOLELIBA (house on wheels) in assembled form makes it a useful example. It is aimed at a higher class of environmentally friendly living and recreation, but provides insights for constructing a modular, relocatable housing unit that could be useful for emergency housing situations.

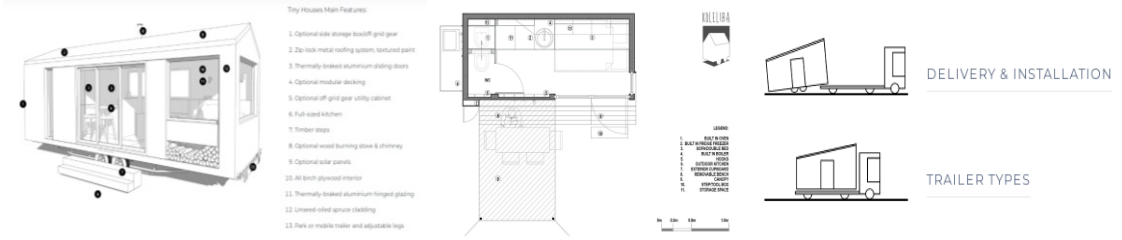



*Fig. 3 - KOLELIBA – model, floor plan and transportation options, M. Arch. Hristina Hristova; source: https://www.koleliba.com/tiny-houses [24.10.2023]*

The measurements are in accordance with the potential of transportation in a ready-to-use structure - 4.50×2 m or a surface area of 9 $m^2$. The plain design has received acclaim globally from numerous architectural publications (dezeen.com, archello.com, archdaily.com) [3]. The module is arranged in a compact and functional way, with a height of around 2.40 m, a sloping roof, and is constructed with oiled pine timber. The product has some drawbacks, including complicated logistics and storage difficulties due to its assembled form requiring more space. Additionally, the richly glazed facade, while suitable for outdoor recreation, can present challenges in achieving privacy within groups.

- **Emergency Housing by Shigeru Ban**

Shigeru Ban is a world-renowned architect, having great experience on the topic with more than 40 projects around the world since 1995 [4]. The designs of his different possible accommodation types could be grouped in three main categories, presented in the table below - **a. multiple sleeping units under the roof of an existing building b. standalone sleeping units with shared toilets** and **c. micro living units** (Tab. 1).

The first group is situated in large pre-existing structures, which limits the adaptability in situations where no shelter is available. The second group, we refer to as the "sleeping units," comprises variously sized shelters that only provide sleeping areas for a specific number of people. Other activities are clustered in shared toilets and dining spaces. Consequently, they lack essential amenities and comfort. The third grouping comprises "housing modules" or "micro homes." They are classified as a form of

accommodation for an extended duration due to their provision of kitchens and bathrooms, which are the most relevant aspects of comfort according to recent research.

*Table 1 – main emergency housing types defined by authors, based on the work of Shigeru Ban; source: https://shigerubanarchitects.com*

| MULTIPLE SLEEPING UNITS UNDER THE ROOF OF AN EXISTING BUILDING | STANDALONE SLEEPING UNITS WITH SHARED TOILETS | MICRO LIVING UNITS |
|---|---|---|
|  |  |  |
| 2022, Ukraine Refugee Assistance Project | 2023, Relief for Noto, Japan | 2011,Onagawa Container House |

- **Basic principles derived for module configuration**

Based on the examples considered, it can be concluded that in order to ensure comfort, the main housing unit should have a minimum size of between 12 $m^2$ and 15 $m^2$. For the research, two variations of the housing unit were taken into account, with a minimum area of A=3.5 $m^2$ per person and a minimum height of h=2.4 m for cold climates and A=4.5 $m^2$, h=2.40 m for hot climates [5].

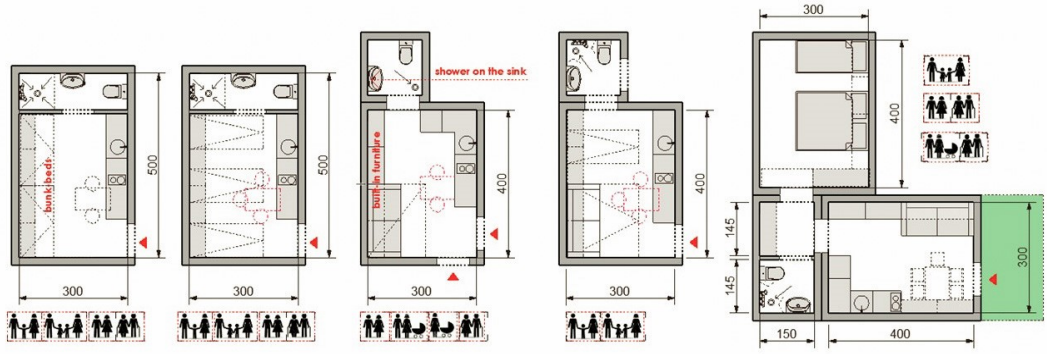



*Fig. 4. a. solutions for a 3×5 m housing module with integrated bathroom - left; b. solutions for a 3×4 m housing module with a separate 1.5×1.5 m bathroom module for different number of occupants - right*

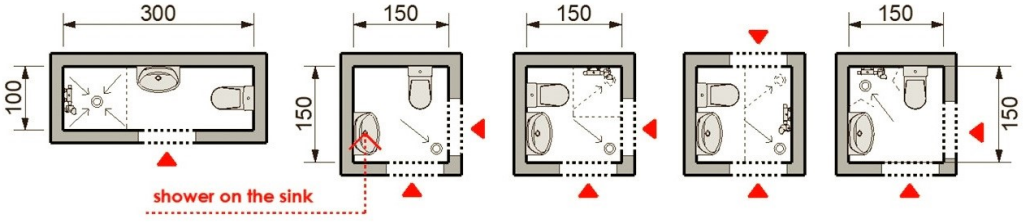



*Fig. 5. a. solutions for a sanitary module integrated in the living room with dimensions 1×3 m - left; b. solutions for a separate sanitary module with variations of furnishing and access - dimensions 1.5×1.5 m – right*

The initial version of the module includes an indoor bathroom with optimal dimensions of 3×5 m (see Fig. 4.a). The second option is a 3×4 m module investigated with a 1.5×1.5 m adjacent bathroom accessible from its interior to facilitate dwelling unit assembly (see Fig. 4.b). The most effective solution would be to install the modular bathroom after prefabrication in the factory.

- **Optimal module construction and materials**

The optimal choices for the load-bearing structure of the modules are two-fold, either a load-bearing skeleton or self-supporting external walls. It is advisable to make use of prefabricated elements made of steel, wood, or aluminum. For the former option, both the enclosing and partitioning components ought to be fashioned from lightweight, sturdy materials that can undergo functional modifications. Conversely, in the latter alternative, this is only applicable to the partition walls. The module should have thermal and acoustic insulation properties ensuring optimal energy efficiency across all temperature variations and seasonal changes in any module configuration.

Viable options include utilizing straw modules (ecocon panels or straw panels [6,7]) set within a timber frame (Fig. 6-A) or steel sandwich panels (PUR/PIR) for enclosure and gypsum board or OSB stud walls for partitioning. The module foundations (Fig. 6-B) need to rise above ground level to enable their use across sites with different soil hardness and composition levels. Ground anchors [8] represent the accessible options on the Bulgarian market and allow the construction of buildings with a maximum height of two storeys. In global practice, gabions have been utilized as foundations, with each gabion serving as a point foundation. Additionally, there have been unconventional proposals such as using beer crates filled with sandbags, suggested by Shigeru Ban. However, implementing these techniques in Bulgaria presents difficulties due to elevated earthquake safety standards.

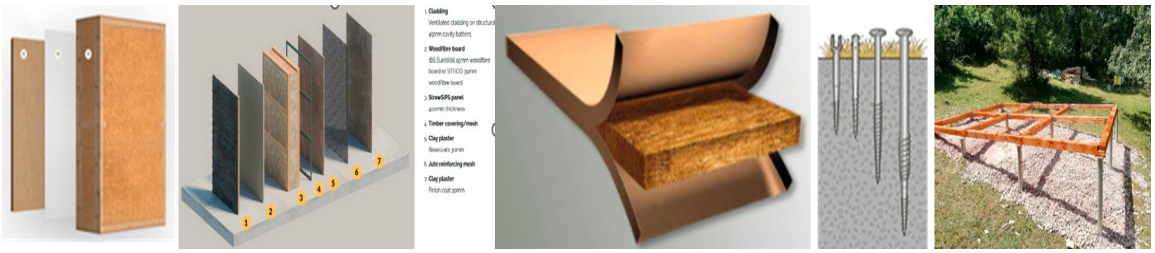


*Fig. 6 - A. solutions for enveloping walls of straw panels [6, 7] - left; B. solutions for foundations with earth anchors - right [8]*

- **Investigating potential spatial connections between modules**

In the context of achieving cost-effectiveness, optimal load-bearing design and rapid assembly without heavy mechanisation, this research concentrates on single-level spatial configurations.

The fundamental designs comprise of point, linear-sided and angular (Fig. 7), which can be implemented in various scenarios based on the displacement, area and shape of the terrain provided. Linear schemes are highly adaptable but may lack variety, which can be addressed by creating spatial breaks through the removal of modules for shared spaces. Point and corner schemes, on the other hand, are more space-efficient although better suited to flatter terrain.

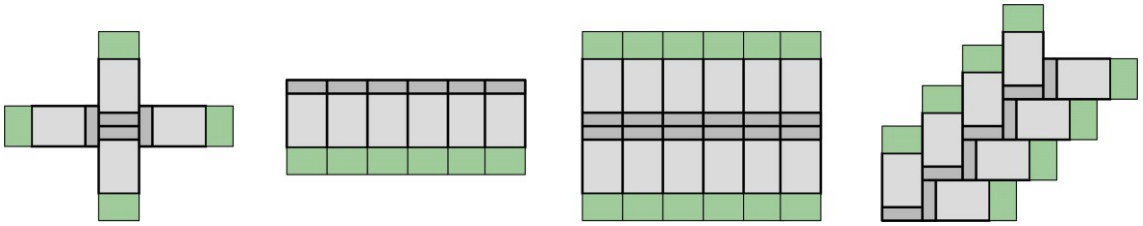


*Fig. 7 – Different types of spatial connections of the modules - point placement, linear connections, bilaterally arranged linearly connected housing units, angular connections*

More complex spatial configurations are L-, T-, U- and E-shaped schemes (Fig. 8), which provide more variety but require more space and relatively flat terrain. At the same time, they offer the greatest opportunities for creating shared social spaces providing mini social communities, which are much needed for psychological comfort and support for victims..

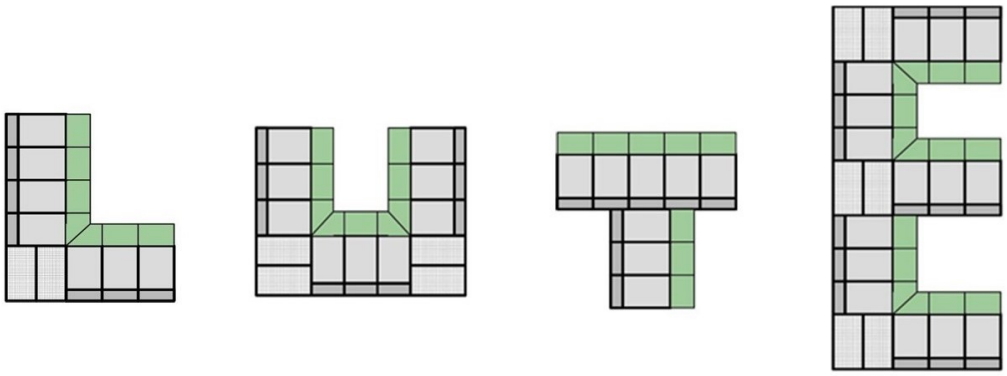


*Fig. 8. L-; U-; T- and E-shaped placement of housing units.*

Spatial combinations of the above-described configurations can be utilized horizontally or vertically to form diverse mixed, compact, or clustered schemes. This increases the intensity of temporary accommodation and enriches the architectural vision.

The compact dwelling, measuring 3×4 m, adopted in the present study, may be integrated into any of the previously described configurations, according to the specifics of the site. (Fig. 9.) The separation of the sanitary unit in a module (Fig. 5.b.), adjacent to the finished unit and accessible from the inside, is considered effective.

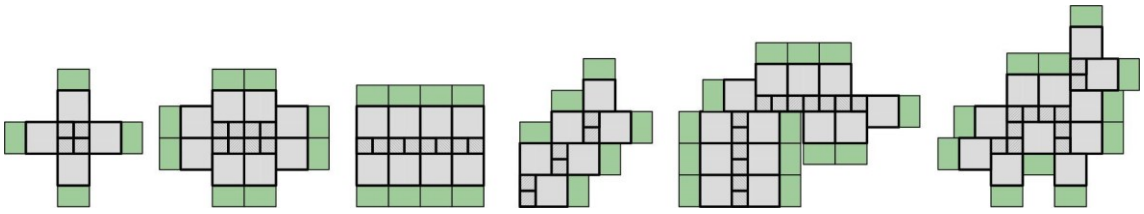


*Fig. 9. – spatial connections between modular units measuring 3×4 m and grouping of adjacent sanitary modules measuring 1.50×1.50 m.*

## RESULTS AND CONCLUSIONS

From the analysis of the examples provided, certain principles are applicable to the design and construction of a future prototype housing module to offer more comfortable temporary accommodation in the long-term. These entail both the individual housing unit and their collection.

- **In relation to the height/floor of the module/structure:**
  - Maximum height of 2-3 storeys - psychological limit in case of earthquakes and floods;
  - Separation from the ground - ecological and fast foundation.
- **In relation to choice of materials used:**
  - In terms of sustainability, use recyclable materials - steel and/or wood pre-treated to withstand wet environments;
  - Limiting the size of the module for ease of transport and storage - a solution of individual elements rather than a whole large module, ecopanel insulation - straw panels up to 20 cm thick, to facilitate grouping and achieve minimum carbon print;
  - OSB partitions - as only the sanitary units are partitioned in the schemes developed.
- **Regarding the functional solution of the module itself:**
  - The sanitary facilities should be a prefabricated module (bathroom unit);
  - All principles of functional densification, defined by the authors in previous studies, are relevant, with the most cost-effective options being **static densification** (horizontal, vertical, requiring greater heights and creating more potential installation and transport difficulties) and **dynamic densification** by means of hidden and transformable furniture;
  - Optimal spatial relationships are: in terms of functional solution - the point ones, in the formation of a cruciform structure (Fig. 10-C); in terms of land use and compactness of the solution - linear, bilaterally oriented configurations (Fig. 10-A); and in terms of volumetric solution and the creation of an above-ground residential scale - combinations of L-shaped groups of two modules. (Fig. 10-B)

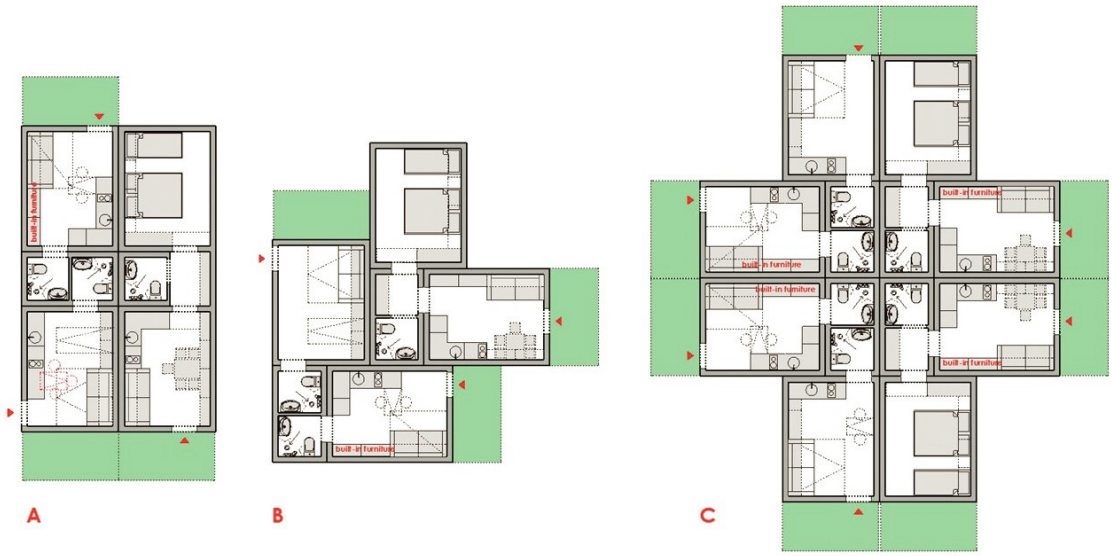



*Fig. 10 - spatial relationships between individual modules with furniture, contact of different groups; A - linear, bilaterally oriented structure; B - L-shaped combination of two modules; C - cruciform combination*

The upcoming project phase involves investigating methods of powering the housing modules via renewable energy sources, implementing grey water recycling, and improving light and air circulation within the units using roof vents, roof construction and material selection in order to achieve sustainable housing goals.

The crucial stages in the recovery and return to normality for individuals affected by natural disasters include: securing residents' comfort by applying principles of functional densification; building a sturdy volumetric structure featuring appealing architectural features; providing necessary amenities such as individual sanitary facilities; ensuring optimal thermal comfort for all seasons incorporating energy-efficient materials and ultimately - using architectural designs to convert shared spaces into mini social communities.

To conclude, the architects' proposals should prioritize not only residential amenities but also the psychological, health, and social comfort of the inhabitants to promote their subsequent social reintegration, while applying ecological solutions for installations and materials to protect the environment.

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# TAX BREAKS FOR THE ENERGY RENOVATION OF ITALIAN BUILT HERITAGE. KEY ISSUES AND FUTURE PROSPECTS FOR A NEW TRANSITION PROCESS.

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## ABSTRACT

The transition of built heritage towards energy efficiency is a complex process, encompassing a wide range of aspects and challenges. Moreover, the ongoing climate change, marked by rising average temperatures and an increase in extreme weather events, amplifies the urgency to improve energy efficiency and the overall performance of buildings. This necessitates the crucial adoption of appropriate solutions. Within this scenario, the discussion brings to light two key elements that define the current situation. The first element pertains to the existing regulatory framework, extending its scope to include the recent proposal for revising the Energy Performance of Building Directive (EPBD), a component of the 'fit for 55' package. The second element concentrates on the energy efficiency measures executed in Italy after the introduction of the 'Superbonus 110%' tax break, offering an initial interpretation of ENEA's data on the measures implemented until 2021. A thorough examination of these two elements allows us to pinpoint crucial factors that can help foster a model that harmoniously integrates the preservation of built heritage with the sustainability and effectiveness of energy efficiency measures.

**Keywords:** energy efficiency, tax breaks, energy transition, built heritage, Italy

## INTRODUCTION

The energy transition process of the Italian building stock is a current and much-debated topic in the scientific and regulatory fields [1, 2]. In Italy, buildings represent a significant part of the architectural and cultural heritage. Their efficiency is a crucial aspect in contributing to reducing greenhouse gas (GHG) emissions and mitigating the effects of climate change. It is important to immediately report here the extent of the assets involved to fully understand the impact and extension the process will produce in the coming years. According to estimates by the National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), buildings of historical-architectural value amount to approximately 5.6 million units, of which 3.2 million are residential properties built before 1945 [3]. To these should be added the buildings that will potentially be affected by total or partial energy improvement interventions. Although the data is limited to only residential buildings, approximately 50% of the building stock was made before 1970 [4] and, therefore, before the first Law on energy containment of 1976, Law N. 373/1976. A good part of this heritage is characterized by a low energy performance due to its nature, the materials, and the

techniques used, which cause a high use of energy with consequent negative impacts on the environment and on the financial balance of the owners and institutions that have their management. A fact that deserves attention concerns the use of final energy: buildings consume 40% of the European Union's total energy [5] and produce 36% of GHG emissions. Furthermore, heating, cooling, and hot water production represent 80% of the energy consumed by families [6].

## MATERIALS AND METHODS

Despite the challenges [7], the energy transition process must address protection needs by opening up to an initial reflection. The Italian legislation on the subject is mainly oriented towards new buildings and on the identification of limit values to be respected in the case of interventions on the existing stock. Law N. 90/2013 and Legislative Decree N. 102/2014 precisely define these limits. The so-called monumental buildings listed under the Code of Cultural Heritage and Landscape (Legislative Decree N. 42/2004) are excluded from these requirements. While slowing down the transition process, the Code protects the heritage from negative consequences on the conservation of its distinctive characteristics. Protected historic buildings represent, however, a small part of the Italian architectural heritage, and it is possible to identify a precise operating area within which to work from an energy point of view. In addition, it is always permitted to make an exception if energy improvement operations are incompatible. On the other hand, the unprotected building stock must face this process directly and without safeguards, clashing only with rigid performance parameters.

At a time when energy improvement interventions have gained more strength thanks to tax breaks at the national level starting from 2006, the Ministero dei Beni e delle Attività Culturali e del Turismo (MiBACT) in 2015 drew up the "Lines for improving energy efficiency in cultural heritage" [8]. This important document represents the first accurate thematic reference accompanying professionals and protection institutions along the way. The guidelines collect a series of indications for energy diagnosis, offer a tool for evaluating interventions, and provide examples divided by typology [9]. The document filled a critical gap but constitutes only a starting point for the numerous interdisciplinary challenges of the transition process. One above all is the definition of a real process that brings together the energy and conservation needs to arrive at a conscious project.

The EN 16883:2017 standard on "Conservation of cultural heritage - Guidelines for improving the energy performance of historic buildings" fits precisely in this direction by proposing a procedure for evaluating and identifying efficiency actions for buildings officially designated as Cultural Heritage and for buildings of all types and ages. It delves into the interventions and their impact concerning the conservation of the elements that characterize the buildings and their value by proposing a risk assessment matrix divided into seven categories. This tries to combine all the needs at stake while remaining not very indicative and leaving too much space for interpretation.

The EPBD revision proposal is part of the Climate package ('Fit for 55' package) of the European legislation on the Green Deal and is included in such a solid and fixed regulatory context from a performance point of view, but, at the same time, still uncertain from a procedural point of view. The objective is a 55% reduction of GHG emissions compared to 1990 levels by 2030 and to achieve a zero-emission building

stock by 2050. The revision follows the first EPBD of 2010 (Directive N. 2010/31 /EU), and the second version of 2018 (Directive N. 2018/884/EU), which had already set pressing limits. This decision arises from buildings' role and the growing awareness of the need for urgent action due to climate change. The revision provides new ways of certifying energy performance to standardize and allow comparisons between properties in European countries. The classes will be remodeled starting from class A for zero-emission buildings up to class G, which must include approximately 15% of the most energy-consuming buildings.

Regarding efficiency measures on buildings, public or non-residential properties must reach class E by 2027 and class D by 2030, while residential buildings will have three more years available. A series of exemptions would apply to monuments, buildings of particular historical-architectural value, technical buildings, places of worship, publicly owned social housing, buildings whose renovation works would lead to rent increases that cannot be compensated with savings in energy bills, buildings for temporary use (less than four months a year) and independent ones with a surface area of less than 50 square meters. Furthermore, individual countries can ask the EU Commission to exempt up to 22% of all residential buildings from the obligations and to extend the deadline to January 2037 for economic reasons and the unavailability of a qualified workforce [6]. Therefore, The civil sector will have to contribute significantly, with a drastic reduction in emissions through in-depth redevelopment interventions. In this scenario, even the existing assets and, in particular, residential properties will be affected by massive energy efficiency interventions to reduce the distance from modern buildings and get closer to the standards of a zero-emission building stock.

It is helpful to report here some data to understand the extension of the interventions if the proposed revision of the EPBD is approved. From the analysis of the Energy Performance Certificates (APE) issued in Italy between 2018 and 2021, it can be seen that 34% of the certifications place the properties in class G, 24% in class F, and 17% in class E, for a total of 75% on the overall. The data for the year 2021 highlight a slight improvement in the energy performance of the Italian stock subjected to certification, with a decrease of approximately one percentage point among properties positioned in classes G, F, and E and an increase of approximately half a percentage point among class A buildings. However, there is a clear difference between the Energy Performance Certificates of residential and non-residential buildings. The former, in fact, places 76% between classes G, F, and E, while the latter reaches a total of 60% in the same categories [10]. The main concern can be understood thanks to a first estimate of the number of buildings that would be affected by energy efficiency interventions following a possible adoption of the EPBD proposed revision. In this scenario, the nature of the problem can be understood by calculating the number of buildings that would be affected by such interventions. Keeping the position of the latest negotiations valid, priority will be on the 15% of the most polluting buildings, equal to approximately 2.25 million buildings, including exempt buildings. According to the National Association of Home Builders (ANCE) forecasts, between 1.4 and 1.8 million buildings would be divided equally between flat complexes and individual residential units [11]. The properties should, therefore, move to class E by 2030 if residential, while by 2027 if non-residential, reaching class D by 2033 or 2030. Furthermore, all the buildings that will be included in classes F and E with the new certification methods will be added. In

these terms, using the current classification, approximately 9 million buildings would need to be redeveloped by 2033 or 2037 if the EU will accept the time extension.

## RESULTS

The panorama that has emerged leads to specific reflections that affect multiple aspects. If we were thinking of energetically renewing the national real estate park even regardless of the possible evolution of the EPBD, an open and multidisciplinary discussion would be necessary to ensure that most of the components can coexist. The 'Rilancio' Decree N. 34/2020, the so-called 'Superbonus 110%' [12], fits into this framework and introduces extraordinary measures to support the recovery of the construction sector with advantageous incentives for interventions aimed at seismic improvement ('SuperSismabonus' ) and/or the energy efficiency of existing buildings ('SuperEcobonus'). The introduction of the first tax breaks in 1998 provided new intensity to the renovation field of the building stock. Starting from that year, it is possible to notice an increase in building recovery interventions. The introduction of incentives for energy requalification in 2007 confirmed the trend [4] thanks to the progressive increase in the amounts deductible, which went from 50% to 65-75% of the total works carried out, stabilizing at an average amount of around 2 billion euros on about 335,000 interventions per year. However, the 'Superbonus' has radically changed the scenario by allowing a 110% tax deduction on the expenses incurred by citizens/managers for energy ('SuperEcobonus') and/or seismic ('SuperSismabonus) improvement interventions through an invoice discount mechanism and the assignment of credit. The eligible operations must fall within the regulations introduced with Law N. 296/2006 and subsequent updates up to the 'Rilancio' Ministerial Decree of 2020. In extreme summary and in order not to dwell here on the details of the Decree, the 'Superbonus' applies to residential buildings, in particular to single-family properties, to the common areas of flat complexes, and to functionally independent residential units. Limiting ourselves here only to energy efficiency needs and, therefore, to the 'SuperEcobonus', the operations falling within the benefit are divided into two categories: the driving interventions and the driven ones. The former include interventions regarding: a. the insulation of the opaque vertical, horizontal, and sloped structures that enclose the building with an incidence greater than 25% of the gross dispersion surface; b. the replacement of winter air conditioning systems with cooling and/or heating systems (for example, condensing boiler, heat pump, micro-cogeneration systems, solar thermal collectors). In addition, so-called driven interventions can also be included as long as at least one of the driving forces is carried out: a. further energy improvement interventions, including the replacement of windows or the equipment of solar thermal panels; b. the installation of photovoltaic solar panels connected to the grid; c. installation of systems for charging electric vehicles. To access the benefits, all the interventions carried out must demonstrate an effective energy improvement of the property between the original situation and the future one. Expressly, the admissibility of the interventions is conditioned on the passage of at least two energy classes [13] to be calculated through the drafting of a statement with the description and technical data of the planned operations and the uploading of additional documentation, including the Performance Certificates Energy (APE) *ante* and *post operam*.

The introduction of the 'SuperEcobonus' offers a broad field of experimentation, which should be useful in generating a document to bring order to the energy transition process and evaluate its effects on the built heritage. ENEA makes available a series of raw and partially processed data that allow further reflections to be developed. The information available to date concerns the year 2021 [14] and records the activation of almost 92,000 construction sites, which involved investments eligible for tax deductions for more than 15.2 billion euros. From a numerical point of view, the interventions concerned 15% flat complexes, 52% single-family buildings, and 33% independent residential units. In terms of investments eligible for deductions, however, 48% was used for operations on flat complexes, 33% on single-family buildings, and 19% on independent residential units with average amounts of approximately €543,000, €108,000, and €96,000 respectively. In general terms, the operations focused on the building envelope (61.7%), the heating systems (17.9%), and the installation of photovoltaic solar systems (16.7%). As regards the interventions on the building shell, it is interesting to analyze the subdivision by type, which is reported in the summary data and highlights a clear distinction. Vertical walls' insulation operations, ceilings and dispersing roofs, and fixtures' replacement represent 88% in economic terms, 86% concerning the renewed surface, and 92% of annual energy savings on the total interventions on the external envelope. The operations to improve the efficiency of floors, horizontal walls, or non-dispersing roofs and the insertion of solar shading represent only 12% in economic terms, 14% in the renewed surface area, and 8% in terms of annual energy savings.

Regarding the activities on the building shell, the most performed intervention is the insulation of the vertical walls, followed by the replacement of the windows and doors. The association of the two interventions is preferred almost exclusively by their high efficiency and the possibility of obtaining the double passage in energy class to access tax deductions. The replacement of the heating system with a condensing boiler or heat pump is often added, as well as the installation of photovoltaic solar systems for electricity production. The document also reports some data referring to 2022, highlighting an exponential growth in activated construction sites, around 250,000, with investments close to 43 billion euros. Regarding 2023, only the indicative number of 60,000 construction sites started is known, with an investment of approximately 17 billion euros. This information is extremely brief and not yet consolidated. We will have to wait until the beginning of 2024 to have a more precise picture of the situation and to go into more detail on the operations carried out.

## DISCUSSION

The EPBD revision proposal is currently under interinstitutional discussion, and its possible approval does not yet seem that close. Not even its actual contents seem consolidated apart from the general framework aimed at reducing consumption and GHG emissions. However, drawing a first picture and evaluating its theoretical applicability in the Italian context is possible. Considering today’s reconstructions, which involve 1.8 million buildings in the worst-case scenario, it can be said that the construction sector would barely be able to deal with the entire process in the required time frame. The 'SuperEcobonus' expressed a particularly high number of construction sites that started considering the interventions' complexity and extension. However, they

would not be sufficient to satisfy the EU's requests. Although affected by the pandemic in 2021, the sector's capacity was approximately 155,000 construction sites per year, also confirmed by the performance of 2023. This would mean completing the planned activities on the 1.8 million buildings in just over 11 years. Trying to improve the estimates by including data before 'SuperEcobonus' and, specifically, regarding the 'EcoBonus' and the 'Bonus Casa' is complex. These measures are characterized by interventions that are less complex on average, shorter, and less energetically effective but decidedly more substantial, as highlighted by the Market Research Centre (CRESME) [4] and ENEA [14]. Although the numbers may seem encouraging, such activities will not be able to contribute, if not minimally, to increase the capacity of the building sector to meet the energy transition needs desired by Europe. Most of these interventions are actually aimed only at the replacement of windows (34.1%) or boilers (32.3%) or the installation of heat pumps (12.5%). These operations affect similar categories but not directly the construction sector and would not allow the double or triple energy class passage. The data published by ENEA, although interesting and helpful in evaluating the performance of the 'SuperEcobonus' from a quantitative, economic, and statistical point of view, do not, however, allow in-depth analysis and, above all, a direct connection with the buildings subjected to the energy retrofit.

Furthermore, it is impossible to read this information in a shared way to the context it belongs to despite the procedure requiring the drafting of an in-depth document [14] aimed at declaring the type of each individual operation with precise indications regarding the energy condition before and after the intervention and the delivery of the two Energy Performance Certificates (APE). Unlike what can be found in the project documents (certification and APE), there is no trace, for example, of data regarding the construction period, the building type, the intended use, the energy sources used, and the climatic zone. These indications, if well systematized and declared together with the data in the report, would allow more targeted analyses and provide valid proposals for professionals and institutions that manage buildings.

## CONCLUSION

Awareness of the problems linked to the energy crisis and pollution, together with the decisions taken at the European and national levels aimed at mitigating the impact of man on the environment by intervening in the construction sector, allow us to confirm the progress of the energy transition process and to highlight its urgency. Tax breaks helped start the process, but it still shows many limitations. The central role within the debate is played exclusively by the performance aspect, the only one that affected the procedure if we consider that the economic one is irrelevant as it is entirely supported by the State and, precisely for this reason, it is the cause of anomalies and contradictions [15]. The operations are actually selected on the basis of the transmittance parameter they guarantee, without considering the material, dimensional, historical-architectural, and cultural characteristics of the building. Although the MiBACT guidelines [8] and the EN 16883:2017 standard propose methodologies and approaches aimed at highlighting these peculiarities and filling this serious procedural gap, in practice, these aspects totally lose importance, leaving them solely to the perception and experience of the designer or manager/administrator. In addition, the interventions carried out show the massive use of traditional insulating materials, which are often too expensive from

an energy point of view and impactful considering their life cycle compared to more innovative or nature-based solutions. The uncertainties highlighted so far demonstrate that they have a negative impact on the concept of sustainability that underlies the energy transition process, undermining its founding pillars: environmental, economic, social, and cultural. The instruments available today and dedicated to the process of energy improvement of buildings are already numerous, such as the indications at the European level, and have contributed to large-scale experimentation on a national scale. Although partial, the outcomes should change and foster a new debate to adopt a multidisciplinary and integrated approach that considers the technological, material, architectural, economic, and conservative aspects with the same level of importance.

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# THE POTENTIAL IMPACT OF BLUE-GREEN INFRASTRUCTURE ON RESTORING BIODIVERSITY IN AN URBANIZED LANDSCAPE.

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## ABSTRACT

Climate change has precipitated severe issues such as soil erosion, intense precipitation and extended droughts, impacting not only agricultural sectors but also ordinary citizens globally. Unfortunately, human interventions frequently exacerbate, rather than mitigate, these problems. Our approach emphasizes the application of environmentally friendly blue-green infrastructure, recognized for its potential in mitigating climate change effects and enhancing living conditions for both the local populace and the indigenous fauna and flora in treated areas. This strategy is a recurrent focus of environmental programs and projects within the European Union and worldwide. The main goal of our research is to determine of the impact of blue-green infrastructure on potential development of biodiversity restoration in selected model territories using development prediction based on the latest scientific results from similar studies and the professional statistical software Statgraphic centurion 2018. We conducted an assessment at three designated locations within the Zavar village cadastre. Our analysis, based on a comprehensive dataset and meticulous observations, anticipates a significant augmentation in both macrobiota and microbiota diversity, as well as improvements in the fungal and plant community structure within the specified sites. Our evaluation addresses the alignment of these modifications with the specified criteria and identifies any deviations and intercorrelations between treatments applied and groups of organisms and plants observed. We provide actionable recommendations and results will be applicable for predicting and optimizing effect of any planned blue-green infrastructure on biodiversity in business practice.

**Keywords:** blue-green infrastructure, biodiversity, microbiota, fauna and flora

## INTRODUCTION

As the global population grows, the demand for food, fodder, fiber, and bioenergy rises. In Europe, this has driven agricultural intensification, simplifying, and specializing agroecosystems through reduced landscape heterogeneity, increased chemical use, and the abandonment of less fertile areas. These processes have diminished habitat quantity and quality, leading to decreased biodiversity across trophic levels and spatial scales. This biodiversity loss has significantly altered European agroecosystems over the last 50 years [1]. Additionally, unchecked building activity and the erosion of blue and green landscape elements threaten existing habitats and wildlife mobility [2]. Urban development also leads to changes of surface cover that disrupt the hydrological cycle in cities. Impermeable surfaces and the removal of vegetation reduce the ability to intercept, store and infiltrate rainwater [3] and has placed increasing pressure on urban

ecosystem services [4]. European urban areas are home to over two-thirds of the population, with a level of urbanisation that is expected to increase to approximately 83.7% in 2050 [5] and can contain rich flora that contribute significantly to biodiversity, but loss and isolation of habitats due to urban sprawl threaten biodiversity and warrant limits on development [6]. We face the challenge of maintaining provisioning services while conserving or enhancing other ecosystem services and biodiversity in urban and agricultural landscapes [7]. There is a growing concern about biodiversity loss and its implications on health, well-being, climate, economy, degradation of nature, and social inequality. Ecosystem degradation erodes nature's ability to support human societies, as ecosystems provide a wide range of services [5], however integrating the outputs of multiple ecosystem service assessments to provide recommendations for nature-based planning remains a challenge [4]. Nature-inspired Blue-Green Infrastructure (BGI), designed initially for sustainable stormwater management and enhancing urban liveability, is now recognized as a local-scale measure with emerging synergies for biodiversity [2], while is designed to address long-term sustainability and create multi-benefits for society, culture, business, and ecology [8]. Urban green-blue infrastructures (GBI) provide a spectrum of services, essential for human survival (e.g., maintaining or restoring hydrological functions for clean water) and enhancing well-being (e.g., facilitating physical activity, mental restoration) [3, 9]. Simultaneously, they support various ecosystem services, yielding health co-benefits. The ecological function of GBI, acting as 'stepping stones' or linear corridors for fragmented habitats, is undisputed. Challenges arise in integrating quantitative models for ecological connectivity in conservation planning with those supporting the planning of GBI, necessitating clear recognition of urban ecological elements and the investigation of GBI's ecosystem service capabilities [10, 11]. Despite GBI's potential to systematically mitigate the impact of urbanization on biodiversity and ecosystem services, challenges persist due to development pressures and complex socio-economic settings in cities, particularly in developing countries [12]. However, recognizing the importance of these benefits, urban restoration through GBI can reconnect residents with nature, enhance ecological literacy, alleviate "nature deficit disorder," and contribute to regional, national, and international biodiversity goals and broader ecological aspirations [8].

## MATERIALS AND METHODS

***Observed area description:*** The village of Zavar is located at the coordinates 48°21′02″S 17°40′22″E at an altitude of 127 m. and belongs to the climatic region of moderate latitudes, the amplitude between the warmest and coldest months ranges from 20°C to 22°C. Agricultural production is the most widespread production in the region. Plant production is mainly focused on the cultivation of densely sown cereals - wheat and barley, corn, sunflower, rapeseed, and potatoes. The Zavar cadastre includes the PSA Peugeot Citroën factory, which was opened in 2006. Thanks to it, Zavar has expanded significantly in recent years and almost doubled its population. New local parts were created, and active construction of new houses took place. This resulted in the loss of several natural sites, but also enabled the new ones that are the subject of our observation. The cadastral territory of the village of Zavar is in the 1st degree of protection and is subject to the all-municipal nature and landscape protection regime according to the second part of Act NR SR No. 543/2002 Coll. on nature and landscape protection as amended by other regulations. In the cadastral territory of the

municipality, no territory from the continuous European system of protected areas NATURA 2000 - a territory of European importance or a protected bird territory - is proposed or declared.

**Table 1.** Basic distribution of the individual area components of the Zavar village cadaster ($m^2$) in the years 1997 - 2022 and the development of the number of permanent residents [13].

| Year | Pop. | Total agri. l. | Pern. grass. agri. l. | Total non-agri. l. | Build-up non-agri. l. | Other non-agri. l. |
|---|---|---|---|---|---|---|
| **1997** | 1717 | 12,637,762 | 7,346 | 1,312,419 | 936,152 | 252,272 |
| **1998** | 1734 | 12,633,123 | 7,346 | 1,317,058 | 940,791 | 252,272 |
| **1999** | 1722 | 12,629,941 | 7,346 | 1,320,240 | 946,269 | 249,976 |
| **2000** | 1734 | 12,494,913 | 307,834 | 1,455,268 | 887,039 | 452,885 |
| **2001** | 1738 | 12,493,718 | 307,834 | 1,456,463 | 886,954 | 454,093 |
| **2002** | 1734 | 12,492,586 | 307,834 | 1,457,595 | 888,191 | 453,988 |
| **2003** | 1743 | 11,469,338 | 282,589 | 2,480,843 | 1,935,004 | 430,725 |
| **2004** | 1755 | 10,965,896 | 274,922 | 2,984,285 | 2,449,311 | 419,860 |
| **2005** | 1767 | 10,952,777 | 274,992 | 2,997,404 | 2,637,259 | 245,031 |
| **2006** | 1852 | 10,860,951 | 274,992 | 3,089,230 | 2,728,667 | 244,999 |
| **2007** | 2078 | 10,859,741 | 275,003 | 3,090,440 | 2,730,930 | 243,946 |
| **2008** | 2262 | 10,852,595 | 275,003 | 3,097,586 | 2,737,972 | 243,823 |
| **2009** | 2327 | 9,498,686 | 275,003 | 4,451,495 | 2,739,651 | 1,597,551 |
| **2010** | 2361 | 9,467,199 | 275,003 | 4,482,982 | 2,739,204 | 1,629,728 |
| **2011** | 2245 | 9,458,956 | 275,033 | 4,491,225 | 2,740,792 | 1,635,985 |
| **2012** | 2218 | 9,453,295 | 274,933 | 4,496,886 | 2,738,015 | 1,637,223 |
| **2013** | 2205 | 9,452,635 | 274,933 | 4,497,546 | 2,738,418 | 1,637,480 |
| **2014** | 2222 | 9,447,998 | 274,928 | 4,502,183 | 2,743,648 | 1636887 |
| **2015** | 2220 | 9,441,882 | 274,928 | 4,508,299 | 2,747,251 | 1,638,616 |
| **2016** | 2275 | 9,428,661 | 274,928 | 4,521,520 | 2,757,672 | 1,641,724 |
| **2017** | 2291 | 9,423,827 | 274,928 | 4,526,354 | 2,762,561 | 1,641,698 |
| **2018** | 2234 | 9,414,986 | 270,383 | 4,535,195 | 2,772,242 | 1,640,858 |
| **2019** | 2230 | 9,409,040 | 270,383 | 4,541,141 | 2,783,011 | 1,636,035 |
| **2020** | 2219 | 9,399,932 | 270,355 | 4,550,249 | 2,788,740 | 1,639,414 |
| **2021** | 2396 | 9,389,887 | 270,261 | 4,560,294 | 2,792,770 | 1,645,428 |
| **2022** | 2492 | 9,381,878 | 270,113 | 4,568,303 | 2,797,904 | 1,648,409 |

**Table info:** Pop. – Permanent population; Total a.l. - Total agricultural land; Grass. agri. l. - Agricultural land, Pern. grass. agri. l. - permanent grassland; Total non-agri. l. - Total non-agricultural land; Build-up non-agri. l. - Non-agricultural land, built-up area and courtyard; Other non-agri. l. - Non-agricultural land - other area

***The total area of the village cadastre represents 13.95 km$^2$**. Arable land decreased during the monitored years from 12,295,341 $m^2$ in 1997 to 8,800,531 $m^2$ in 2022 (difference of 3,494,810 $m^2$). Part of agricultural are land also vineyards - decrease in area from 161 $m^2$ in 1997 to 137 $m^2$ in 2022; orchards - area increase from 0 $m^2$ in 1997 to 18,906 $m^2$ in 2022 and gardens - decrease in area from 334,914 $m^2$ in 1997 to 292191 $m^2$ in 2022. Non-agricultural land (built-up and other areas) also consists of water areas - area decreased from 123,995 $m^2$ in 1997 to 121,990 $m^2$ in 2022. There are no forest areas. This component of the landscape are presented only as naturally developed or artificially planted tree alleys or smaller groves, which are included in the other non-agricultural area.

***Our observations:*** Within this cadastre, 3 localities were selected, where long-term monitoring of the development of biodiversity took place (Picture 2):

1. location: PSA Peugeot-Citroen Logistic Park; 2. location: Polianky housing estate; 3. location: modified section of the melioration canal flowing into the Blava stream.

**Picture 1.** The development of urbanization within the inner city of the village of Zavar in the years 1950 – 2023 (source: Google Eart (2023), gis.tuzvo (2023)).




a) Zavar 1950´ - without: highway, Polianky estate and new housing estates.; b) Zavar 2006´ - without: Polianky estate and new housing estates.; c) Zavar 2011´ - without: new housing estates; d) Zavar 2014´ - without: new housing estates, the beginning of development; e) Zavar 2019´ - fast development of new housing estates; f) Zavar 2023´ - the current state of development.

**Picture 2.** The comparison between the urbanization and BGI within the observed cadastre of the village of Zavar in the years **a) 2006** and **b) 2023** (source: Google Eart (2023)).



b) 


**Map info:** Within each location, several smaller areas where BGI was applied were monitored. ⇩- location 1; ⇩- location 2; ⇩- location 3. Numerical points indicate places where new construction has taken place since 2006. The only difference are highway and the PSA Peugeot-Citroen logistics park, that was largely already built: 0 - The highway; 2 - PSA Peugeot-Citroen logistics park; 3 - Poronda housing estate (Polianky); 4 - New housing development (Polianky); 5 - A new, developing district of the village

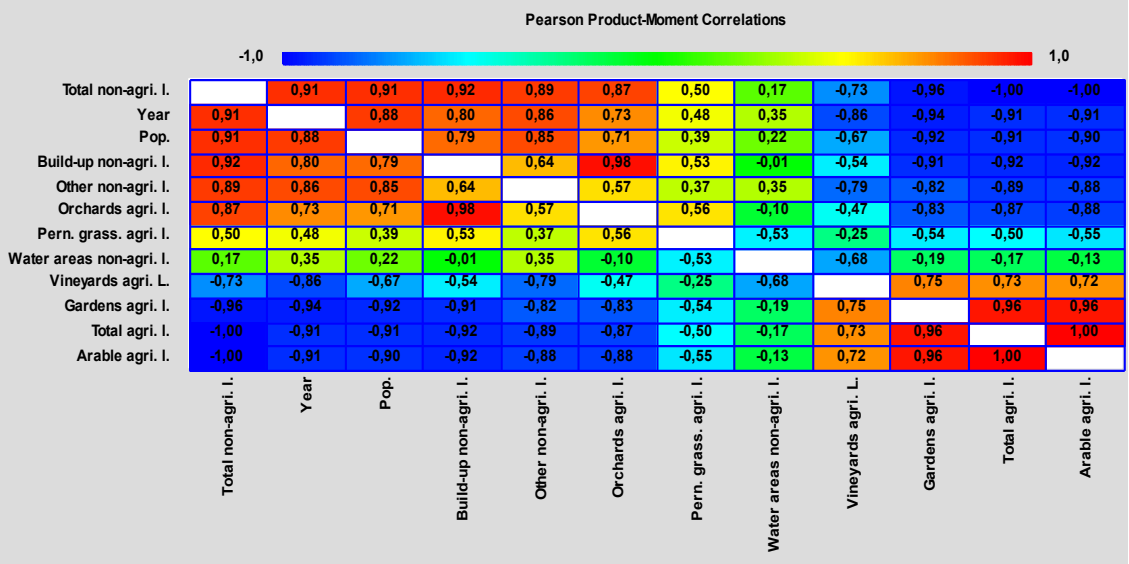
The Pearson correlation relation was used in the Statgraphics Centurion XVIII program (StatPoint Inc. USA) for the statistical evaluation of the correlation relations between the observed parameters.
The source of the maps was Google Earth, 2023.

## RESULTS

***1. location:*** Observed in the years 2001 – 2023. Together with the logistic park construction (2002 – nowadays), the land with only minimal human intervention were created and ditches were built to catch excess rainwater. The gradual development of meadow and forest biodiversity was observed in several separated sections. These blue-green infrastructural interventions greatly supported the development of biodiversity in the area in a natural way and a species-rich meadow flora was gradually created. This supported the development of species- and genera-rich soil microbiota, especially bacteria and microscopic fungi, or invertebrates linked to the occurring species of plants and animals. Although a similar biotope is natural for the given area, it is rare due to the long-term intensive use of the land for agricultural purposes. And to a significant extent, it helped to increase the population of today's significantly receding species of ground-nesting birds such as *Perdix perdix* or *Galerida cristata*. It had a similar meaning for today's more rare species of game such as *Phasianus colchicus* or *Lepus europaeus*. In a later phase, part of the meadow biotope was naturally afforested with invasive trees such as: *Acer campestre*, *Rosa canina*, *Elaeagnus angustifolia*, *Populus nigra*, *Populus alba*, *Salix alba*, which created natural tree lines along communication roads or fences.
***2. location:*** Observed in the years 1997 – 2023. The construction of a new housing estate (2003 – 2005), located on an area with agricultural land, but also grassy areas with naturally high groundwater. Along with the housing estate, a BGI was also built, including a meadow biotope and the improvement of an amelioration channel, which also fulfils a drainage function in the event of excessive rainfall or groundwater level. A forest biotope was left around it consists of woody plants such as *Alnus glutinosa*, *Salix alba*, *Populus nigra* with shrubby sections of *Prunus spinosa*. It thus provides space for nesting of *Strix aluco*, *Buteo buteo* or *Falco tinnunculus*, which have an irreplaceable

useful function in the agricultural landscape. It also creates space for several types of amphibians protected by law, such as *Pelophylax kl. esculentus*, *Pelophylax lessonae* or *Bufo viridis*, as it maintains a wetland character all year round in several sections.
***3. location:*** Observed in years 2020 – 2023. Melioration canal on the edge of the village was used for agriculture, but in 2021 a section was set aside within it where a flower meadow was sown. It is a habitat that is still developing. Based on our field observations, it provides living space for several useful insect species, such as solitary bees of the genus *Andrena* or the species *Colletes cunicularius*. This location is connected with the Blava stream, which represent the majoority of the water area within the cadastre. Based on the observation, it can be concluded that with proper BGI interventions in this section, the given melioration channel could also provide a suitable spawning ground for several now rare species of fish found in the Blava stream, such as *Misgurnus fossilis*, *Rhodeus amarus*, *Tinca tinca* or *Sabanejewia aurata* that occur in the area and thus contribute to saving their population.

**Graphic 1:** The Pearson correlation between the observed parts of Zavar cadastre, the population in the area and the years of observation (Statgraphics Centurion XVIII).




The correlation analysis, conducted using Pearson's product-moment correlation diagram, revealed notable relationships, particularly with total non-agricultural land. Positive relationships (> 0.75) were observed between the year of observation and the population of the village with permanent residence, total non-agricultural land, built-up area within the cadastre, and other areas. Strongly negative correlation values (< -0.85) were noted in cases such as the decline in total agricultural land, arable land, and the gradual loss of gardens and vineyards compared to the observed year. Over time and with an increasing population, agricultural land decreased, while built-up areas and other non-agricultural land, including newly created tree stands through applied BGI, increased. Importantly, permanent grassland increased during the monitored years.

## DISCUSION

Presently, significant focus centres on fostering biodiversity amid increasing urbanization and agriculture. A promising, yet underexplored solution is the use of green-blue infrastructure, with scientifically proven positive impacts on biodiversity, as confirmed by various studies. For instance, in [10] analyses have shown that selecting BGI options that specifically target vegetation cover, quality and soil moisture retention present the greatest opportunity in creating conditions suitable for regional biodiversity enhancement. In [2], an integrated assessment approach was demonstrated to enhance biodiversity assessment in human-dominated landscapes, specifically targeting the strategic planning of Blue-Green Infrastructure. While confirming its positive effect, it's noted that the interpretation and impact of BGI can vary. Understanding innovative Green-Blue Infrastructure (GBI) strategies in various cities is crucial in addressing escalating urban challenges exacerbated by the impacts of climate change, biodiversity loss, and the pursuit of other sustainable development goals [12]. A novel approach involves the combined application of remote sensing, landscape metrics, least-cost analysis, gravity model, and graph theory for developing urban green space networks dedicated to biodiversity conservation [6]. Analysis indicated that the ecosystem services provided by urbanized GBI were influenced by factors such as park area, the total value of normalized difference vegetation index and normalized water body index, and distance from the city centre [11]. Even in the case of our observations can be assumed, that the area on which the BGI was built can be insufficient. Based on the observation of [8], recommend native tree planting in private and public land to achieve the minimum target of 10% indigenous (forest) cover in urban areas. In this case the valued habitats would better benefit the species selected here and attract other interdependent native species along with human-cultural relationships.

## CONCLUSION

In conclusion, our extensive field observations spanning from 1997 to 2023 provide valuable insights into the intricate relationship between human development and biodiversity across three distinct locations.

The interventions within 1. location supported the development of diverse meadow flora, benefiting soil microbiota and ground-nesting bird species. Within the Polianky housing estate, the field observations indicate, that these areas serve essential functions for recreation, microclimate, sustainability, and rare biodiversity development within the village's cadaster. Hoover reserving or supporting their development is crucial. Within the observations on 3. location, the providing of habitat for useful insect species was recorded. With proper BGI interventions, this area could serve as a spawning ground for rare fish species in the Blava stream, contributing to their conservation.

In summary, our findings highlight the complex dynamics between urbanization, land use changes, and biodiversity. The successful integration of blue-green infrastructure and strategic land management practices can contribute significantly to the preservation and enhancement of biodiversity, serving as a blueprint for sustainable development in similar landscapes. But these results also point to the importance of preserving and restoring natural habitats within an urbanized landscape and can serve as a basis for more detailed research in the future.

**ACKNOWLEDGEMENTS**

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# THE RELEVANCE OF THE BIOREGION IN LATVIA FOR THE PRESERVATION OF BIOLOGICAL DIVERSITY

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## ABSTRACT

A study of the bioregion paradigm reveals that bioregions can be diverse. A bioregion is a construct of the human mind that evolves to resist the continued destruction of wild systems in an effort to restore ecological balance. Latvia has a diverse landscape, relatively rich biological diversity and cultural heritage, which provides a solid foundation for the creation of potential bioregions. The UN Sustainable Development Goals, the European Green Deal and other documents offer solutions to make our farming more sustainable in the long term. Recently, these ideas have also been brought up to date in Latvia, where one of the topical issues is the identification of bioregion development opportunities. The research results reveal that, in general, the bioregion approach is suitable for the development of rural areas, as it promotes ecological sustainability, social justice, and place identity. In accordance with the procedure established by the legislation, 658 specially protected nature territories have been established in Latvia and their management is carried out by four regional structural units of the Nature Conservation Agency. By actualizing bioregions, it is potentially possible to solve various problems related to the full use of the economic potential of these territories. The purpose of the article is to emphasize the importance of biological diversity and protected areas in the creation of bioregions in Latvia.

**Keywords:** bioregion, biological diversity, sustainability, specially protected nature territories.

## INTRODUCTION

The common objective of any regional development policy is related to improvement of the quality of the living environment of a particular society. In general, regional development is a key aspect for sustainable development, creating balanced and prosperous communities in rural areas. The opportunity to promote sustainable business development through bioregions is highlighted in the new Action Plan for the Development of Organic Production in the European Union (EU) presented by the European Commission in March 2021. It defines a bioregion as a geographical area where farmers, society, tourism operators, non-governmental organizations and municipalities conclude an agreement on sustainable management of local resources based on organic practices and principles. The European Commission's vision for the development of rural areas in the EU identifies the challenges and concerns facing rural

areas and highlights some of the potential opportunities to be realised in these regions [1].

The current trends in the development of bioregion concept in Latvia are partly related to the implementation of guidelines of the European Commission for the identification and creation of bioregions. The Ministry of Agriculture in Latvia is working on a national Action Plan for the Development of Organic Production, in which it is planned to define criteria for the establishment of bioregions in Latvia. It is important to compile the development indicators already available and their tendencies, which could form the basis for the development of guidelines for the determination of territorial zoning and operational principles of bioregions. Latvia has a diverse landscape, rich biodiversity and cultural heritage that provides a solid foundation for the creation of potential bioregions. Latvia's sustainable Development Strategy 2030 sets the goal of being the EU leader in the conservation, increase and sustainable use of natural capital. The strategy emphasizes that Latvia is one of the greenest countries in the EU, given the relatively small population density [2]. In order to emphasise the importance of biodiversity and specially protected nature territories (SPNT) for the creation of bioregions in Latvia, the authors of the paper define their research aim: to analyse publicly available documents to reveal the topicality of the bioregion concept in Latvia in the context of the preservation of biological diversity.

## MATERIALS AND METHODS

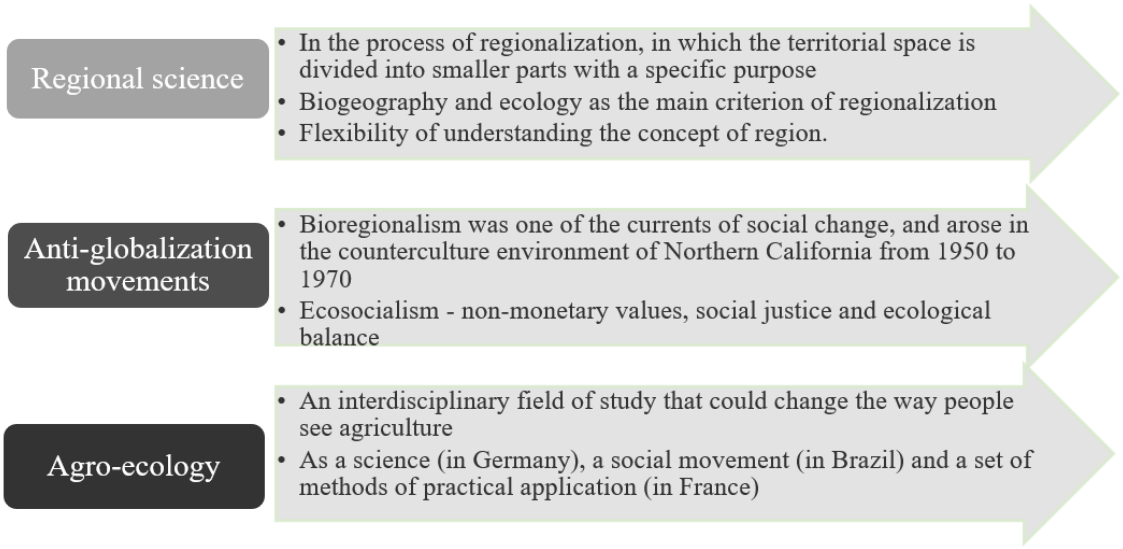
The study of the bioregion paradigm in the context of biodiversity conservation and regionalisation was based on a document analysis what was done to analyse the role of biodiversity and SPNT in the creation of bioregions as well as topicality of the bioregion idea in Latvia. An analysis of the documents (development policy strategies, laws, records of relevant public discussions, and scientific publications) provided an overview of the awareness and ways of promoting the bioregion topic.

According to the administrative division of Latvia, Latvia has 43 administrative territories, of which seven are the state cities. These administrative territories are divided into five planning regions – Kurzeme, Zemgale, Latgale, Vidzeme and Riga. Planning regions are designed to ensure regional development planning and coordination [3]. The authors of the paper therefore assumed that the development possibilities of potential bioregions could most effectively be determined using available data on planning regions. The purpose of the application of the method was to analyse information regarding the possibilities for the creation of bioregions in the context of biodiversity conservation and also organic farming in Latvia. The documents for analysis were selected if they comprised the following concepts: bioregion, biodiversity, organic farms, specially protected nature territories.

## RESULTS

In regional science, the understanding of the concept of a region varies according to the purpose and general perception of the territory. Conventional understanding is stressed by Watson [4], Meinig [5] and Barnes [6], pointing out that the region is associated with the cultural, economic and political processes taking place there. Climate change, which has occurred under the influence of human action and puts under the threat existence of the entire planet are a reason to seek new development opportunities [7]. In the 21st

century, regional science incorporates the new idea of sustainability and bioregionalism, where an integrated planning framework is highlighted that allows people to map territories and develop strategies for a variety of topical areas, such as planning food systems and green infrastructure [8]. Taylor [9] sees bioregionalism as an environmental movement and social philosophy that envisages decentralized community self-government within political boundaries emphasizing the ethics of the place, sustainable human societies that would be in harmony with the nature and consistent with the rise of all native species [9]. Conservation of biodiversity is also highlighted in the context of agroecology, which can be defined as both science and movement related to environmentally friendly farming in rural areas. Ecological concepts and principles are applied to the design and management of sustainable agro-systems [10]. Bioregion combines the Greek word for life (*bios*) as a term with the Latin word for territory (*regia*) and the Latin term for rule/administration (*regere*). Thus, a bioregion means "territory of life" or "place of residence" [11]. Despite the many different arguments for establishing a bioregion, theoretical discussions show three main directions (Figure 1).




*Source: developed by the authors based on an analysis of scientific publications.*

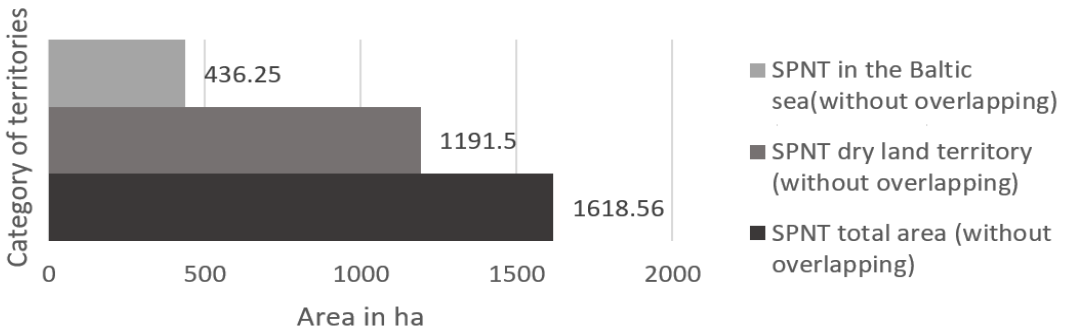
**Fig. 1. Development of bioregion paradigm.**

There are also efforts in Latvia to establish balanced development of rural regions; local governments and regional entrepreneurs are looking for opportunities to promote realization of economic potential, while preserving the importance of natural resources and local culture. However, policy makers lack a conceptual view, at the same time that is consistent with the reality of Latvia, of the development of these many different territories. Modern neoliberal societies should be directed towards more environmentally friendly actions, green governance should cover the whole set of socio-ecological system interactions across all sectors, both in terms of environmental management and consumption, including food [12]. Bioregions already operating in EU Member States, such as the Cilento bio-district in Italy, which brings together 400 organic farmers from 38 municipalities and biologically manages more than 30% of agricultural land in the region, are a good example. Biological regions are often designed to protect and promote economic development in SPNTs, thus protecting rare

species of animals and plants, preserving biodiversity while promoting economic activity. In Latvia, there is a first attempt to develop a bioregion linked to the SPNT in the Gauja National park.

Under the Nature Conservation Agency, SPNT (Figure 2) are geographically defined areas for which specific national protection has been established in order to preserve and protect natural diversity. SPNT shall be considered as:

1) rare and typical ecosystems,
2) the living environment of protected species, unique, beautiful and Latvia-specific landscapes,
3) geological and geomorphological formations,
4) dendrological plantations and great trees,
5) areas of importance for public recreation, education and upbringing [13].



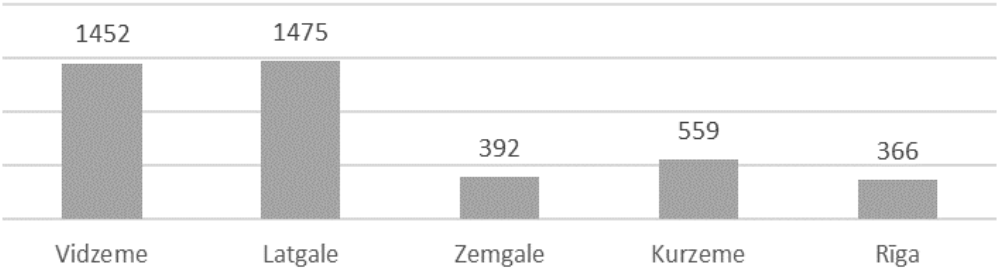

*Source: developed by the authors based on the data of the Central Statistical Bureau of Latvia [14].*

**Fig. 2. Specially protected nature territories in Latvia, 2023 (ha).**

According to the procedures laid down in the legislation, 658 SPNT have been established in Latvia, except for protected trees and protected boulders. Each of the SPNT falls within one of the eight categories of protected areas, in accordance with the objectives of their establishment and protection [15]. In 1993, the Law of the Republic of Latvia on specially protected nature territories was adopted. On the basis of this Law, several Cabinet of Ministers regulations have been issued, which in most cases determine the regulations for the use and protection of territories. The main provisions to be taken into account when carrying out any economic activity in the SPNT, including agriculture, are defined by the Cabinet of Ministers [16]. Although the existing regulatory framework lays down rules for the use of agricultural land, it does not include the specific rules necessary for the approach to the establishment of bioregions. Both the SPNT and the bioregion approach focus on conservation of nature and biodiversity, as well as the sustainable use of land resources.

Looking at the bioregion as a potentially effective spatial development strategy, it is necessary to assess the land resources available in the region and the factors affecting them. Land use available (for which purpose existing land resources are used) and human resources managing land resources are important factors. The interaction between these two factors has an impact on production volume, types, farming system (biological, conventional), employment, business development and environmental impact. According to the data of the Ministry of Agriculture [17] and the Central

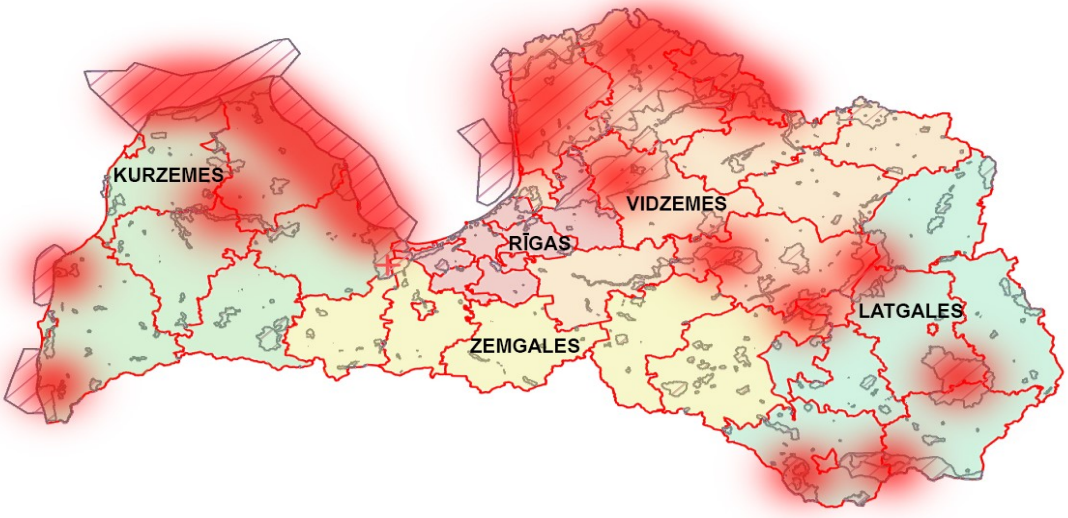
Statistical Bureau of Latvia, over a period of 20 years the biologically certified area of agricultural land in Latvia has increased 18 times: from 17 thousand ha in 2001 to 304 thousand ha in 2021 [14]. As a positive aspect, the organic arable land area of the 1st transitional period has increased significantly in 2021, indicating the growing interest of farmers in organic farming. According to data compiled in 2023, a total of 4244 certified organic farms have been registered in Latvia [14] (Figure 3).




*Source: authors' calculations.*

**Fig. 3. Registered organic farms in Latvian planning regions in 2023.**

Taking into account the borders of the planning regions of Latvia, the largest number of SPNT is located in Vidzeme planning region, followed by Latgale and Kurzeme planning regions. According to the data, possible bioregion creation sites in Latvia are indicated in the Figure 4.




*Source: authors based on the data of Latvian Geospatial Information Agency [18] and the Ministry of Environmental Protection and Regional Development [19].*

**Fig. 4. Prospective territorial zoning of bioregions in Latvia.**

The authors propose to form groups of bioregions within planning regions, assuming that precise boundaries of planning regions may not be respected. The approach shown in the Figure 4 would allow economic development interests to be defined while ensuring that environmental needs are met and that natural diversity is preserved. This would underpin the inclusion in the sustainable governance framework of business

principles relating to the effective structuring of sustainable entrepreneurship, sociocultural principles relating to the social aspects of workers and society, as well as respect for and protection of cultural heritage and living culture, and environmental principles relating to aspects of business management affecting the use of natural resources, ecosystems, biodiversity and climate change.

## CONCLUSIONS

Regionalisation is seen as a dynamic process, but the region is widely recognised as an innovation policy field where new development areas can be developed depending on defined criteria. Overall, the paradigm of bioregions is suited to the development of rural areas as it contributes to ecological sustainability.

The regulatory framework at all levels of policy planning impact is essential to ensure the sustainable use and conservation of natural resources, as well as to promote regional cooperation and sustainable development. Given that bioregion is a new concept in Latvia, it currently has no specific regulatory framework, however, the creation of potential bioregions is largely influenced by the development of protected areas and the regulatory enactments regulating organic farming.

The SPNT in Latvia acts as a tool for environmental protection and there are certain restrictions on economic activity in these areas, while the bioregion acts as a tool for the conservation of biodiversity based on biological management methods, so the creation of bioregions in SPNT areas would contribute to the full exploitation of economic potential, which would otherwise not be possible. Taking into account the location of the SPNT, the number and location of biologically certified farms, as well as other considerations, the authors proposes to form groups of bioregions in Vidzeme, Kurzeme and Latgale planning regions, assuming that the boundaries of precise planning regions may not be respected.

## ACKNOWLEDGEMENTS

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# THE ROLE OF LANDSCAPE DESIGN AND URBAN NATURE-BASED SOLUTIONS IN ADDRESSING GLOBAL CHALLENGES

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## ABSTRACT

Urban green infrastructure has historically been used for aesthetical purposes, making landscapes visually pleasant. More recently the green infrastructure in urban and sub-urban areas has started to be applied to help solve a broad range of problems that city dwellers are facing. These problems include natural disasters, overheating, excessive flows rainwater, biodiversity decline and food insecurity among others. Thus, implementation of green infrastructure as Nature-based Solutions has become increasingly popular. This trend is likely to continue in future as global climate change poses more and more changes to city dwellers. Urbanization and population growth are likely to drive implementation of urban green infrastructure across the globe and especially in the Global South, where impacts of the global heating are likely to be particularly strong. Implementation of green infrastructure may be developed either mostly for aesthetical purposes or as Nature-based Solutions or both. It is, however, important to note that the integrated approach is likely to have the greatest potential to meet global challenges. Furthermore, implementation of the green infrastructure is likely to have the greatest positive impact when implemented as a holistic system rather than individual elements. Thus, there is a clear need to evaluate opportunities for green infrastructure development that could simultaneously maximize aesthetic impact and effectiveness of the urban green and blue spaces to act as Nature-based Solutions. Here we illustrate some of the variables and system dynamics involved. Using a simplified model with specific focus on food supply, system structural analysis suggests that the urbanization variables commonly considered in urban design models are not closely connected with those that would provide long-term sustainability. The positive feedback between variables within each of these two groups and negative impacts between groups create barriers to their combined assessment for practical mitigation actions. The various sub-systems and an expanded variable set can ideally be addressed with a similar methodology. We also discuss opportunities for integration and provide recommendations for policy makers, city planners and other stakeholders involved in construction and management of urban landscapes.

**Keywords:** sustainable development, food security, climate change, system model

## INTRODUCTION

Urbanization is an ongoing trend, with half of the global population already living in cities and about two-thirds expected to be inhabiting urban environments by 2050 [1]. The process of urbanization may have negative environmental impacts, including deforestation, freshwater source depletion, habitat and biodiversity loss, pollution and climate change [2]. The negative effects of urbanisation may also undermine public health. Implementation of Nature-based Solutions and greening of urban landscapes provide some opportunities to mitigate these negative effects and may even have the capacity to make urban environments positively contribute to environmental conservation and human health. Nature-based Solutions "are actions to protect, sustainably manage, and restore natural and modified ecosystems that address societal challenges effectively and adaptively, simultaneously benefiting people and nature" [3]. Urban Nature-based Solutions that may be implemented in cities are diverse and include, for example, green spaces, green built environment, green transportation infrastructure, natural and semi-natural water storage, harvesting and supply, urban farming, blue-green space establishment and/or restoration [4]. Landscape design can make green spaces aesthetically appealing, thereby attracting public support to development of the green infrastructure and delivering cultural ecosystem services. However, green spaces may be developed solely for aesthetical purposes, ignoring or giving very limited attention to ecological aspects. Alternately, in some cases green infrastructure may be developed as Nature-based Solutions, not considering aesthetics. In both cases the outcomes may fail to deliver sustainable outcomes. In addition to that lack of holistic evaluation of risks, costs and benefits associated with development of the green infrastructure may ultimately lead to problems that may either undermine biodiversity conservation goals or have negative effects on human-wellbeing.

One of the most significant issues that may be easily overlooked by urban planners and landscape designers is the fact that for green infrastructure to have a significant contribution to biodiversity conservation a multi-scale approach needs to be applied. This approach implies that the blue-green infrastructure across urban areas should be planned as a holistic landscape rather than as individual parks or Nature-based Solutions. Furthermore, the urban blue-green infrastructure needs to be seen as a part of larger landscape, within which the city is imbedded. Such approach is rarely taken, with a few examples available globally. However, application of the multi-scale approach and holistic view of the urban blue-green infrastructure have demonstrated effectiveness in delivering biodiversity benefits and contribution to human-wellbeing. These positive effects can be explained by consideration of landscape ecology principles. Given the ongoing expansion of urban areas and construction of new cities, there is a clear need for consideration of integrated, multi-scale approaches to urban planning. Thus, in present paper we first illustrate some of the variables and system dynamics involved using a simplified model with specific focus on food supply system structural analysis. Then we review benefits of application of integrated and multi-scale approaches. We also discuss opportunities for integration and provide recommendations for policy makers, city planners and other stakeholders involved in construction and management of urban landscapes.

## METHODS

Here we apply System Structural Analysis methodology. The analytical phase in holistic modelling is largely aimed at understanding the system components and their interaction. It is often necessary to subdivide the system, limiting the scope of the model but increasing the relationship specificity and basis for modelling. The initial set of variables was suggested from our combined knowledge of similar system descriptions. We decided to focus on the food production in connection with urbanization, although we recognize the critical importance of other issues, such as energy use, water resources and space limitations, which could be addressed using a similar approach.

As part of this system structural analysis, impact matrixes are used here to identify key variables and system dynamics. The impact values can be derived from empirical or theoretical arguments, and in this case authors have jointly estimated the values using both personal and literature-based information. The relative values, using a 0-4 scale, are unavoidably dependent on our judgement, but the transparency of the method allows their re-evaluation and refinement in an iterative process that is motivated with increased information regarding the consistency of the model. The model refinement and system understanding are further useful in communication between researchers and other stakeholders.

The overall impact of each variable on others and the combined influence of the other variables on each specific variable can be plotted using the row and column sums of the impact matrix. These are plotted on a “Cause and Effect” diagram. Relationships between individual variables are also separately illustrated and, together with the specific values in the impact matrix, used to identify groups of closely related variables and possible feedback between the variables. A more detailed discussion of the methodology is provided by Hudson [5], Scholz & Tietje [6] and Vester [7].

## SYSTEM DYNAMICS

In the simplified, urban system, defined with food production as a sub-system for this exemplification, the plotted variables on the Cause-Effect diagram (Fig. 1) indicate that all have a significant role, impacting on and being influenced by the other variables. This is expected because we have intentionally selected variables considered to be important. Although Wellbeing has the least impact on the other components, societal rather than individual considerations on the long-term effects might increase its importance. Rural food production is least affected by other variables, which reflects the current societal dependence on extensive transport.

Considering the strongest, pairwise relationships from the impact matrix, there are two distinct groups: variables commonly identified in urban dynamics and others, less commonly used, but that could add long-term sustainability (Fig. 2). Each of these groups has its main positive impacts internally. Especially in the case of typical variables of urbanization, the positive feedback loops involving two or more variables are part of the system dynamics, providing continued growth of the components involved and, possibly, creating a degree of instability in the system as a whole. Positive feedback loops can also include two negative impacts, for instance, between the variables for Green and Built infrastructures and Built infrastructure and Ecological balance. An increase in one is expected to decrease the other, which can continue to affect similar changes. Since the two groups of variables are mainly related by negative impact relationships, there is a conflict in their simultaneous development, which can be

particularly difficult to avoid if this is strengthen by positive feedback loops involving the negative impacts.

Thus, one challenge regarding the implementation of new, urban design features that would be favorable for sustainability is that these need to be integrated with the existing variables that strongly drive the functional relationships in the urban areas, i.e. increasing the positive impacts between the two groups of variables in this example. Other essential resources, such as water, energy, and space, that have not been illustrated here can be expected to have similar limitations on sustainable development, and understanding these sub-system relationships can be aided by separate system structural analyses before they are to be combined within more complex and inclusive models.

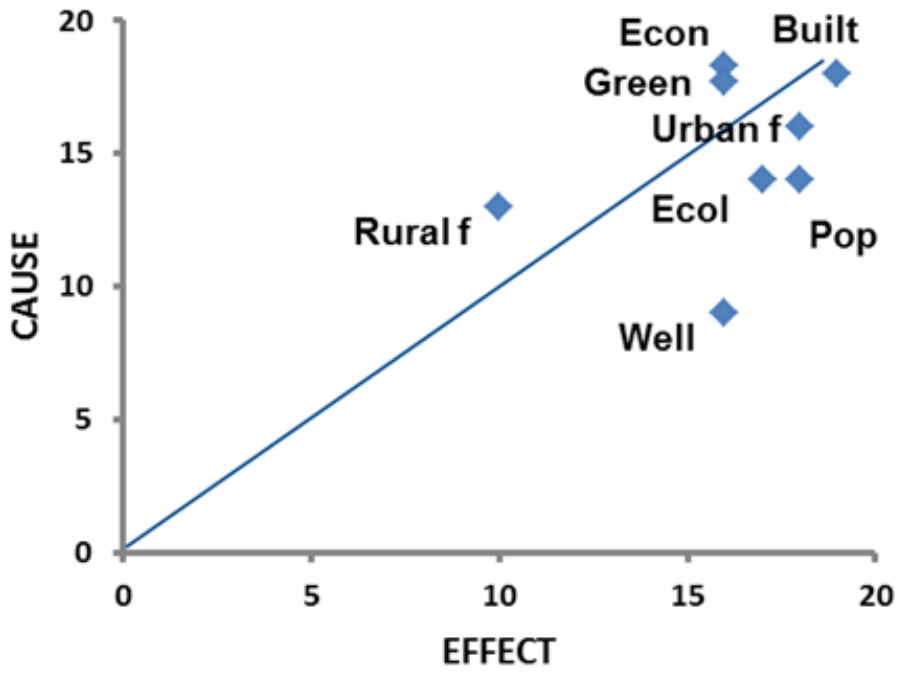



**Fig. 1. The combined impacts of variable in the urban sub-system dealing with food supply. Variables used in the graph are: rural food production (Rural f), urban food production (Urban f), green infrastructure (Green), built infrastructure (Built), economy (Econ), ecological balance (Ecol), wellbeing (Well) and population (Pop).**

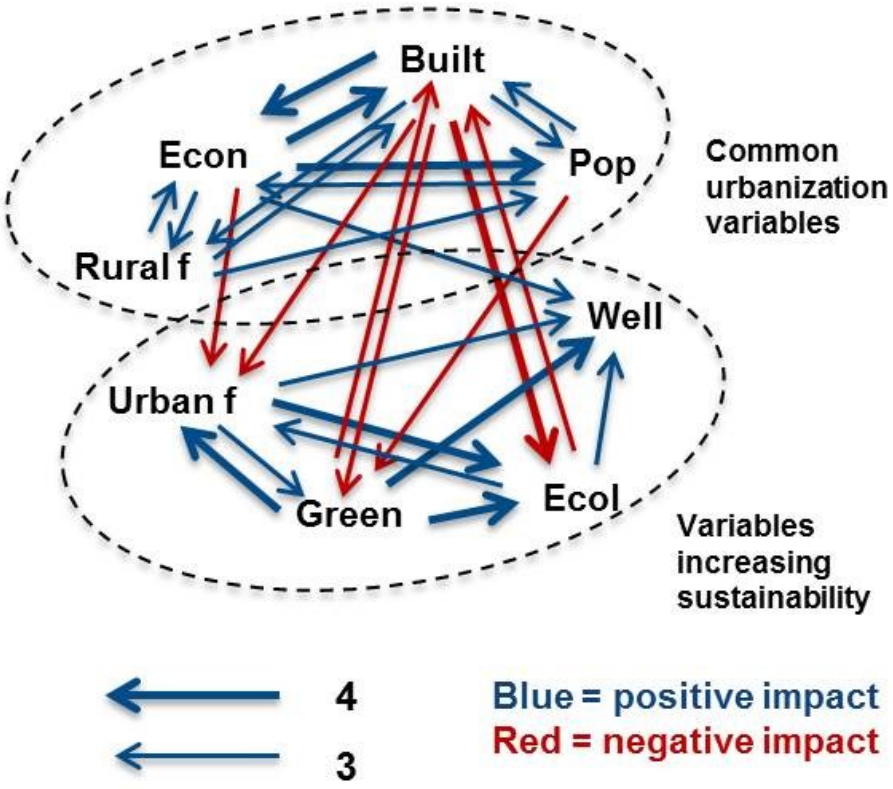



**Fig. 2. The strongest impacts (positive and negative) between individual variables.**

## INTEGRATED VIEW

Our modelling suggests that a holistic view on planning and development of urban green infrastructure are essential for maximisation of its positive impacts. Benefits associated with green infrastructure development can be maximised by integration of ecological considerations, functions of the blue-green spaces for human wellbeing and aesthetical aspects. Landscape diversity, which may be formed through combining blue and green infrastructure as well as different elements of landscape design, tends to be associated with higher levels of biodiversity than homogenous landscapes [8]. Increasing the area of water bodies and landscapes combining trees, shrubs and flowers is likely to have many positive contributions to human health and wellbeing. At the same time aesthetical value can be increased through diversification of the landscape composition [9]. Possible negative feedbacks also need to be considered during urban planning process. These may include, for example, human allergies in some urban dwellers caused by bloom of certain tree, shrub or flower species. Some tree species are also highly prone to windfall (e.g., poplar), posing risks to urban dwellers. Integration of such species into design of the green infrastructure needs to be avoided.

Some aspects of landscape design may be less straightforward. For instance, the choice between usage of native versus exotic plant species may be especially problematic. On the one hand native species tend to provide greater benefits to urban biodiversity and their use excludes the risk of invasion of non-native species into natural ecosystems beyond urban areas. However, differences in climatic conditions between urban areas and surrounding landscapes as well as global warming may create

conditions in which use of exotic species may make urban ecosystems more resilient. In such complex situations uncertainty may be reduced by predictive modelling and scenario development.

## MULTI-SCALE APPROACH

Another critically important aspect of maximising benefits derived from development of the green infrastructure and urban Nature-based Solutions is a multi-scale approach. The approach implies considering all urban green and blue areas as a holistic ecosystem rather than focusing on composition of individual parks or other elements of green infrastructure (i.e., green roofs, green walls, Nature-based Solutions etc.). Furthermore, the multi-scale approach allows considering how urban green infrastructure as an ecosystem integrates into larger landscape. The approach has multiple significant advantages. Furst of all, the individual elements of the blue-green infrastructure and Nature-based Solutions can have greater contribution to biodiversity conservation and climate adaptation when implemented at a large spatial scale. There are many examples of this. An excellent example is the Swedish city of Augustenborg, which was constructed as a holistic ecosystem composed of blue and green spaces in order to both buffer the city dwellers from flooding and maintain a high level of biodiversity. Another interesting example is the system of Montreal's Green Alleyways, which is a 5,000 kilometre network of greenways occupying large proportion of this Canadian city, maintaining biodiversity and positively contributing to citizen health. Bishan-AMK Park in Singapore is yet another successful example of a large-scale, blue-green infrastructure. The city's largest park is developed around the river that has proved to be an effective Nature-based Solution serving for recreation, climate adaptation and maintaining high levels of biodiversity.

Considering how urban green infrastructure as a whole integrates into a broader landscape context can be particularly important for ensuring its positive effects on human wellbeing and biodiversity. Urban and peri-urban areas cab harbour high levels of biodiversity [10], which in turn can provide numerous ecosystem services both to urban dwellers and inhabitants of surrounding landscapes. For instance, species rich pollinator assemblages may inhabit urban landscapes, spilling over into adjacent farmlands. However, urban landscapes themselves need to be connected to natural and semi-natural ecosystems that would ensure influx of species into urban habitats. Connecting urban green infrastructure to natural habitats is also critical for ensuring resilience of urban ecosystems, which is particularly important in the context of global climate change. In urban green infrastructure design, it is also important to consider risks that may take effect at larger spatial scales, including for example rea of invasive species from urban areas into natural ecosystems. Mitigation measures, such as avoiding use of potentially invasive species in the design of green infrastructure and Nature-based Solutions, need to be considered. It is also useful to consider impacts of urban green infrastructure in regional and even global contexts. For instance, returning to the urban food production example, above, it is important to consider how this may impact global food supply and whether impacts on biodiverse ecosystems in other parts of the world may be significant. Overall, taking holistic multi-scale approaches can help navigating complexity, maximising benefits and reducing negative externalities.

## CONCLUSIONS

Results of system dynamics analysis indicate that in complex systems, such as urban areas implementation of green infrastructure and Nature-based Solutions may have multiple effects, including both positive contributions to human well-being, economy and biodiversity, and negative effects and risks. This suggests that approaches to urban design are not always closely connected with those that would provide long-term sustainability. This problem can be addressed through design and implementation of urban green infrastructure that aim at long-term sustainability. Here we demonstrate that an integrated approach to landscape design and implementation of the green infrastructure for the society well-being purposes has the potential to increase the benefits. Furthermore, it is critically important to take an integrated multi-scale approach to planning and design of blue-green infrastructure, considering urban landscapes as an ecosystem consisting of individual elements. Moreover, the urban landscape with the networks of the green infrastructure should be considered in a broader landscape context. The approach allows evaluation of benefits and risks, not only within the city but also in a regional context. This in turn can help maximising positive effects and reducing risks and trade-offs. Ideally, urban infrastructure and Nature-based Solutions planning should take into account both regional and global positive and negative impacts. The impacts may significantly differ depending on the local context, such as tropical versus temperate ecosystems may have significant differences between the effects of green infrastructure on human well-being and the local economy. Taking a holistic integrated multi-scale approach would ensure achievement of truly sustainable urban development.

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# VENTILATION INTENSITY AS A MARKER OF INDOOR ENVIRONMENTAL QUALITY IN BUILDINGS

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## ABSTRACT

Ventilation plays a key role in maintaining the indoor environmental quality of buildings. This contribution focuses on the significance of ventilation intensity as a crucial indicator of air quality within buildings. The aim is to analyze the impact of ventilation intensity on various aspects of the indoor environment, including thermal comfort, pollutant concentrations, and overall occupant comfort. The research is centered around methods for measuring ventilation intensity and ways to optimize this value to achieve optimal outcomes in the realm of air quality and comfort. Emphasis will be placed on technological innovations that enable more efficient and intelligent ventilation control, including the use of sensors, automation, and energy-efficient systems. The results of this study could have a significant impact on building design and operation, especially in the context of growing awareness of the importance of a healthy indoor environment and the need for energy efficiency. Ventilation intensity is becoming a pivotal factor in achieving these goals, and its analysis and optimization are essential for the future of sustainable buildings.

**Keywords:** ventilation, ventilation intensity, indoor environmental quality, air quality, pollutant concentrations

## INTRODUCTION

The indoor environment of buildings plays a pivotal role in the daily lives of individuals, encompassing workplace settings, residential spaces, and recreational areas. The quality of indoor air within these structures significantly influences the health and well-being of their occupants [1]. As a result, an increasing number of scientific and industrial sectors are focusing on the monitoring and enhancement of indoor air quality [2-4]. One of the key indicators that assumes paramount importance in this context is ventilation intensity.

The building sector is a significant contributor to energy consumption [5]. Current trends aim to increase the airtightness of the building envelope and reduce uncontrolled air exchange in buildings. Ventilation, as the mechanism for air exchange within buildings, is indispensable in eliminating pollutants, maintaining optimal temperature and humidity levels, and contributing to the overall comfort and health of occupants [6]. This research article delves into the role of ventilation intensity as an indicator of indoor building environment quality. We will explore how ventilation intensity impacts the concentrations of harmful substances, microorganisms, and the comfort of occupants. Additionally, we will examine the technologies and strategies that can be employed to

optimize ventilation and ensure the best possible conditions for building inhabitants. Carbon dioxide (CO2) serves as an indicator of indoor environmental quality, and its concentration in indoor air should not exceed the value of 1500 ppm.

This paper thus becomes a significant contribution to the discussion on improving indoor air quality and enhancing the comfort of building users. Given the escalating challenges associated with climate change and air pollution, it is imperative to seek innovative solutions that will lead to an improvement in the quality of life and the protection of health in the environments where we spend most of our time.

## MATERIALS AND METHODS

The case study presents an analysis of the natural ventilation system with respect to a typical residential building. The construction of the monitored family house is situated in the South Bohemian region, in the statutory city of České Budějovice, Czech Republic. For the presentation of the results, a reference residential room was selected. The corner room is located on the 1st floor of the family house. The non-transit room is oriented to the southwest. Access to the space is provided from the kitchen through wooden veneered doors without sealing. The doors are closed almost throughout the day.

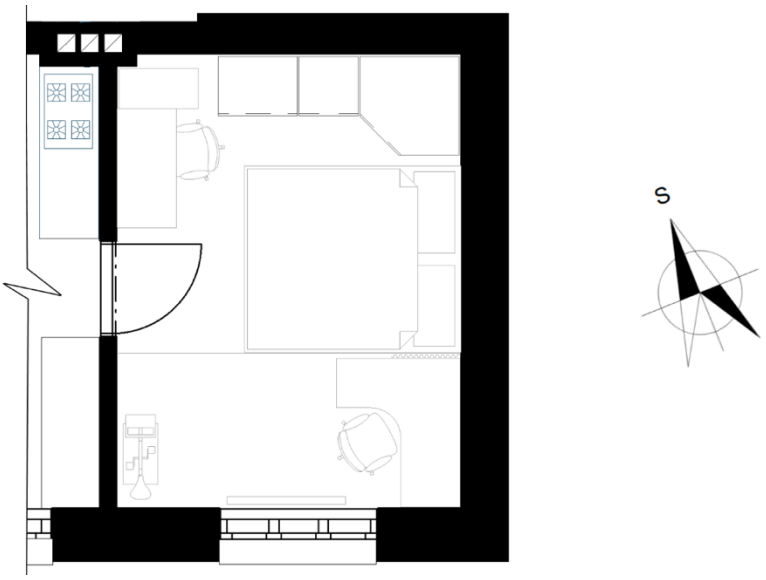
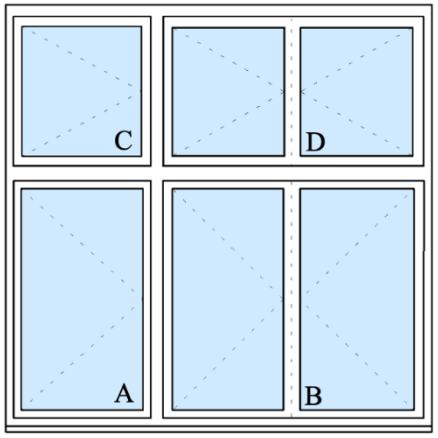



**Figure 1:** Floor plan of monitored room.

The space is naturally ventilated and illuminated by a casement window. The window is wooden and original, with the capability to open in 4 main ways. In total, the window has 4 single window sashes and 4 double windows sashes. All window leaves open into the interior. The windows do not have any sealing. Large windows sashes are located at the bottom part of the structure, and small window sashes at the upper part. The filling of the window openings consists of clear pane glass, darkened into the wooden structure. The window sashes are opened and closed using brass handles. The space between the exterior and interior construction of the casement window is 110 mm. The window structure is somewhat permeable, allowing air infiltration through the frame. The interior windowsill, at a height of 880 mm from the floor level, is also made of wood. There is a crack between the windowsill and the frame. This area is colder (compared to the surrounding surfaces) in the autumn and winter periods. The room's clear height is 3 meters, the floor area is

approximately 16.07 $m^2$, and the room volume is 48.21 $m^3$. The bottom edge of the window, in relation to the sidewalk in the public space in front of the building, is approximately 2.5 meters. The window is 4.7 meters away from a single-lane road with longitudinal parking. The space between the window and the opposite building is just under 16 meters.




Main window dimension: 1800 x 1800 mm

Window opening dimension A: 490 x 955 mm

Window opening dimension B:1000 x 955 mm

Window opening dimension C: 490 x 590 mm

Window opening dimension D: 1000 x 590 mm

**Figure 2:** The diagram of window leaves of a jalousie window.

There are currently 2 adults living in the room (sleeping, working, exercising, cleaning, etc.). Person A is female and 26 years old. Person B is a 25-year-old male. Both people spend in the room only the so-called working week (from early Sunday evening or Monday to Friday afternoon).

**Table 1:** Basic time points of the daily schedule in the room

| | |
|---|---|
| 06:00 AM | Person B leaves for work. |
| 09:00 AM | Person A wakes up, tidies up, and refreshes. |
| 09:30 AM | Person A works from home, possibly commutes to work, or engages in off-site studies. |
| 01:00 PM | Person A leaves the room, takes care of a family member. |
| 02:30 PM | Person A returns to work. |
| 03:15 PM | Person B returns from work. |
| (Person A works sporadically in the afternoon, and later in the evening, typically from 09:00 PM to 02:00 AM, based on the necessary time and work schedule.) | |
| 11:00 PM | Person B sleeps, while Person A continues to work. |
| Note: This is a simplified daily schedule for an understanding of the individuals' presence in the observed environment. A cat occasionally has access to the room. | |

The monitored room is equipped with basic furniture (beds, wardrobes, work desks), followed by exercise equipment and a stationary bicycle. During the activities of person A, a blackout curtain is drawn, preventing uniform ventilation across the entire room.

The Testo 480 device was placed on a work desk at a height of 780 mm above floor level and approximately 1000 mm away from the window. The instrument was also positioned in the library, approximately 1.5 m above the clean floor level, with the probe directed towards the center of the room.

The room is naturally ventilated according to the users' preferences. In the summer, the window is almost constantly open, while in the winter, it is the opposite. Ventilation is most commonly done through the window opening marked as A, or alternatively, B.

During the analysis of indoor air quality and comfort, the frequency and duration of ventilation are recorded.

## RESULTS

The measurements were conducted during the summer, autumn, and predominantly winter seasons. Particularly in the winter months, air exchange was found to be insufficient. The device, along with the probe, was positioned on a shelf in the library, approximately 1.5 meters above the clean floor level. The measuring probe was directed into the room. The research was conducted at a single point, a specific location. The measuring device was programmed with three monitored parameters. Parameters measured by the TESTO 480 device:

- Concentration of $CO_2$ [ppm],
- Relative humidity [%],
- Air temperature [°C].

In the case of ventilating a living room, it can be assumed that there is a certain behavior of air currents when doors are open or, conversely, closed. In the room, there is one door, an inward-opening flush door. The only window in the room is located on the west side of the building and is also wooden with casement opening. The positions of the wall containing the window and the wall containing the door are not opposite each other. The walls with the door and the window (the objects are approximately halfway along the walls) are almost perpendicular to each other.

Ventilation is not as effective as with openings (doors, windows) placed directly opposite each other. The anticipated airflow is dependent not only on the size of the window sash but also on the position of the door sash. In the case of an open door sash, for example, at a 90° angle, there is an influence on the fresh air current. Airflow enters from the exterior into the interior and then moves towards the open door. The fresh air current bypasses the corner of the room, where there is a corner wardrobe, leading to uneven ventilation.

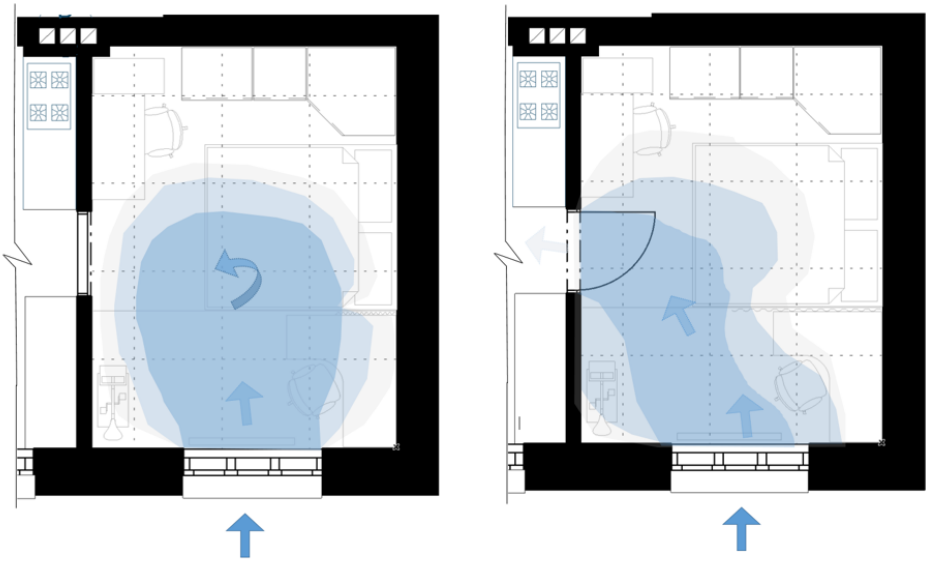


**Figure 3:** Predicted airflow with closed and open doors in the room.

The concentration of carbon dioxide depends not only on the ventilation intensity but also on the sources of carbon dioxide in the indoor environment. In the case of the studied family house, the main sources of carbon dioxide are individuals. Below are shown only the results for a few reference days.

With one person in the children's room, there is a slower increase in the concentration of carbon dioxide compared to when there are two people in the room. This is evidenced by day 1, the reference day when there was one person and occasionally one cat in the room.

On Saturday, February 4, 2023, the measurements commenced at 13:45:50. The weather was partly cloudy to sunny, with a temperature around 2 °C. The measurement of the assessed day lasted for 10 hours, during which not a single instance occurred where the concentration of carbon dioxide exceeded the threshold of 1500 ppm. The room was ventilated a total of 3 times. Initially, ventilation occurred through window leaf A with curtains, followed by window leaf A without curtains. The final ventilation was performed through the large window with curtains. The highest recorded value of carbon dioxide in the children's room on February 4, 2023, was 1359 ppm. The lowest concentration of carbon dioxide on the mentioned day was 852 ppm.

On the following day, i.e., February 5, 2023, the carbon dioxide concentration exceeded the limit of 1500 ppm. There were 2 people in the room. An assessment was conducted over the same time period as on February 4, 2023. The carbon dioxide concentration reached 2226 ppm, with the lowest concentration at 731 ppm. According to the results, it was found that in the presence of 2 people, there is a 1.64-fold increase in carbon dioxide compared to the initial concentration. There is no twofold increase in carbon dioxide.

The graph below illustrates carbon dioxide concentrations on reference days. The brown line represents the development for 1 person, while the red line depicts concentrations for 2 people in the monitored room.

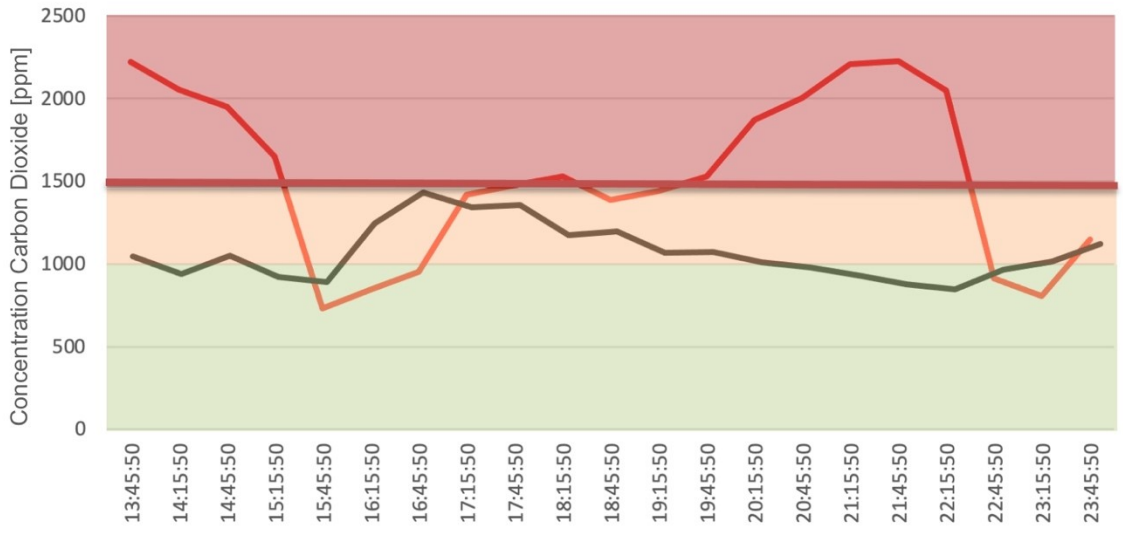



**Figure 4:** The carbon Dioxide Concentration [ppm] in the monitored room for the reference days.

## DISCUSSION

During the research on environmental quality and ventilation, it was found that the monitored room would exceed the threshold value of 1500 ppm (set for the need for air exchange). Theoretical calculations indicated that the room should have ventilation at a rate of 55.0 $m^3$/h, with a ventilation intensity of 1.0 $h^{-1}$. Using a measuring device, it was determined that to achieve the required air exchange, ventilation for 17 minutes is needed when window sash A (window area 0.47 $m^2$) is open. Window sash B has a 103% larger area than window sash A (0.955 $m^2$), resulting in a faster air exchange, taking only 7 minutes. If both window sashes A and B are opened simultaneously, ventilation will last only 5 minutes.

To ensure optimal climatic conditions indoors (temperature, relative humidity, and carbon dioxide concentration), four variants are proposed for the monitored room. The first three variants utilize natural ventilation, while the fourth belongs to forced ventilation. The proposed variants are:

1. Variant – Timed ventilation,
2. Variant – Air quality monitoring,
3. Variant – Installation of a new window with automatic opening,
4. Variant – Installation of a ventilation recovery unit.

In table number 2, proposed solutions are listed that could maintain a high-quality environment in the room through sufficient ventilation. The best (most effective) value is assigned 4 points, while the lowest (least effective) is assigned 1 point.

**Table 2:** Multicriteria analysis.

| | **Timed ventilation** | **Air quality monitoring** | **Installation of a new window with automatic opening** | **Installation of a ventilation recovery unit** |
|---|---|---|---|---|
| Price | 4 | 3 | 2 | 1 |
| Maintenance | 4 | 3 | 2 | 1 |
| Implementation complexity | 4 | 3 | 2 | 1 |
| Effectiveness | 1 | 2 | 3 | 4 |
| CO2 level control | 1 | 4 | 4 | 4 |
| User comfort | 1 | 2 | 3 | 4 |
| **Total** | **15** | **17** | **16** | **15** |

According to the parameters in table, the air quality monitor emerges as the most advantageous option. Continuously and retrospectively measured values will be displayed using a mobile phone or, if necessary, a laptop. One AirThings Wave plus device is proposed for the room. The total investment for room will be 4000 CZK (160 EUR). The installation of sensors and software is relatively undemanding, and the sensor significantly does not disrupt the interior of the rooms.

The second-best option based on scoring is the installation of a new window in the room, followed by the installation of automatic electric openers. The third-place is occupied by two options – timed ventilation and installation of a recovery unit. The major drawback

of installing a recovery unit is primarily the acquisition cost and subsequent maintenance, but it ensures the highest efficiency in providing fresh air. The proposed timing of ventilation is the least comfortable for users in the ventilated room. The exact values of carbon dioxide, relative humidity, or interior air temperature cannot be determined. Users are constantly subliminally pressured to adhere to planned ventilation. In the case of possible user sleep during the scheduled ventilation, air exchange will not occur, and suitable values cannot be guaranteed even after subsequent room ventilation.

## CONCLUSION

This study emphasizes the pivotal role of ventilation in sustaining indoor environmental quality. Focusing on ventilation intensity as a key indicator of air quality, the research aims to analyze its impact on thermal comfort, pollutant concentrations, and overall occupant well-being. Technological innovations, including sensors and automation, are explored for efficient ventilation control.

Results highlight the growing significance of ventilation intensity in achieving sustainable building goals, emphasizing the need for analysis and optimization in future designs. The case study, centered on a typical residential building, provides practical insights into challenges related to natural ventilation.

Measurements conducted over reference days reveal the impact of occupancy on carbon dioxide concentrations, emphasizing the necessity for effective ventilation strategies. Multicriteria analysis suggests an air quality monitor as the most advantageous solution, providing continuous measurements for optimal user comfort.

In conclusion, this research significantly contributes to discussions on improving indoor air quality and enhancing user comfort. As climate change and air pollution challenges escalate, innovative solutions, such as the proposed ventilation strategies, are imperative for fostering healthier and more sustainable living environments.

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# SECTION ANIMALS & SOCIETY

# DISPELLING MISCONCEPTIONS ABOUT ANIMALS AND HUMAN-ANIMAL INTERACTIONS IN ROMANIA

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## ABSTRACT

The article delves into the prevalent misconceptions surrounding animals and their interactions with humans in Romania, with the primary goal of fostering a more accurate understanding and dispelling commonly held myths. A widespread misconception among many individuals is the erroneous belief that all animals inherently pose harm or threats when encountering humans. Our aim is to illuminate the reality that animals exhibit diverse behaviors and intentions, and in most cases, they prefer to avoid confrontations with humans.

Within the article, alongside expert insights and scientific viewpoints, readers will find practical advice on coexisting safely with wildlife. This advice encompasses general precautions and behaviors that individuals can adopt to reduce the likelihood of conflicts or accidents in encounters with animals. Furthermore, the article equips readers with essential techniques and strategies for peacefully coexisting with potentially unpredictable animals. These practical tips guide individuals on the appropriate actions to minimize risks when interacting with animals in various settings.

In conclusion, this paper presents a valuable initiative directed at enhancing awareness, dispelling unfounded fears, and nurturing a deeper appreciation for the animal kingdom in Romania. Through education and heightened awareness, readers gain valuable knowledge on achieving harmonious coexistence with wildlife while minimizing potential risks. This comprehensive approach plays a pivotal role in rectifying misconceptions and contributes to the development of a society that respects and values its natural environment.

**Keywords:** animal misconceptions, human-animal interactions, wildlife coexistence, awareness

## INTRODUCTION

The complex web of interactions between humans and animals has been an integral part of human history for thousands of years. Throughout time, these interactions have evolved, spanning from mutualistic partnerships to confrontational encounters, shaping the narratives of cultures and societies around the world. In the heart of Eastern Europe, Romania stands as a testament to this age-old relationship, where the diverse fauna of this nation has coexisted with its human inhabitants for centuries. However, this

coexistence has not always been harmonious, and the pages of history are often stained with tales of misunderstandings, unfounded fears, and misconceptions about the animal kingdom. Common misconceptions about animals in Eastern Europe countries can be influenced by a variety of factors, including genetic data, media coverage, and historical perceptions. Additionally, media coverage often emphasizes the rarity of human fatalities caused by certain animals, potentially leading to misunderstandings about the actual risks posed by these animals [1]. Furthermore, historical viewpoints and regional cooperation endeavors may contribute to the propagation of false information about animals in Eastern Europe [2]. One common belief is that all animals inherently pose harm or threats when encountering humans. This misconception is often driven by inherent fear and dread of wildlife, leading to hostility towards animals. Media coverage of rare but highly publicized incidents, such as animal attacks, can also contribute to the perception of animals as universally dangerous. Additionally, the lack of experience living alongside wild animals can contribute to heightened fear, while those with such experience tend to be less fearful of animals [3]. Erroneous beliefs regarding the potential hazards posed by animals can also be shaped by social and cultural elements. Effectively addressing these fallacies necessitates a multifaceted approach that takes into account psychological, social, and cultural factors, alongside the promotion of accurate and unbiased media coverage of human-animal interactions.

The article offers a thorough examination of the prevalent fears associated with wildlife interactions among the residents of Romania. Through meticulous observation and analysis, it uncovers the enduring and significant factors that impact daily life for both people and animals, often resulting in adverse consequences for the latter. Furthermore, the article delves into the primary species that evoke fear and repulsion within the population, accompanied by scientific insights into these species' behaviors and characteristics. Additionally, key recommendations are presented to facilitate safe and informed interactions with these species, promoting a sense of coexistence and mutual respect between humans and wildlife.

## MATERIALS AND METHODS

The data collection commenced with a systematic and comprehensive approach, involving the use of questionnaires that encouraged discussions with individuals. These questionnaires were disseminated across a broad spectrum of social, ethnic, and age groups, ensuring representation from diverse demographics within the country. The data collection period spanned from January to December 2022, providing an extensive dataset for subsequent rigorous analysis. The selection of this method was deliberate, as it enabled the precise targeting and engagement of specific demographic groups, establishing a robust foundation for subsequent in-depth examination. The questionnaire itself was meticulously constructed to capture a wide spectrum of responses, incorporating a combination of closed, open, and mixed questions, thoughtfully designed to accommodate the varied preferences and experiences of our surveyed participants [4].

The data collection process also involved extensive fieldwork, which included numerous trips throughout Romania. This allowed us to connect with people from various counties, each characterized by its customs and traditions. Romania's regional diversity played a pivotal role in shaping the survey, with each county representing a unique microcosm of cultural heritage. By engaging respondents from diverse regions,

our survey embraced a wide spectrum of local beliefs, customs, and traditions. This multifaceted approach enriched our dataset, fostering a more comprehensive understanding of Romania's cultural complexity. In summary, our data collection methodology combined the efficiency of questionnaires with the depth of fieldwork, accommodating the diverse demographics, customs, and traditions found across the regions of Romania. This comprehensive approach laid the foundation for rigorous analysis in our research.

## RESULTS

In this section, the empirical findings obtained from the comprehensive survey are presented, which gathered responses from a diverse sample of individuals across multiple counties within the country. A total of 4262 participants, representing various age groups and educational levels, completed the questionnaire on myths and beliefs pertaining to animals (Figure 1).

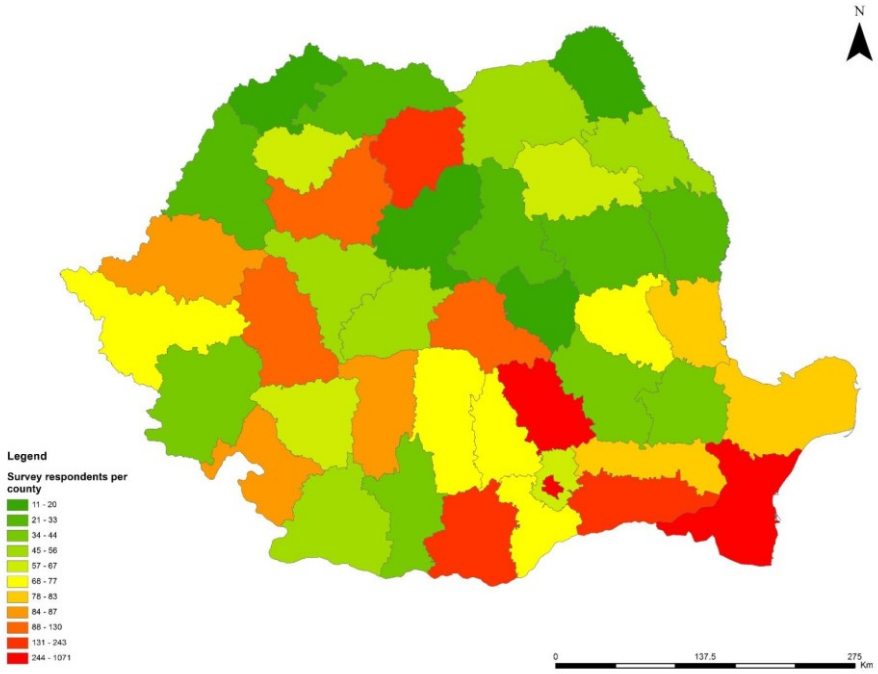



Figure 1. Survey respondents per county at national level

The diagrams included in Figure 2 illustrate different aspects of the study, including the participant count, the average age and educational levels. The decision to limit survey participation to individuals over 18 years of age was made to ensure that respondents possessed the necessary maturity and life experience to provide meaningful and informed insights into the topic of myths and beliefs related to animal-human interactions in Romania. The demographic breakdown indicates that respondents spanned a wide range of age groups, with substantial representation from young adults, middle-aged individuals, and seniors. This diversity in age demographics suggests that myths and beliefs about animals hold significance across generations, underscoring the enduring cultural significance of these narratives. Furthermore, the educational background of the respondents displayed a diverse range of qualifications, from those with bachelor's and master's degrees to those with upper secondary education and even PhD holders. This diversity in educational levels underscores that myths and beliefs

regarding animals are not confined to specific educational backgrounds but permeate various strata of society.

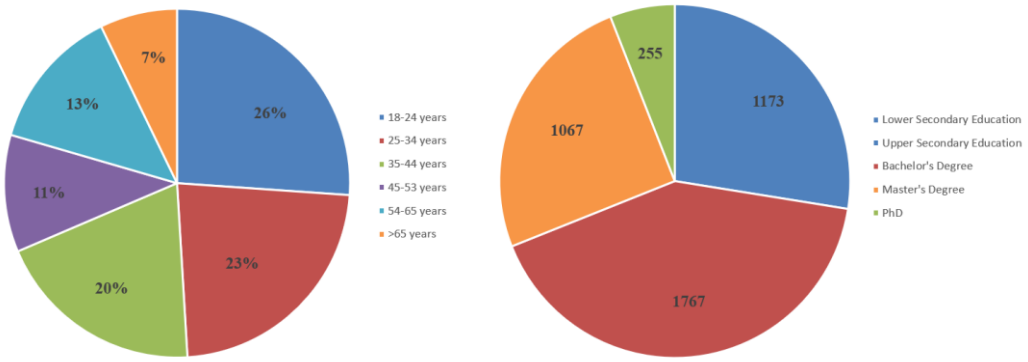



Figure 2. The average age (left) and the educational level (right) of survey respondents

Through the comprehensive collection and analysis of data obtained from the survey participants, a quantitative and objective groundwork was established for comprehending people's perception and engagement with animal-related beliefs and myths. This extensive body of empirical evidence plays a pivotal role in elucidating the intricacies from the relationship between humans and wildlife. Our findings highlight the diversity of cultural interpretations and superstitions surrounding animal species within the regional context. Consequently, it offers valuable insights into the intricate dynamics characterizing human-animal interactions in Romania. In summary of our findings, the following predominant beliefs have surfaced:

- Regarding snakes, common themes include a fear of snake venom, beliefs in snake magic, snake-related superstitions, and even weather predictions associated with snakes. Due to these beliefs, many people are inclined to kill snakes on sight, regardless of their actual threat;
- Bats have a complex array of beliefs surrounding them, from getting tangled in hair to being metamorphosed vampires. Some associate bats with bloodsucking, which is inaccurate as they primarily feed on insects. COVID-19 has also been wrongly attributed to bats in some beliefs, contributing to negative associations;
- Frogs, too, have a range of beliefs associated with them, such as causing skin conditions or warts when touched, the potential for transformation into princes, and their role as symbols of luck and blessings. Some associate them with witchcraft and curses;
- Owls hold a special place in Romanian folklore, with beliefs ranging from omen of death to symbols of wisdom and intelligence. Some view them as predictors of death, while others consider them bringers of good luck. Owl hooting is associated with various omens, including death and pregnancy prediction;
- Weasels are believed to be venomous and aggressive. There are also misconceptions that they can attack women's breasts and braid horse tails, and they are generally seen as bad omens.

These beliefs illustrate the intricate and diverse cultural interpretations of these animals within Romanian traditions and folklore, reflecting the complexity of human-animal

relationships at regional level. Approximately 80% of respondents were familiar with certain myths surrounding these animals, while about 50% expressed belief in these myths.

The participants in the study were also inquired about the sources from which they acquired knowledge about these myths and beliefs. The findings indicated that the primary sources were family members, followed by friends, with a smaller number mentioning school as the origin of their knowledge.

## DISCUSSION

It's important to note that these popular beliefs can vary widely among communities and may be influenced by specific cultural, religious, or local traditions. Promoting education and awareness is crucial to counter these beliefs and to help protect and conserve snakes and other wildlife species. Additionally, we present recommendations and practical guidelines aimed at minimizing conflicts with the wildlife species emphasized in the study, particularly those provoking intense reactions from individuals, both on a national and broader scale.

In Romania, there are 10 species of snakes. It is essential to note that these species are not systematically included in the national educational curriculum due to a clear lack of knowledge regarding this taxonomic group. Additionally, there is a genetically inherited fear of these animals, resulting in most individuals displaying disgust, fear, and, in some cases, even causing harm to snakes without a valid reason. Considering the distribution of snake species in Romania (Figure 3) and the lower reporting frequency of viper species at the national level, it can be inferred that most encounters between people and snakes likely involve species belonging to the families Colubridae and Natricidae. These families comprise non-venomous snake species such as the grass snake (*Natrix natrix*), dice snake (*Natrix tessellata*), smooth snake (*Coronella austriaca*), Aesculapian snake (*Zamenis longissimus*), and Caspian whipsnake (*Dolicophis caspius*). Furthermore, some individuals interviewed described incidents or presented photographic evidence of snakes they believed to be vipers, which, in reality, belonged to the species *Anguis colchica* (slowworm), a legless lizard with a distinctive appearance. Isolated encounters with venomous snakes (of which three species inhabit Romania, namely the common European adder (*Vipera berus*), nose-horned viper (*Vipera ammodytes*), and meadow viper (*Vipera ursinii*)) require knowledge and precautions. All these species, however, are legally protected throughout Europe. It is advisable to merely photograph them and subsequently seek information, under no circumstances resorting to harming them. Unfortunately, this behavior is not limited to Romania alone. The initial human response is often to rid themselves of the snake. Additionally, religious beliefs promote aversion to snakes, compounded by the fact that many individuals harbor phobias related to them.

Conquering fear or disgust regarding snakes is a multifaceted process that involves education, gradual exposure, changing perceptions, and adopting safety precautions. Understanding and appreciating the importance of snakes in ecosystems can significantly contribute to overcoming these negative feelings. Snakes, including vipers and non-venomous species, are part of Romania's biodiversity, and distinguishing between them is essential for safe and informed encounters. Learning about the diversity of snake species, their habits, and their ecological significance can replace fear

with fascination. Gradual exposure is an effective strategy for managing snake-related fear or disgust. Controlled encounters in safe environments, such as zoos, nature centers, or guided hikes with knowledgeable experts, can help individuals become more comfortable around snakes. Always keeping a safe distance from snakes, whether they are vipers or non-venomous species, is vital. Attempting to handle them should be avoided, as snakes require space to move freely. By respecting their need for space and understanding their role in controlling rodent populations and maintaining biodiversity, people can foster empathy and respect. Moreover, wearing suitable footwear and exercising caution when exploring natural areas, especially in tall grass or underbrush, can help minimize the risk of accidental snake encounters. These combined efforts promote a more positive and informed attitude toward snakes while ensuring personal safety in their presence.

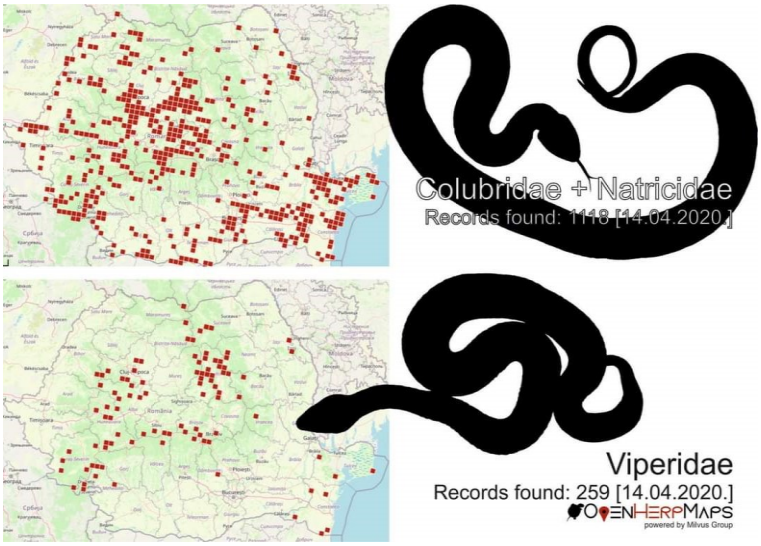



Figure 3. The distribution of snake species in Romania [5]

In Romania, 32 species of bats inhabit the territory, all of which are legally protected. Among these, three species are frequently encountered in the capital city: the noctule bat (*Nyctalus noctula*), Kuhl's pipistrelle (*Pipistrellus kuhlii*), and the parti-colored bat (*Vespertilio murinus*). Bats do not become entangled in human hair; they utilize echolocation to navigate and easily avoid obstacles. They do not feed on blood, and the bat species in our country are strictly insectivorous, playing a crucial ecological role in controlling populations of harmful insects and limiting the spread of pathogens transmitted by certain insect vectors. Generally, all myths surrounding bats lack scientific foundation and are unfounded. To minimize potential health risks, individuals encountering bats should maintain a safe distance and refrain from direct physical contact. Bats, especially those displaying abnormal behavior, may carry zoonotic diseases, including rabies, making avoidance of contact essential. When engaging in research or conservation efforts involving bats, proper personal protective equipment, such as gloves, should be utilized. Additionally, any encounter with a sick or injured bat should be promptly reported to local wildlife authorities to ensure safe and appropriate handling and to mitigate potential risks of disease transmission. These measures contribute to both public safety and the preservation of bat populations.

When referring to frogs that might transmit skin diseases, most people describe green frogs with warts on their backs. Considering the highest species density among frogs that can inhabit a wide range of habitats, displaying adaptability to anthropogenic conditions, thereby increasing encounters with humans, along with the provided descriptions, it can be inferred that people are referring to the species such as the marsh frog (*Pelophylax ridibundus*), green toad (*Bufotes viridis*), common toad (*Bufo bufo*), and yellow-bellied toad (*Bombina variegata*). What most people are unaware of is the highly significant role these species play in nature, warranting their protection (*Bombina variegata* is listed in Annexes II and IV of the Habitats Directive). Promoting positive human-frog interactions involves a multifaceted approach. First, it's crucial to prioritize habitat conservation by minimizing disturbance to wetlands and aquatic areas where frogs breed, and by avoiding the use of chemicals in these habitats. Second, education and engagement are vital components. This entails raising public awareness about frogs' ecological roles, unique life cycles, and their significance in controlling insect populations. Encouraging participation in community-driven conservation efforts or citizen science programs centered on frogs fosters a sense of connection and shared responsibility for their protection.

While owls are revered as symbols of wisdom, they are also associated with death, witchcraft, and superstitions. These contrasting beliefs have influenced human interactions with owls and have implications for owl conservation and cultural understanding. Among the 10 owl species living in Romania, the species most frequently encountered are typically those that exhibit greater adaptability to human-altered landscapes or those that reside in urban and suburban areas. Little owls (*Athene noctua*) are often seen in rural and suburban areas, including farmlands, villages, and even cities. Being partially drawn to human environments, the species is lured by the gatherings of insects that accumulate in illuminated areas. Historically, it has been linked with death and has often faced persecution, a practice that continues to this day. This association arises from its presence in places where there have been recent deaths and where people gather for vigils, owing to the attraction of insects it preys upon due to the overnight lighting.

Weasels are sometimes associated with cunning or trickery in various cultures, which can further contribute to the belief in their involvement in mysterious events. While they are skilled hunters and agile animals, there is no scientific basis to support the idea that they intentionally braid horses' tails. Tangles in horse tails can occur for various reasons, including natural behaviors of horses or environmental factors, and attributing them to weasels is likely a result of cultural or anecdotal explanations rather than factual evidence. In Romania, where both *Mustela nivalis* (weasel) and *Mustela erminea* (stoat) are present, such beliefs may have roots in local folklore or traditions. They play important roles in ecosystems, such as controlling small rodent populations. Understanding the natural behaviors and ecological significance of these animals is crucial for dispelling myths and superstitions surrounding them.

Notably, the high level of familiarity with these myths, with approximately 80% of respondents having heard of them, and the fact that about 50% of participants expressed belief in these narratives, demonstrates the enduring presence and impact of these beliefs in contemporary Romanian society. These statistics emphasize the need for comprehensive educational efforts and public awareness campaigns that not only debunk myths but also foster a greater understanding of wildlife, ultimately promoting

responsible and informed interactions with animals. The survey results serve as a valuable foundation for future initiatives aimed at bridging the gap between traditional beliefs and scientific knowledge, contributing to a more harmonious coexistence between humans and wildlife in Romania.

## CONCLUSION

In conclusion, this study has provided valuable insights into the intricate dynamics of human-animal interactions in Romania by examining prevalent beliefs and myths surrounding various animal species. Through the collection and analysis of data from survey participants, a quantitative and objective foundation was established to comprehend people's perceptions and engagement with these beliefs. The findings underscore the diversity of cultural interpretations and superstitions surrounding animals in the regional context, shedding light on the complex relationship between humans and wildlife.

Specifically, the study revealed a range of beliefs associated with snakes, bats, frogs, owls, and weasels, reflecting the rich tapestry of Romanian folklore. These beliefs vary widely, from fear of snake venom to associations of owls with wisdom and intelligence. Family members and friends were identified as primary sources of knowledge for these myths and beliefs, emphasizing the role of interpersonal transmission in perpetuating these cultural narratives.

To address misconceptions and promote responsible interactions with wildlife, recommendations include comprehensive educational reforms, public awareness campaigns, responsible media portrayal, strengthened laws regarding animal well-being, collaboration with animal welfare organizations, and ongoing research to assess the impact of educational efforts. International cooperation and a sustained commitment to fostering empathy and informed approaches to animal behavior and ethical treatment are essential for the continued progress in this field.

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# DIVERSITY OF SOIL NEMATODES IN THE THREATENED ECOSYSTEM OF PONICKA CAVE

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## ABSTRACT

Ponicka Cave, situated in central Slovakia, is a natural wonder, known for its stunning stalagmites, stalactites, and underground lakes. This unique cave system has a hydrological connection to the Earth's surface, representing a very vulnerable habitat. Long-lasting intensive livestock grazing in the aquifer area of Ponicka cave and insufficiently insulated manure dump were the main reasons for the contamination of this cave. Soil nematodes are the most abundant multicellular organisms on Earth with respect to both species' richness and abundance. They are ubiquitous invertebrates in the soil that include a variety of feeding types, and they perform key functions in soil food webs. They are considered as one of the best soils bioindicators. Although the mesofauna of caves in Slovakia is relatively well described, information about soil nematodes are missing. In this study, we present the first data on the fauna of free-living and plant-parasitic nematode species from soil taken in the Karst region (karst spring, ponor, sink hole) and manure. Totally we identified 54 nematode species across 43 genera, 28 families, and 9 orders. The highest number of identified nematode species (44) was observed in Ponor, while 33 species were observed in both the karst springs and in a sinkhole and only 8 species were found in manure. Bacterivores were the dominant trophic group in all sites. Differences in nematode species composition in sites around Ponicka Cave, showing the differences in the environmental conditions nearby and site characteristic. This study emphasizes how important it is to know, understand and protect the soil biota that plays an important role in keeping the cave and the surrounding environment healthy.

**Keywords:** Soil nematodes, communities, Ponicka cave

## INTRODUCTION

The Ponicka Cave is part of the karst region known as Ponicky kras. Historical information about the presence of cave spaces within the limestone massif of Drienok was confirmed in 1981. Characteristic features of the cave include calcite formations such as stalagmites, stalactites, and underground lakes. The cave is characterized by a close hydrological connection with the earth's surface, thus representing a very vulnerable habitat. Long-lasting intensive livestock grazing in the aquifer area of Ponicka cave and insufficiently insulated manure dump were the main sources of pollution of this cave [1].

The soil biota of cave is diverse and complex ecosystems that plays important role in the overall health and function. The soil in caves is inhabited by a variety of microorganisms, including bacteria, fungi and protozoa, as well as larger organisms such as nematodes, springtails and mites. These environments are unique, supporting highly specialized species, some of which can only be found within cave ecosystems [2]. Nevertheless, soil biota in and around the caves remain poorly studied as many caves are still unexplored, sensitive to anthropogenic disturbance and/or present severe physical challenges for taking samples. Thus, information relating to cave-dwelling faunal groups including soil nematodes is very limited. A review study of nematodes in cave ecosystems reported only 41 scientific papers over 138 years of studies and identified 295 nematode taxa from 72 caves in 18 countries around the world [3].

Soil nematodes are ubiquitous and the most abundant soil organisms that occur in all as well as extreme and disturbed ecosystems. Soil nematodes based on the structure of their oral cavity and food preferences can by divided into trophic groups. Soil nematodes have major roles in many soil processes such as mineralisation and decomposition, and are highly sensitive to changes in their surroundings. Therefore, nematodes are considered as one of the best ecological bioindicators [4]. This study presents the first data on the fauna of free-living and plant parasite nematode species from soil taken in karst spring, ponors, sinkhole and manure around Ponicka cave. We evaluated species distribution, abundance, and dominance of soil nematodes in selected sites.

## MATERIALS AND METHODS

The Ponicka Cave (48°42′47.6′′N 19°15‘43.8′′E) is situated in the Banska Bystrica district in the middle of Slovakia. For nematode communities’ study we chose eight sites around Ponicka cave: two sites were located near Karst spring; three sites were near ponor; one near sinkhole and one near former manure pit. Each sample consist of five soil sub-samples. Obtained soil samples were transported in plastic bags to the laboratory for nematode extraction and identification.

Soil nematodes were isolated from 100 g of soil sample using modified Baermann’s technique [5]. Isolated nematodes were killed in a water bath (60-70 °C) and counted using a Leica S8APO stereomicroscope (Germany). All isolated nematode individuals were identified to the species level using an Eclipse 90i light microscope (Nikon, Japan) at magnifications of 100, 200, 400 and 600× and taxonomic and systematic monographic works. We calculated species dominance (D%) as $D\ (\%) = (n/s) \times 100$, where $n$ is the total abundance of a species and $s$ is the total abundance of the species in a sample. Identified species were classified into five trophic groups [6].

## RESULTS

The total nematode communities consist of 54 species belonging to 43 genera, 28 families and 9 orders. Specifically, our findings indicate that 44 nematode species were observed in Ponor, while 33 species were identified in both the karst springs and sinkhole. Notably, an additional set of 8 species were observed in manure pit, reflecting the adaptability of nematode species to diverse sites.

Different nematode species dominated at each site. In the karst spring, the dominated species were *Cervidellus vexilliger*, *Rhabditis producta*, *Eucephalobus striatus* and *Filenchus vulgaris*. In the ponor, the dominated species were *Cephalobus persegnis*, *Acrobeloides nanus*, *E. striatus*, *E. oxyuroides* and *Aphelenchus avenae*. In the sinkhole, we identified a dominated species: *A. nanus*, *R. producta*, *A. avenae*, *E. striatus*, *Helicotylenchus pseudorobustus* and *C. persegnis*. Within the manure, the dominated species were *R. producta, A. avenae, E. striatus* and *Panagrolaimus rigidus* (Table 1). These observed variations in dominant species reflected complex interactions between nematodes and their surroundings, including soil characteristics, vegetation, and microclimate conditions.

**Table 1** Check-list of nematode species at four localities around Ponicka cave with their dominance (%). TG - allocation of species to trophic groups B (bacterivores), F (fungivores), H (herbivores), P (predators) and O (omnivores).

| Nematode species | TG | Karst spring | Ponor | Sinkhole | Manure |
|---|---|---|---|---|---|
| ARAEOLAIMIDA | | | | | |
| **Aulolaimidae** | | | | | |
| *Aulolaimus oxycephalus* de Man, 1880 | B | 3.9 | 0.8 | - | - |
| **Plectidae** | | | | | |
| *Plectus communis* Bütschli, 1873 | B | 1.7 | 1.6 | 0.6 | - |
| *Plectus parvus* Bastian, 1865 | B | - | - | 0.6 | - |
| *Plectus opisthocirculus* Andrassy, 1952 | B | 4.7 | - | 1.2 | - |
| *Wilsonema schuurmansstekhoveni* (De Coninck, 1931) | B | 0.8 | 3.0 | 0.6 | - |
| RHABDITIDA | | | | | |
| **Teratocephalidae** | | | | | |
| *Teratocephalus tenuis* Andrássy, 1956 | B | 0.4 | 0.6 | - | - |
| **Cephalobidae** | | - | - | - | - |
| *Cephalobus persegnis* Bastian, 1865 | B | 1.0 | 18.9 | 6.6 | 8.9 |
| *Heterocephalobus elongatus* (de Man, 1880) | B | 2.3 | - | - | - |
| *Eucephalobus oxyuroides* (de Man, 1876) | B | - | 6.2 | - | - |
| *Eucephalobus striatus* (Bastian, 1865) | B | 9.1 | 8.5 | 9.0 | 15.2 |
| *Acrobeloides buetschlii* (de Man, 1884) | B | - | 2.2 | - | - |
| *Acrobeloides nanus* (de Man, 1880) | B | 1.0 | 11.5 | 13.9 | 7.0 |
| *Chiloplacus propinquus* (de Man, 1921) | B | - | 0.8 | 2.4 | 12 |
| *Cervidellus vexilliger* (de Man, 1880) | B | 14.0 | 1.9 | 1.2 | - |
| **Panagrolaimidae** | | | | | |
| *Panagrolaimus rigidus* (Schneider, 1866) | B | - | - | - | 15.2 |
| **Rhabditidae** | | | | | |
| *Rhabditis producta* (Schneider, 1886) | B | 8.9 | 1.6 | 13.3 | 20.9 |
| **Diploscapteridae** | | | | | |
| *Diploscapter rhizophilus* Rahm, 1928 | B | - | 0.8 | - | - |
| **Steinernematidae** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *Steinernema affine* (Bovien, 1937) | B | 1.6 | - | - | - |
| APHELENCHIDA | | | | | |
| **Aphelenchidae** | | | | | |
| *Aphelenchus avenae* Bastian, 1865 | F | 1.4 | 5.1 | 10.2 | 3.2 |
| **Aphelenchoididae** | | | | | |
| *Aphelenchoides composticola* Franklin, 1957 | F | 3.5 | 3.0 | - | 17.7 |
| *Aphelenchoides fragariae* (Ritzema Bos, 1890) | F | - | - | 1.8 | - |
| *Aphelenchoides parietinus* (Bastian, 1865) | F | - | 0.6 | - | - |
| TYLENCHIDA | | | | | |
| **Tylenchidae** | | | | | |
| *Filenchus vulgaris* (Brzeski, 1963) | F | 6.6 | 1.4 | 2.4 | - |
| *Malenchus bryophilus* (Steiner, 1914) | H | - | 2.0 | 1.2 | - |
| **Pratylenchidae** | | | | | |
| *Pratylenchus crenatus* Loof, 1960 | H | - | - | 1.2 | - |
| *Pratylenchus penetrans* (Cobb, 1917) | H | - | 1.6 | 1.2 | - |
| **Hoplolaimidae** | | | | | |
| *Helicotylenchus canadensis* Waseem, 1961 | H | - | 3.4 | - | - |
| *Helicotylenchus digonicus* Perry in Perry, Darling & Thorne, 1959 | H | - | - | 1.2 | - |
| *Helicotylenchus pseudorobustus* (Steiner, 1914) | H | - | 1.2 | 9.0 | - |
| *Rotylenchus goodeyi* Loof & Oostenbrink, 1958 | H | - | 1.4 | 2.4 | - |
| *Rotylenchus robustus* (de Man, 1876) | H | - | 0.8 | 1.2 | - |
| **Paratylenchidae** | | | | | |
| *Gracilacus goodeyi* (Oostenbrink, 1953) | H | - | 1.6 | - | - |
| *Paratylenchus projectus* Jenkins, 1956 | H | - | - | 1.8 | - |
| ENOPLIDA | | | | | |
| **Prismatolaimidae** | | | | | |
| *Prismatolaimus dolichurus* de Man, 1880 | B | 2.3 | 0.8 | - | - |
| *Prismatolaimus intermedius* (Bütschli, 1873) | B | - | 3.9 | 1.8 | - |
| **Tripylidae** | | | | | |
| *Tripyla filicaudata* de Man, 1880 | P | 0.8 | 2.3 | 1.2 | - |
| ALAIMIDA | | | | | |
| **Alaimidae** | | | | | |
| *Alaimus primitivus* de Man, 1880 | B | 3.3 | 2.6 | 1.8 | - |
| DIPHTHEROPHORIDA | | | | | |
| **Diphtherophoridae** | | | | | |
| *Diphtherophora communis* de Man, 1880 | F | 2.7 | 0.8 | - | - |
| **Trichodoridae** | | | | | |
| *Trichodorus primitivus* (de Man, 1880) | H | 2.7 | 0.3 | - | - |
| *Trichodorus sparsus* Szczygiel, 1968 | H | 1.6 | 0.3 | - | - |
| MONONCHIDA | | | | | |
| **Mylonchulidae** | | | | | |
| *Mylonchulus brachyuris* (Bütschli, 1873) | P | 3.5 | 0.6 | 2.4 | - |
| **Anatonchidae** | | - | - | - | - |
| *Miconchus studeri* (Steiner, 1914) | P | 0.4 | 0.6 | - | - |
| *Anatonchus tridentatus* (de Man, 1876) | P | 1.2 | 0.6 | - | - |

| | | | | | |
|---|---|---|---|---|---|
| DORYLAIMIDA | | | | | |
| **Dorylaimidae** | | | | | |
| *Mesodorylaimus bastiani* (Bütschli, 1873) | O | 5.6 | 0.5 | 1.2 | - |
| **Qudsianematidae** | | - | - | - | - |
| *Crassolabium ettersbergense* (de Man, 1885) | O | 0.4 | 0.8 | - | - |
| *Eudorylaimus iners* (Bastian, 1865) | O | 2.7 | 0.8 | 0.6 | - |
| *Eudorylaimus similis* (de Man, 1876) | O | - | 0.6 | 1.2 | - |
| *Ecumenicus monohystera* (de Man, 1880) | O | 0.2 | - | 1.8 | - |
| **Aporcelaimidae** | | | | | |
| *Aporcelaimellus obtusicaudatus* (Bastian, 1865) | O | 1.2 | 0.3 | - | - |
| **Nordiidae** | | | | | |
| *Longidorella microdorus* (de Man, 1880) | H | 5.2 | 1.2 | 0.6 | - |
| *Enchodelus macrodorus* (de Man, 1880) | P | - | 1.2 | 2.4 | - |
| **Swangeriidae** | | | | | - |
| *Oxydirus oxycephalus* (de Man, 1885) | P | 5.0 | 0.8 | 1.2 | - |
| **Mydonomidae** | | | | | |
| *Dorylaimoides micoletzkyi* (de Man, 1921) | F | 0.2 | 0.3 | 0.6 | - |
| **Tylencholaimellidae** | | | | | |
| *Doryllium zeelandicum* (de Man, 1876) | F | 0.2 | 0.3 | - | - |
| **Total number of identified nematode species** | | **33** | **44** | **33** | **8** |

The number of isolated nematodes individuals in 100g soil samples varied from 99 to 364. The quantification of nematode abundance displayed differences among the different sites. The highest nematode abundance was observed in the karst spring (107±80). Ponor presented the lowest nematode abundance (91±115). These differences in nematode abundance may be attributed to local environmental factors, such as soil moisture, temperature, and nutrient availability.

The assessment of nematode trophic groups found bacterivores as the dominant trophic group with more than 50% proportion in all four sites (Figure 1). In Ponor and sinkhole were bacterivores followed by herbivores, fungivores, predators, and omnivores. In Kast spring were bacterivores followed by fungivores, predator omnivores and herbivores. These findings highlight the essential roles nematodes play in nutrient cycling and the decomposition of organic matter in these ecosystems. In contrast, in the manure, we exclusively identified microbial-feeding nematodes, which demonstrates the specialization of nematode communities in response to distinct nutrient sources and trophic soil food web.

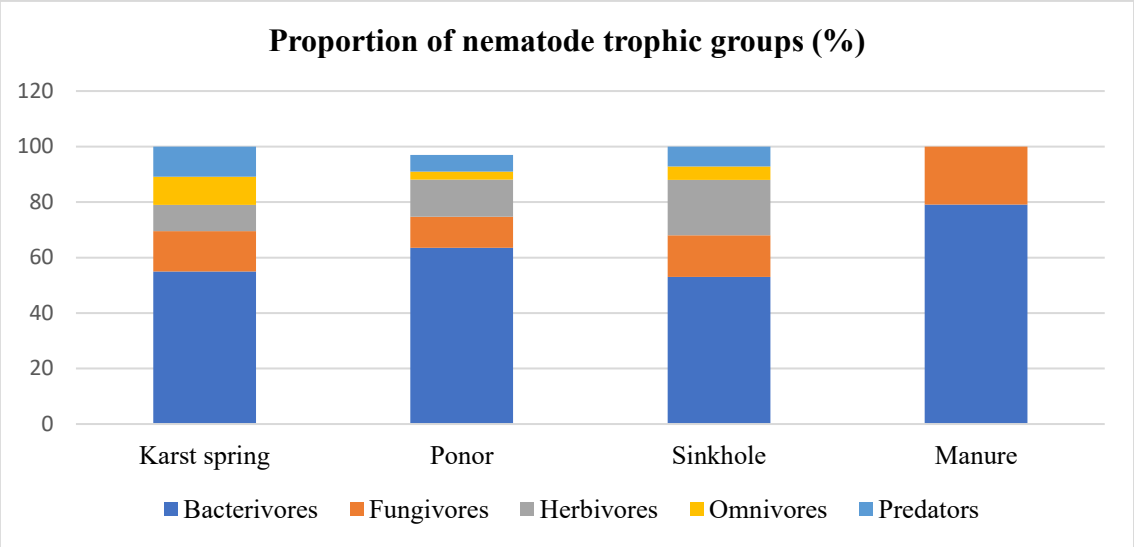



**Figure 1** Percentual proportion of nematode trophic groups in four sites (karst spring, ponor, sinkhole and manure) around Ponicka cave.

## DISCUSSION

Ponicka Cave in central Slovakia highlights its vulnerability due to intensive livestock grazing and inadequate waste management. The study provides the first data on soil nematodes and their taxonomic diversity, abundance, and trophic structure in the Karst region, revealing a rich diversity of species across different sites. The presence of 54 nematode species indicates a diverse and dynamic ecosystem, with variations in abundance linked to local environmental conditions. The highest nematode abundance in the karst spring and the lowest in ponor suggest that local environmental conditions and resource availability may significantly influence nematode populations. The most diverse and abundant nematode communities were observed in ponor. Higher nematode richness is considered a good indicator of the decomposition function in soil. More diverse nematode assemblages contribute to more resilient ecosystem services [7]. Thus, our data suggested fast nutrient cycling in ponor site.

The presence of all nematode trophic groups in the karst spring, ponor, and sinkhole suggests a consistent trophic structure, with each group playing a specific role in nutrient cycling and ecosystem functioning. Via sinking streams, sinkholes and other karst features can be soil nematodes transported into subterranean environments following soil erosion by water [8]. Microbial feeding nematode species can survive in cave systems and they in turn serve as a food source for omnivores and predators. Bacterivores, which feed on bacteria, typically play a critical role in nutrient cycling and ecosystem functioning. Fungivores are important in the decomposition of organic matter and nutrient recycling, while the presence of predators and omnivores points to more complex food web interactions in these ecosystems [4]. According to some authors [9], the herbivores are almost certainly accidental inhabitants of caves. Certain caves often face disruptions like the entry of biota and organic material. This leads to changes in the underground community and brings in accidental inhabitants that might only survive there for a short time [10]. The representation of the trophic group in cave ecosystems reveals that bacterivores dominated (mostly 50%) followed by omnivores (20%), predators (17%) and plant-parasitic nematodes (12%). The fungivores accounted for less than 5% of the

reported taxa, consistent with the study by Preez [3]. Similar to our results, bacterivores also dominated in the most of the sampling sites in Bakwena Cave in Irene in South Africa, while fungivores, omnivores, as well as herbivore nematodes were also found [11]. Bacterivore nematodes are most tolerant of environmental disturbance and changes and they are early colonisers that quickly multiply in a new environment with favourable conditions for their population growth [12].

The identification of nematode species and their different dominance in each site shows the importance of understanding the specific ecological functions and interactions of these species within their respective habitats. Studying these ecosystems that host diverse nematode species will result in the discovery of new species. Furthermore, cave ecosystems provide an ideal opportunity to investigate unique ecological networks and extreme evolutionary pathways.

## CONCLUSION

The study presents the first data about nematode communities from the cave ecosystem in Slovakia. In the assessment conducted on sites surrounding Ponicka cave, we examined the communities of free-living and plant parasite nematodes. This evaluation consisted of a detailed analysis of their species composition, dominance, and trophic structures. Within isolated materials, we identified a total of 54 nematode species from 43 genera. These findings contribute to our knowledge and understanding of nematode diversity and community structure in cave environments. They lay the groundwork for future research with a particular emphasis on the ecological significance of specific nematode species within these unique habitats. Such studies promise to provide more comprehensive insights into the soil food web prevalent in these specialized environments. Evaluation of abundance data on nematode species, a topic of further study, can throw more light on the actual functioning of nematode communities in the caves.

## ACKNOWLEDGMENT

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# HYSTOLOGICAL INVESTIGATION OF HYPOSPADIAS IN A CROSSBRED CALF WITH SYNDROMIC EVOLUTION

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## ABSTRACT

Hypospadias is a developmental abnormality in which the male urethra opens on the underside of the penis or on the perineum and can be accompanied by penile aplasia or hypoplasia, hypoplasia of the corpus cavernosum, incomplete formation of the prepuce, or cryptorchidism [1]. In animals, it rarely appears in horses, sheep, goats, cattle, dogs, and cats as isolated cases with urogenital anomaly or with other abnormalities [2,3]. The aim of this paper is to present the hystological results in a crossbred calf seen with anterior hypospadias with syndromic evolution. Clinically, the penile urethra ventrally opened at approximately 4 cm caudal of the normal opening of the urethra, also, the prepuce was incompletely developed and had failed to fuse normally. The Hematoxylin-Eosin method was used for hystological examination of the urethra. Microscopically, the urethra wall was composed of mucosa with epithelium and lamina propria, vascular layer, thin muscle layer, and adventitia, a lax connective tissue. The formation that should be the corpus cavernosum was separated from the body of the corpus spongiosum and urethra. In the center of the structure there was a connective tissue strand made of converging trabeculae. However, between the trabeculae, where the cavernous spaces should be, there was an abundance of monolocular adipocytes. In conclusion, histological examination revealed the normal structure of the urethra with adipose degeneration into the corpus cavernosum.

**Keywords:** hypospadias, congenital abnormalities, syndromic evolution, urethra.

## INTRODUCTION

The frequency of congenital defects in cattle is estimated to be 0.25% to 3.6% and can be caused by genetic factors, environmental factors, or genetic-environmental interaction during embryonic development [4]. Hypospadias (OMIA 001187-9913) is a developmental abnormality in which the male urethra opens on the underside of the penis or on the perineum and can be accompanied by incomplete formation of the prepuce [1,5].

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Abnormal development of the penile urethra is caused by a disruption of closure of the external male urethra through the fusion of the urogenital folds in the midline of normal penile development [5]. Physiologically the fusion process begins proximally in the perineal region and extends distally toward the glans penis at the solid urethral plate [2]. Based on where the fusion of the urogenital folds failed, hypospadias can be classified as *balanitic/glandular* – the urethra opens in the region of the gland penis, *penile* - the urethra opens ventral and caudal to the gland penis and cranial to the scrotum, and could be proximal, distal or in the mid shaft of the penis, *scrotal* – the urethra opens between the halves of the divided scrotum or at the scrotum, *perineal* – the urethra opens in the perineum, ventral the anus or *anal* – the urethra opens in the anal region [2,4].

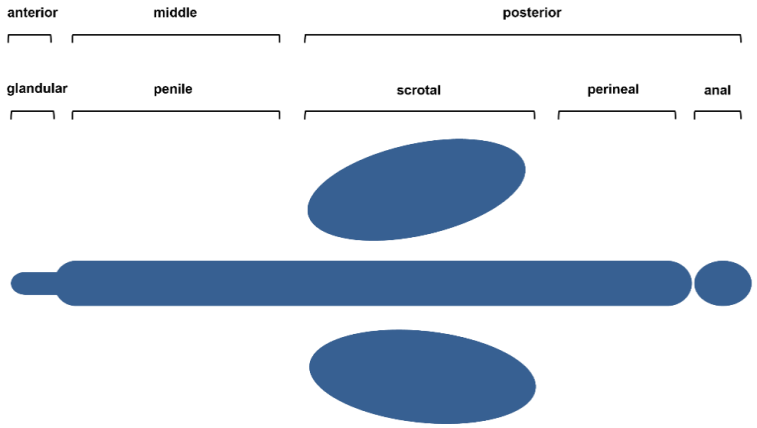



**Figure 1.** Clinical classification of the hypospadias according to the position of the urethral meatus, based on [1,2,6].

The incidence of hypospadias in animals is rare and occurs in horses [7], sheep, goats, cattle [2,4,8], dogs, and cats [9,10]. The molecular mechanism of hypospadias is complex and genetic, endocrinological, environmental factors and even epigenetic alteration of the genetic material are incriminated in the etiology of hypospadias [3,5,6]. For example, different polymorphisms of *MAMLD1*, *SRD5A2* and *AR* genes were identified in dogs with XY DSD, whereas some of the dogs had hypospadias, but without any association between the polymorphisms of the genes studied and hypospadias or cryptorchidism, because the distribution of the polymorphic variants in the affected dogs and control group was nod significantly different [9,11].

This article is intended to describe the hystological findings in this calf with penial hypospadias and to enhance the literature data on congenital defects that can affect animals.

## MATERIALS AND METHODS

The case study was performed in compliance with national (Romanian Low 43/2014) and European guidelines (Directive 63/2010) for using animals in for scientific purposes, and in accordance with the internal SOP of USAMVBT-PG-001-R021 and Ethical Statement no 73 / 2020.

The male calf Piemontese crossbreed was born alive after a normal pregnancy period of a heifer on September 5th, 2020 in the Didactic Farm of University of Life Sciences ‘’King Mihai I’’ from Timişoara. His mother was a primiparous Piemontese crossbreed (a product between Holstein female and Piemontese male) and the father was Wagyu breed. It was the first case with such congenital abnormalities in our Research Farm. The calf was

euthanized few days later due to severe neonatal diarrhea and anemia, clinically was not possible to feed himself properly with colostrum and he could not be maintained in a quadrupedal stance. Euthanasia was performed with Ketamidor 100 mg/ml and Xylazin Bio 20 mg/ml, and followed by T61 1ml/10kg, intravenous.

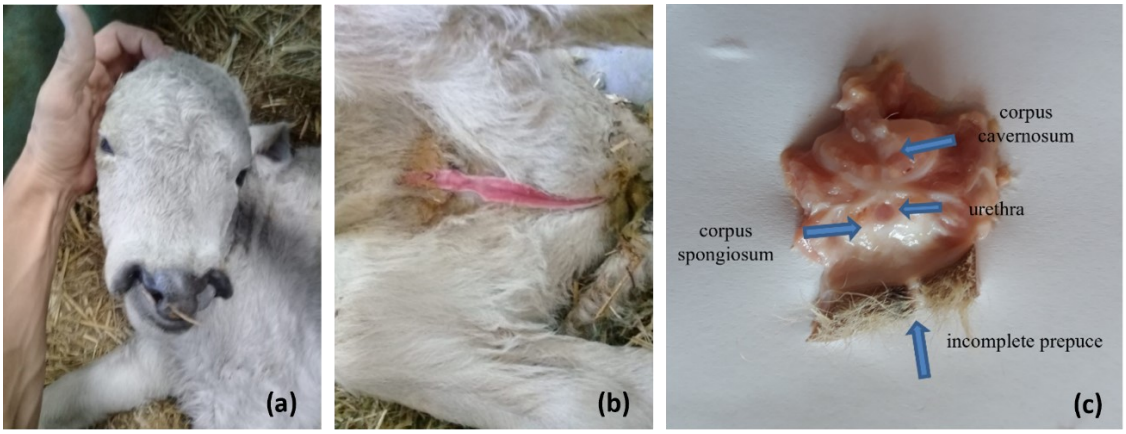



**Figure 2.** Clinical aspects of the calf (a) head view with bilateral cheilognatoschisis, (b) penial region with hypospadias, (c) tissue sample used for histological sections.

At birth, the affected calf presented a cleft lip and jaw (OMIA 001714-9913) and hypospadias (OMIA 001187-9913). On the andrological examination, the calf showed a penile hypospadias, the penile urethra ventrally opened at approximately 4 cm caudal of the normal opening of the urethra and the prepuce was incompletely developed and had failed to fuse normally.

### *Histological analyses*

Tissue samples were dissected from the adjacent tissues and prepared for paraffin (Paraffin for histology, Merck, Germany) embedding, dehydration being achieved by bathing the tissue samples in ethanol (Sigma-Aldrich, Germany) increasing concentrations and then clarifying them in Toluen (Lach-Ner, Czech Republic). The paraffin blocks, with embedded tissue samples, were cut on 5 mm thickness using microtome (Cut 4062 Slee Mainz, Germany), and the sections were then placed on glass slides. Hematoxylin-Eosin method was used for staining the slides, which were examined by a Olympus microscope with digital camera (Olympus life and material science CX41RF) and software for images analysis (QuickPHOTO Micro 2.2.).

## RESULTS

The sample for histological examination was taken from nearby the open urethra. Microscopic examination of histological slides revealed the normal structure of urethra. The wall of urethra is composed of mucosa with epithelium and lamina propria, vascular layer, thin muscle layer and adventitia, a lax connective tissue (Figure 3a-3b). The mucosa of urethra is lined with transitional epithelium, a stratified epithelium, consisting of polygonal cells, found in basal layer, a pear-like cells located in the middle layer, and dome-shaped cells forming the superficial layer of the epithelium. The underlying lamina propria continues with the connective tissue of vascular layer (Figure 3c) called corpus spongiosum represented by small, different in shape, cavernous spaces separated by the trabeculae of connective tissue, rich in collagen fibers. The cavernous sinuses are lined with squamous endothelial cells. In the structure of trabeculae besides elastic fibers,

collagen fibers, smooth muscle cells, the nerves also can be seen. All these structures form the core of cavernous sinuses (Figure 3c). The cavernous spaces from the vascular layer are supplied by numerous arterial and venous branches.
On both sides of the urethra sinusoidal architecture similar to corpus spongiosum is observed, one that resembles corpus cavernosum. Sinusoidal capillaries, even though they are very wide, are interconnected and separated by trabeculae composed of smooth myocytes, collagen, and elastin. Arteries and cavernous nerves can also be seen (Figure 3c and 5).

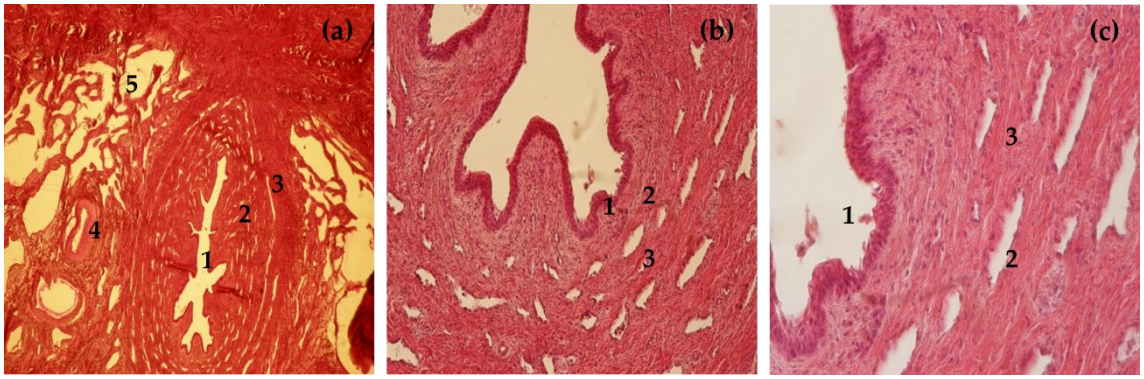



**Figure 3.** Histological section through the urethra of the calf (**a**) 1 – urethra, 2 – vascular layer, 3 –tunica adventitia, 4 – blood vessel, 5 – corpus cavernosum 4X and (**b**) 1 – epithelia, 2 – lamina propria, 3 – corpus spongiosum, 20X, and (**c**) 1 – urethra, 2 – cavernous sinuses, 3 – trabeculae, 40X, H.E. stain.

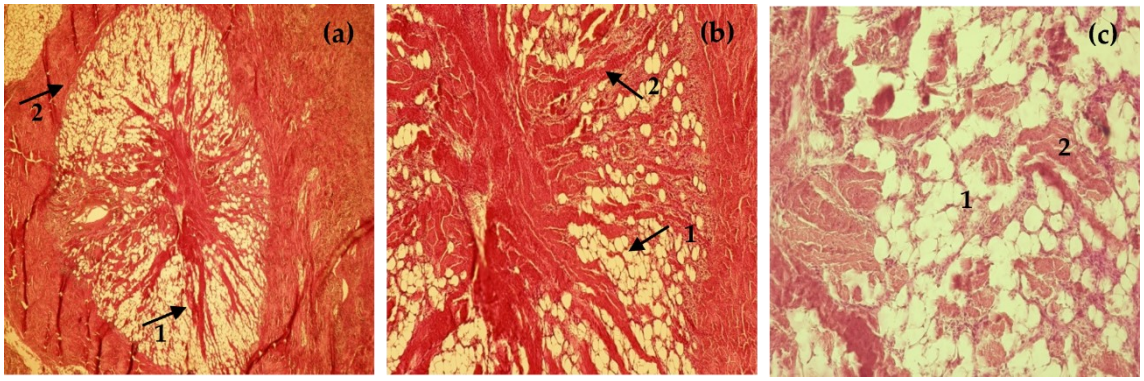



**Figure 4.** Histological section through the penis of the calf **(a)** where 1 – trabeculae, 2 – albuginea, 4X, corpus cavernosum of calf (**b** and **c**) where 1 – adipocytes, 2 – trabeculae, 10X-20X, H.E. stain.

However, the cavernous spaces, although a few and small, are present in corpus cavernosum, and are lined by endothelial cells (Figure 4c). Overall, connective tissue cells and fibers predominate in the structure of corpus cavernosum, which is characteristic of ruminants. The presence of adipose tissue, even its abundance, allows us to say that the corpus cavernosum has undergone a process of adipose degeneration.

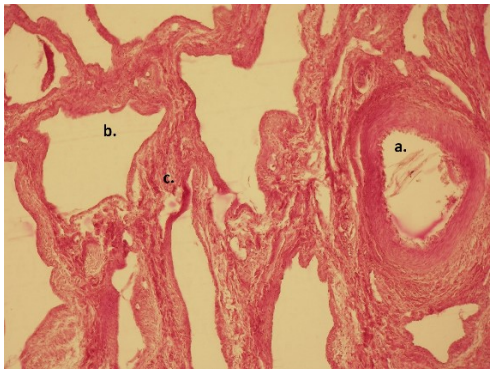



**Figure 5.** Histological section through corpus spongiosum of calf, a – artery, b – cavernous sinuses, c – trabeculae, H.E. stain, ob. 40X.

## DISCUSSION

Hypospadias can occur, in large and small ruminants, as single cases associated with a urogenital anomaly (penile aplasia or hypoplasia, preputial aplasia or hypoplasia, cryptorchidism) [3] or with other abnormalities such as omphalophlebities, atresia recti, atresia ani, growth retardation, and ventricular septal defect, absence of tail, hermaphrodism, rudimentary left kidney and left urethral anomaly [2].

The histological findings in this case are quite interesting. Therefore, if in normal conditions, the body of the penis consists of two corpus cavernosum located dorsally, and one corpus spongiosum that surrounds the urethra located ventrally and tunica albuginea, a connective tissue capsule covers the corpus cavernosum. In this case presented here, the formation that should be the corpus cavernosum is separated from the spongy body and urethra. In the center of the structure there is a connective tissue strand made of converging trabeculae. The albuginea is also present. But between the trabeculae, where cavernous spaces should be, there is an abundance of monolocular adipocytes (Figure 4a-4b).

Literature data from human medicine regarding the histology of the urethral plate from boys with hypospadias, reports the epithelium overlying connective tissue, well vascularized with smooth muscle and collagen, with no dysplasic tissue [12]. As described in introduction section, abnormal penile urethra development is caused by a disruption of the closure of the external male urethra through the fusion of the urogenital folds in the midline of normal penile development [5]. Other data indicate that hypospadias and the abnormalities associated with it have different causes, namely maldevelopment of a complex of primordial fascial and vascular tissue proximal to the urethral orifice, with disproportionately strong proliferation that causes the urethral orifice to shift distalward [13]. In a different report on human hypospadias, was observed hypoplastic corpus spogiosum with a disorganized irregular distribution of smooth muscle fibres. The presence of abnormal large blood sinusoids could explain the low healing process in cases with surgically treated ventral penis [14]. The genomic analyses done on this case, based on whole genome sequencing, identified 13 mutations with a predicted high impact on the following overlapped genes: *EGF, IRF6, CCDC8, ACVR1, PHC2, MYBPHL, BMPRIB, BHMT2, MMP14, CDH1, F13A1, ADGRA2* and *GSTP1*. The *ADGRA2*, *EGF*, *F13A1*, *IRF6*, and *GSTP1* genes showed homozygous mutations, the common consequence being a stop-gained (premature stop codon) causing the transcript to be shorter than normal [15]. The protein function of *EGF, IRF6,* or *CDH1* in various tissues is to stimulate the growth of epidermal and epithelial tissues or to sustain cell-cell

adhesions, so mutation in them can induce congenital abnormalities in the skin in different anatomical regions, as in this case.

## CONCLUSION

Histological examination of the tissue revealed the normal structure of the urethra, where the mucosa of the urethra was lined with transitional epithelium, found in the basal layer, a pear-like cells located in the middle layer, and dome-shaped cells that form the superficial layer of the epithelium. In corpus cavernosum, few and small cavernous spaces were observed, with connective tissue cells and fibres, but with abundance adipose tissue. Corpus spongiosum was observed on both sides of the urethra as sinusoidal architecture interconnected and separated by trabeculae composed of smooth myocytes, collagen, and elastin. Further research on this case is required to specify at the genomic level by sequencing and at the proteomic level by bioinformatic algorithms which of the high-risk genes identified in the case may be responsible for the identified changes.

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# IDENTIFICATION OF INTESTINAL BACTERIAL FLORA ISOLATED FROM JACKALS IN WESTERN ROMANIA

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## ABSTRACT

The golden jackal (*Canis aureus*), a mesocarnivore, is currently spreading from eastern to western Europe. In Europe, the golden jackal is considered an invasive species, as in recent years it has managed to populate extensive territories in Greece, Turkey, Slovenia, Serbia and Hungary. Bulgaria is considered the country with the largest population of jackals in Europe. In Romania it is frequently found from the plains of Dobrogea to Banat. The present work aimed to study and observe the newly developed antibiotic resistance profiles of intestinal bacterial flora isolated from these wild species and to compare our data with those existing in the literature.

Duodenum samples from golden jackals were collected for primary bacteriological examinations. This study used common identification and isolation methods and modern identification techniques, such as proteomics and MALDI-TOF MS technique, for highly accurate information on the bacterial flora of the digestive system.

Antibiograms were performed using the disc-diffusimetric method using antibiotics and chemotherapeutics belonging to several groups.

Several bacterial species were isolated from the jackals studied: *Serratia liquefaciens, Staphylococcus hominis* and *Aeromonas salmonicida*. In *Aeromonas salmonicida* strains, antibiograms showed significant percentages of intermediate strains to most of the antimicrobials used and overall resistance to levofloxacin. The same behaviour was recorded for colistin and strains belonging to the *Seratia* and *Streptococcus* genera.

Interestingly, some bacterial strains isolated showed resistance to levofloxacin, a third-generation fluoroquinolone, as this antimicrobial is banned for veterinary use in EU countries. The results are a wake-up call, as antibiotic resistance has been shown to be present in wild animal populations in Romania, with risk to public health.

**Keywords:** antibiotic resistance, golden jackal, *Serratia spp., Staphylococcus spp., Aeromonas spp.,* MALDI-TOF Ms.

## INTRODUCTION

The phenomenon of antimicrobial resistance in both humans and domestic animals is growing and has reached alarming levels, making the presence of bacteria carrying

antimicrobial resistance (AMR) genes an increasingly serious and complex threat. Today, due to the expansion of urban populations, this event is spreading and contact between humans, domestic animals, and wildlife is very close. It is therefore of utmost importance to study this aspect of antimicrobial resistance from a "One Health" perspective. This perspective must be a multidisciplinary, integrated approach, with particular emphasis on issues related to identifying key priorities for combating antimicrobial resistance. Even if wild animals are unlikely to be treated with antibiotics, the overlap between wildlife, domestic animal, and human habitats inevitably increases the transmission of antibiotic-resistant bacteria. In Romania, there is very little research on aspects of the antibiotic resistance phenomenon in wild animals.

However, even though many studies in the literature from other countries state that wild animals are reservoirs and dispersers of antimicrobial-resistant bacteria, this role is not yet well established. To make this claim, including in our country, further epidemiological research is needed, because the mere fact of being carriers of AMR bacteria does not mean that they can be a vehicle of contagiousness for humans or other animals. Therefore, we aimed through this work to contribute to the establishment of antibiotic resistance profiles of our isolated bacterial flora, comparing our data with those already existing in the literature. Our rationale was to understand the relationship between these wild animals and their gut microbiota, as well as to know the potential associated microbial risks that may occur as a result of these antibiotic resistance phenomena.

## MATERIALS AND METHODS

To reach the purposed aim, bacterial strains were isolated from the duodenum provided by 3 golden jackals (*Canis aureus*). The gastrointestinal mass (duodenum) origin from the carcasses of 3 golden jackals aged about 2-3 years, found in a wooded area near a village in Timis County. The carcasses were examined at the Faculty of Veterinary Medicine Timisoara. The cause of death has not been established.

The primary isolation and biochemical characterization were done on different culture media: Columbia blood agar (Oxoid), Uriselect 4 (Bio-Rad, chromogen agar), Mueller-Hinton agar (Oxoid), Nutritive broth (Oxoid), Selective culture media: EMB, MIU, TSI and XLD (Oxoid). To inhibit the invasion phenomena of *Proteus spp* and to confirm the isolates' purity the samples were also cultivated on chromogenic media. Proteomic identification with the MALDI-ToF-Ms technique was realized based on the related methodology described further:

- Each dispersion from the initial plate was examined by minimum 2 examinators and one sample from each isolated colony, with individual morphological aspect, resulting after incubation at 37ºC, 18-24 hours, was taken and applied on the MALDI-TOF-MS plate in duplicate.
- After drying, was applied 1 µL matrix 4-*HCCA (acid α-Cyano-4-hydroxycinnamic, α-Cyano-4-hydroxycinnamic acid*) and waited till dry again. The plate has been put to analysis for identification using MALDI-ToF-MS Bruker mark, model auto Flex Speed.

Sensibility testing on antimicrobial substances was realized on disk-diffusion method (Kirby-Bauer) using primary culture or subculture following standard procedure, adopted by the Clinical and Laboratory Standard Institute (CLSI), USA.

For testing was selected next substances:

1. Amoxicillin (AML)
2. Ticarcillin (TIC)
3. Piperacillin (PRL)
4. Amoxicillin-Clavulanate (AMC)
5. Ticarcillin-Clavulanate (TCC)
6. Piperacilin-Tazobactame (TZP)
7. Cefoxitin (FOX)
8. Moxalactam (MOX)
9. Mecilinam (MEL)
10. Cefotaxime (CTX)
11. Ceftazidime (CAZ)
12. Cefepime (FEP)
13. Temocillin (TEM)
14. Aztreonam (ATM)
15. Ertapenem (ETP)
16. Meropenem (MEM)
17. Imipenem (IMP)
18. Ceftazidime-Avibactam (CZA)
19. Ceftolozan-Tazobactam (C/T)
20. Gentamicin (CN)
21. Tobramycin (TOB)
22. Amikacin (AK)
23. Netilmicin (NET)
24. Ciprofloxacin (CIP)
25. Levofloxacin (LEV)
26. Rifampicin (RD)
27. Chloramphenicol (C)
28. Nitrofurantoin (F)
29. Fosfomycin (FOS)
30. Trimethoprim-sulf.(SXT)
31. Colistin (CT)

## RESULTS

After the incubation, the cultures were analyzed and interpreted based on the cultural aspects. Were isolated and identified, in total, 7 isolates*, Serratia liquefaciens* – 3 isolates, *Staphylococcus homminis* – 2 isolates and *Aeromonas salmonicida* - 3 isolates. Figure 1 presents characteristic aspects of *Serratia liquefaciens.*

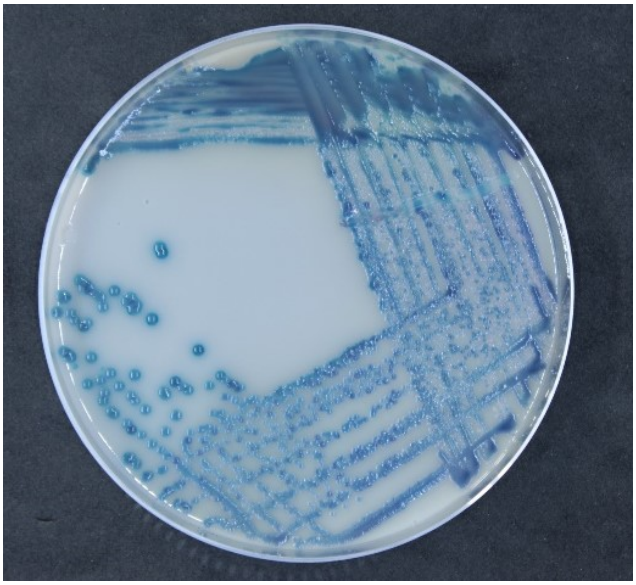


Figure 1. The characteristic aspect of *Serratia liquefaciens* strain isolated from golden jackals (*Canis aureus*) on chromogenic agar

Identification and classification strains were made based on morphological, biochemical, and tinctorial characteristics. Since, based on the cultural aspects on the usual and special culture media, it was not possible to precisely identify the bacterial genera, the API 20E test (bioMerieux, France) was used for the biochemical differentiation of bacteria from the fam. *Enterobacteriaceae* isolated from the duodenum of golden jackals.

For a better differentiation between the *Serratia liquefaciens*, *Staphylococcus hominis* and *Aeromonas salmonicida* strains were used chromogen media and proteomic technique MALDI-TOF MS.

Antibiotic resistance is a global problem that is rising continuously. The past period revealed facts like some bacteria, regarding commensal enterococci, *Serratia spp*. and *Staphylococcus spp*., could be used as efficient indicators to monitor antibiotics resistance in different bacterial populations and to evaluate the transfer of resistant bacteria in different environments [1, 2, 3, 4, 7, 9]. The results of antibiograms were presented in Table 1.

Table 1. Resistance patterns of bacterial strains isolated from golden jackals

| **Antibiotic** | ***S. liquefaciens*** | ***S. liquefaciens*** | ***S. liquefaciens*** | ***S. homminis*** | ***S. homminis*** | ***A. salmonicida*** | ***A. salmonicida*** | ***A. salmonicida*** |
|---|---|---|---|---|---|---|---|---|
| Amoxicilim-ac. clavulanic | R | R | I | S | S | I | I | I |
| Mecilinam | I | I | I | I | I | I | I | I |
| Cefoxitin | S | S | S | S | S | I | I | I |
| Cefotaxime | S | S | S | S | S | I | I | I |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Temocillin | I | I | I | S | S | I | I | I |
| Ceftolozan-Tazobactam | S | S | S | S | S | I | I | I |
| Gentamycin | S | S | S | I | I | I | I | I |
| Moxalactam | I | I | I | S | S | I | I | I |
| Amikacin | S | S | S | S | S | I | I | I |
| Tobramycin | S | S | S | I | I | I | I | I |
| Netilmicin | S | S | S | S | S | I | I | I |
| Nitrofurantoin | S | S | S | I | I | I | I | I |
| Tigecycline | I | I | I | S | S | I | I | I |
| Fosfomycin | I | I | I | I | I | I | I | I |
| Colistin | I | I | I | S | S | I | I | I |
| Rifampicin | S | S | S | S | S | I | I | I |
| Fusidic acid | S | S | S | R | R | R | S | S |
| Levofloxacin | S | S | S | S | S | R | R | R |

The *Serratia liquefaciens* isolates present resistance to amoxicillin-clavulanic acid and most of the isolates were intermediate to mecillinam, temocillin, moxalactam, tygecyline, fosfomycin, and colistin, this could be an aware signal because this may be an increased tendency of the resistant isolates in wild animals [5, 6, 8, 10].

The *Staphylococcus hominis* isolates present resistance to fucsidic acid and most of the isolates were intermediate to mecillinam, gentamycin, tobramycin, nitrofurantoin, and fosfomycin. Isolates of *Aeromonas salmonicida* show resistance to levofloxacin and most isolates were intermediate to 16 of the antibiotics tested in this study.

## DISCUSSION

The faecal microbiota of the golden jackal is influenced by several factors, including host characteristics, feeding habits and pathogen load. Understanding the golden jackal microbiota could inform the study of potential animal and human health risks and inform control measures [11, 12, 13].

In this study were isolated three species of *Serratia liquefaciens, Staphylococcus hominis and Aeromonas salmonicida* from golden jackal. Our results are in concordance with other results obtained by other researchers from Slovenia [5, 17]. In a study conducted by Di Francesco in Italy it was found that intestinal flora isolated from jackals in Italy showed resistance genes to tetracyclines and beta lactam antibiotics [12]. *Serratia* colonizes the gastrointestinal tract of many animal species like wild carnivores and also will constitute a reservoir of antimicrobial resistance genes which reveal an epidemiological role in spreading the resistance [14, 15]. *Staphylococcus hominis* is one of the major *Staphylococcus* species found on the human skin and is mainly found in the

axillae, perineal and inguinal areas. *Aeromanas salmonicida* is considered to be a fish pathogen but is considered to be non-pathogenic for humans. The presence of bacteria that already show varying degrees of resistance to different antibiotics or groups of antibiotics in the duodenum of jackals from western Romanian is of major medical importance

This low-level resistance to antibiotics may be the result of a low infectious pressure in wild carnivores, compared with domestic carnivores. Our data is in concordance with data obtained by other researchers [16, 17], even if other researchers detected multiple antibiotic resistance bacteria strains with can be a real problem when we refer to the dissemination of these strains from wild animals to domestic animals and humans [12, 18].

## CONCLUSION

The present study completes the existing data in literature regarding the bacterial flora isolated from wild carnivores. Interestingly, some bacterial strains isolated showed resistance to levofloxacin, a third-generation fluoroquinolone, as this antimicrobial is banned for veterinary use in EU countries. The results are a wake-up call, as antibiotic resistance has been shown to be present in wild animal populations in Romania, with risk to public health.

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# SURGICAL MANAGEMENT OF A CASE OF MATURE CATARACT IN A DOG COMPLICATED BY IRIS-ANTERIOR LENS CAPSULE ADHESION USING PHACOEMULSIFICATION

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## ABSTRACT

Cataracts occur frequently in older dogs and can lead to partial or complete vision loss. In the present study, a 10-year-old male German shepherd was presented for eye evaluation because of his complete blindness. The diagnosis was bilateral mature cataracts with multiple iris adhesions to the anterior lens capsule in the right eye. This case report examines the process of performing complicated cataract surgery using phacoemulsification method for the patient's right eye. We began to perform surgery as an uncomplicated one, but curvilinear capsulorrhexis was performed only after careful surgical debridement of all adhesion, as close as possible to the anterior lens capsule to prevent iris blood vessel rupture. The hardened cataract lens was removed by phacoemulsification. The surgery was completed by inserting a high performance An-vision Fo-X-14 artificial intraocular lens (IOL), first canine IOL with extended depth of focus. Follow-up surgery the dog gained a better quality of life by becoming visual in the right eye. We checked the intraocular pressure for 1 month after surgery, which remained stable throughout this period. Although iris-anterior lens capsule adhesion can rarely occur in dogs, if debridement can be done carefully during surgery for good capsulorrhexis with preservation of the lenticular capsular bag, phacoemulsification of mature cataracts can also be done successfully. Knowing the eyes at risk, good management of complications is an important point for obtaining a good visual outcome after complicated cataract surgery in dogs.

**Keywords:** cataract, IOL, Dog, An-vision Fo-X, phacoemulsification, Phaco, synechiae

## INTRODUCTION

Cataracts have been reported to be associated with various risk factors such as age, environment, genetics, diabetes mellitus, uveitis, trauma and nutritional status, and this eye disease is characterized by the cloudiness or opacity of the eye lens or the capsule bag [7]. When the opacity covers about 60% of the total lens area, visual impairment often becomes apparent. If the opacity progresses to 100% of the lens, in which case the cataract is fully developed (mature), the dog will be functionally blind in the affected eye [6]. In terms of the degree of opacity, cataract maturity is classified into four stages: incipient, immature, mature, and hyper mature [3].

Mature cataract looks like a white disk behind dog's iris, and it led to partial loss of vision if it is unilateral, or to total blindness if it is bilateral. Cataracts are not typically

brought to the attention of a veterinarian until owners report their dogs bumping into different kind of things or furniture, especially in dim lighting [9]. When the dog's quality of life is being significantly affected by the loss of vision resulting from cataracts, the only effective and recommended treatment is surgery [1]. Cataract surgery has been performed mainly on human eyes, and it has become a commonly used surgical procedure since phacoemulsification (Phaco) was introduced in 1967 [12].

A previous study reported that good clinical results were not obtained in 45 out of 290 dogs that underwent cataract surgery, showing a success rate of 84.5% [10]. Since cataract surgery is reported to have a high failure rate of 15% or more, it is important to recommend that dog owners should arrange for their dogs to receive cataract treatment or surgery in an early stage of cataracts, as more advanced cataracts (mature and hyper-mature) are more likely to cause lens-induced intraocular inflammation, lens instability, and loss of lens capsule integrity [7]. Phaco method requires small incisions in the eyeball, less than 2.2 mm, so the use of this surgical method can decrease damage to the eyeball, the post operatory astigmatism and reduce the time required for surgery, thereby decreasing the stress of the patient. Also, Phaco makes it possible to maintain the intraocular pressure (IOP) of the anterior chamber below a specific level after surgery, and thus it helps to minimize postoperative sequelae [2]. After the damaged eye lens is removed, implantation of an IOL is performed. A-scan biometry demonstrated very different parameters necessary to achieve emmetropia in aphakic dogs [8]. The evolution of IOLs for dogs, however, has closely followed advances in the human field, a canine posterior chamber IOL is 40.00 to 41.00 D, with a biconvex optic measuring 6 to 7 mm in diameter, a peripheral edge to limit posterior capsular opacification (PCO), and a haptic-to-haptic length of 12 to 16 mm. There is a limited number of IOL manufacturers, and considerations related to volume and cost restrict the sizes and styles of lenses available to surgeons. Most stock only a few types of IOLs and use a “one size fits all” approach, which corrects most dogs’ vision to within a satisfactory range of 1.00 to 2.00 D of emmetropia [8]. More specifically, artificial eye lenses for dogs have 4 ring-shaped supports on four sides, and the diameters of artificial eye lenses for dogs are divided into 5 types: 11 mm for very small dogs with a body weight of 2 kg or less, 12 mm for small dogs, 13 mm for medium-sized and large dogs, 14 mm for large dogs, and 15 mm for very large dogs. Although there are many IOLs available now on veterinary medical market, the an-Vision developed the first canine IOLs with extended depth of focus.

Synechiae and adhesions associated with cataracts are rarely reported and can appear after the cataract surgical removal with IOL implantation [14,15]. The purpose of our case presentation is to study whether adequate surgical management of this type of cataract can be performed, while lens-induced uveitis can be managed. Also, with a performance IOL implantation after cataract removal using phaco, it can lead to a good visual surgical outcome.

## MATERIALS AND METHODS

The patient of this case presentation is a male old dog, a 10-year-old male German shepherd breed, who was presented for eye evaluation because of his complete blindness. The quality of his life has a very low score, because of his blindness on both eyes, he was injuring himself with the objects around, his behaviour changed and

became retired, as his owner reported. An ophthalmological examination was performed, and the dog was diagnosed with bilateral mature cataracts, and in the right eye (OD), multiple iris adhesions to the anterior lens capsule were observed.

This case report examines the process of performing cataract surgery and solving complicated adhesion problems for the patient's right eye. Figure 1 represents the external conditions of the right eyeball of the patient, and it shows the cloudy and white appearance of the eye lenses and vision loss, also the adhesions inside the eye. For the therapeutical surgical cataract treatment of dog's right eye, we chosen phaco method which implies lens extraction by phacoemulsification technique cataract surgery. For a good visualization of the anatomical components of the eyes we used, a surgical microscope, with magnification in 5 steps, with two 10x tilting binoculars with wide field, 50W LED lighting system, with articulated mobile arm and with the possibility of horizontal and vertical movement. The phacoemulsification equipment we used was a Bausch and Lomb Stellaris. The surgical instruments we used for both surgical methods were the ophthalmic surgery instruments (Figure 2). We used also a single-use IOL implanter and a soft acrylic haptic IOL. For surgery we needed sterile surgical fields, sterile tampons, single-use syringe and single-use eye cannulas, viscoelastic substance preloaded single-use syringe.

## RESULTS

Adequate history of the case and general examination was done in this patient to reveal systemic diseases accompanied by cataract (like diabetes) and to discover any underlying ocular or systemic disease, that would affect the surgery or anaesthesia protocol. Complete blood count showed that both RBC and WBC levels were in the normal range without any specific symptoms. After clinical examination of the patient, preoperative examination included also a complete ophthalmologic exam. Intraocular pressures (IOP) for both eyes were checked to see for underlying glaucoma or uveitis, IOP levels were in the normal range with 17 mmHg for OD and 22 mmHg for OS Both eyes had normal IOP values in this case. The results of the Shirmer tear test, were 18 mm/min for OD and 19 mm/min for left eye (OS). The patient showed no responses in the menace response test, the dazzle reflex test, and the maze test, but exhibited both palpebral reflexes and corneal reflexes. Thorough slit lamp examination, which is a method used to observe and assess the opacity and cataract maturity of eye lenses, the exam showed that the patient had a mature cataract in both OD and left eye (OS) and we also checked the possible visual interference in cornea (opacity, oedema, pigmentation) or anterior lens capsule and the state of suspensory apparatus of the lens (signs of lens subluxation). Regarding the topical treatment of the eyeball, anti-inflammatory nonsteroidal were instilled in the eye for 7 days before surgery. Before starting the surgery technique, we induced mydriasis with topical tropicamide 20-to-30-minute prior the surgery. The eyeball was fixed with an eye speculum that allows easy access to operative area with less injury. Eyeball was covered with drapes. As for pretreatment agents for the induction of anaesthesia, 0.03 mg/kg of Atropine (Atropine sulphate 0.5 mg/kg) was administered subcutaneous. In addition, 0.5 mg/kg of Diazepham (Diazepham inj 10 mg/2 mL), 0.1 mg/kg of Butorphanol (Butorphanol 1 mg/1 mL) and 6 mg/kg of Propofol were administered IV. To maintain anesthesia, isoflurane gas was used as an anesthetic agent, and the ringer lactate solution was used to maintain adequate perfusion during surgery (Figure 3).

**Cataract surgery.** Nowadays, the method of choice in most cataract cases is phacoemulsification with removal of lens material through small incision by fragmentation, emulsification and aspiration [5]. The only disadvantages are the costs of equipment and materials of this procedure. We began to perform surgery as an uncomplicated one. During Phaco technique, we started with a small incision at the edge of the clear cornea to enter in the anterior eye chamber. We injected trypan blue using a canula in the anterior chamber for better visualization of the lens capsule and then with a viscoelastic substance we filled the entire anterior chamber of the eye (Figure 4, 5). We carefully checked the adhesions that we saw prior to surgery and carefully we made the surgical debridement of all adhesion, as close as possible to



Figure 1. Aspect of the right eye of the



Fgure 2. Ophthalmological surgical instruments

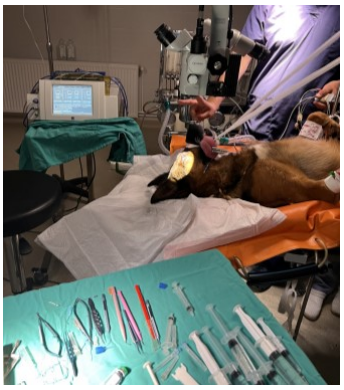


Figure 3. Preparing the operation theatre

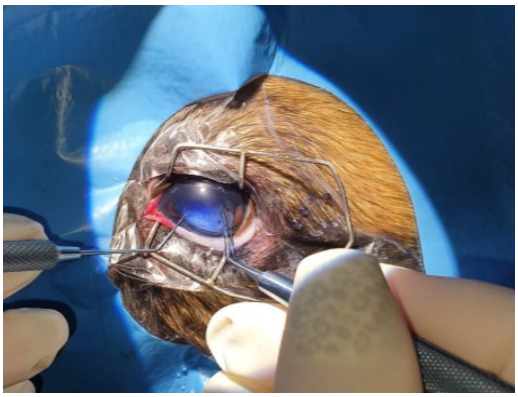


Figure 4. Aspect after trypan-blue coloration

the anterior lens capsule to prevent iris blood vessel rupture (Figure 6). Some iris fragments remained attached to the anterior lens capsule, but their position in the center of the capsula did not affect the next step of the technique, the capsulorhexis. Little haemorrhage from the iris occurred. After we manage to separate the anterior lens capsule from the iris, curvilinear capsulorrhexis was performed continuously in the equatorial area, with the aid of a Utrata forceps and a capsulorhexis cystotome, then followed by hydrodisection, rotation and phacoemulsification of the lens core with the phaco device sonde. The fracture technique used was phaco-chop and the phacoemulsification parameters were set in 60% of ultrasonic power, 40 cc/min, 12 pulses/sec. The aspiration of the cortical waste was performed with an irrigation/aspiration pen from the device (Figure 7). Because the posterior lens capsule,

that we managed to examine after the removal of all lens fragments, was opacified on some areas, we tried to polish it as best as possible for a better post operatory vision (figure 8). The surgery was completed by inserting a high performance An-vision Fo-X-14 artificial intraocular lens (IOL), first canine IOL with extended depth of focus, according with the right size of the dog breed, The Fo-X IOL came sterile with single-use preloaded injector for easy handling and insertion. We inserted it through the small corneal incision (2.5-3.5 mm) (figure 9). into the capsular bag from where we removed the mature lens, after we previously folded the IOL using an IOL-holding forceps in the IOL cartridge. Finally, it was performed the aspiration of the viscoelastic substance and the injection of an air bubble on the anterior chamber and the hydration of the surgical borders. A two stitches suture using 8-0 mononylon was made on the main incision. At the end of the left eye surgical technique, dog received an intraconjuctival injection with gentamycin and dexamethasone, and his owner was recommended to follow a schedule of dosage and prescribed with dexamethasone and enrofloxacin for initial one-week general treatment after surgery and topical eye drops with gentamycin and dexamethasone.

The dog was examined next day (Figure 10), at 7 and 14 days and one month after the surgery was performed. At day 7, a small corneal inflammation was noticed at the main incision area and small hyphema in the anterior chamber. The hyphema and the corneal inflammation were gone by day 14. No other post-surgical complication was seen until day 30.

## DISCUSSIONS

Cataracts are the leading cause of blindness, accounting for 47.8% of global blindness, and old age is the single most common risk factor for cataract [11]. The symptoms of cataracts progress slowly over years. Thus, although the images of objects are blurred and unclear and there is some visual discomfort, patients may frequently not recognize the symptoms. The degree of visual impairment varies depending on the degree of opacity of the eye lens and the location of the lesion and in case of partial opacity, the images of objects may overlap even in the vision of one eye [13]. Usually, only when dogs have undergone a considerable degree of vision loss and the eye lenses have become apparently white, dog owners recognize cataracts in their dogs and take them to a veterinary ophthalmologist. In the mature stage, the vision of the affected eye has been almost completely lost, and the patient does not perceive light in some cases. Also, all the complications may preclude surgery from being performed, may lead to a poorer prognosis for vision and comfort following surgery, and may preclude placement of a synthetic intraocular lens. In addition to vision loss, chronic cataracts can cause excruciatingly painful, unmanageable secondary issues within the eye, for which the only recourse is enucleation, or surgical removal of the eye. This happens because the lens continuously degenerates over time, sparking chronic inflammatory changes around it. These secondary issues include lens-induced uveitis, glaucoma (increased intraocular pressures), lens luxation (dislocation of the lens), different kind of synechia's or adhesion. Cataracts can also cause retinal detachment, which can result in blindness. Cataract surgery is the only effective treatment of choice nowadays for restoring vision in dogs with cataract. IOL implantation improves the optics of the aphakic eye and reduces the formation of posterior capsular opacity (PCO) after surgery. Since cataract surgery is reported to have a high failure rate of 15% or more, it is important to

recommend that dog owners should arrange for their dogs to receive cataract treatment or surgery in an early stage of cataracts [8]. In our case, the patient was a 10-year-old senior dog. The dog was found and save from the streets, so no history about his eyes was available. Both eyes were diagnosed with a hypermature cataract and showed severe cataract symptoms, with multiple iris adhesions to the anterior lens capsule in the right eye, but since the IOP levels were in the normal range and had no uveitis undergoing, the decision to perform surgery was made, choosing phaco surgery for OD.



Figure 5. Capsular coloration



Figure 6. Debriding of the adhesions



Figure 7. Aspect during phaco



Figure 8. Posterior capsule before polish



Figure 9. The IOL an-Vision implant



Figure 10. First examination after surgery

No uveitis post operatory was observed at recontrol days. Other advantages of phaco chop include reduction of zonular and capsular stress because forces are directed toward an opposing instrument and the phaco tip is kept in a central 'safe zone' in the middle of the pupil. But, because of the mature cataract, the time of this technique was longer, because of the difficulty of ultrasound chopping of the cataract by its hardness. If the vacuum level and the ultrasonic power level are set at high levels, phaco time can be reduced but corneal endothelial damage or the rupture of the posterior chamber may occur. Thus, even though phaco time is increased, the vacuum level and the ultrasonic power level should be appropriately set to facilitate the surgical process and the management of postoperative complications. To maintain the external shape of the cornea during cataract surgery, intraocular injections of visco-elastic agents are performed. If visco-elastic agents are not used, the intraocular area tends to collapse as soon as a corneal incision is made, making it impossible to perform surgery. These visco-elastic agents must be completely removed after surgery, but some residues of the agents tend to remain in the eyeball. In our case, a little hemorrhage happened because of the tear of iris blood vessels, but we tried to prevent this as much as we could by cutting the adhesion's closer to the anterior lens capsule. The hyphema noticed after the surgery was all gone by the day 14 at the recontrol. Gentle debriding is also very important, in order not to set much pressure on the zonular ligaments, because there is a risk of tearing them with lens luxation after. A lack of proper inflammation management after cataract surgery may result in vision loss due to uveitis caused by bacterial infections, so eye drops with antibiotics were used to prevent uveitis.

## CONCLUSION

Similar to humans, the loss of the lens functions causes a loss of visual sharpness and dogs benefited from cataract surgery because it allowed them to be able to move around without the fear of bumping on objects and even if dogs may not have completely normal vision after surgery, they regain some vision without affected lens. All complicated cataracts can be challenging for the surgeon because intraoperative problems can appear during the phacoemulsification procedure. The multiple adhesion iris-anterior lens capsule of a cataract is rarely reported but can easily lead to lens luxation that become an emergencies surgery. Follow-up surgery, our dog case presentation boost confidence, avoid life-threatening accidents and gained a better quality of life by becoming visual in the right eye, with good IOP for 1 month after surgery, which remained stable throughout this period. Our study shows that if debridement can be carefully and successfully done during surgery for good capsulorhexis, with preservation of the lenticular capsular bag and prevention of hyphema, Phaco of mature cataracts can also be done successfully. Knowing the eyes at risk, good management of complications is an important point for obtaining a good visual outcome after phacoemulsification surgery for mature complicated cataracts with synechia's in dogs, but further research is needed to know what complication can appear in order to prevent them and not be an intraoperative surprise.

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# TECHNOLOGICAL INNOVATIONS AND LIVESTOCK ANIMAL WELFARE THE CASE OF THE DAIRY COW

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## ABSTRACT

Technological devices, which promote animal welfare in livestock farming and contribute to a reduction in environmental impact, have known increasing interest in the European Union (EU). For dairy cows, the contact technologies (sensors) on the body of animals not only facilitate animal welfare, but also support the milk productivity and better quality, thus economic production efficiency.
According with available statistical data, showing a boost on productivity, the aim of the present work was to test whether the increase in the realised levels of raw milk could derive from technological factors. To validate this hypothesis a study on the evolutionary development of the reported statistical indicators of the two biologically related variables (cow, raw milk) was presented. The method chosen, made statistically significant, i.e. comparable in the statistical unit of measurement (coefficient of variation) to establish the strength of the linear relationship between the two variables and the corresponding evolution $r^2$, relied on the intensity of the correlation (correlation coefficient $r$) between two statistical indicators, dairy cattle (independent variable) and raw milk (dependent variable).
The results showed that EU quantity of raw cows's milk realised in EU dairy farms, even in the presence of breaking events, never stopped growing. The representation of the variables' trends showed, a part of an exception, situations where the respective evolution were not perfectly linear between them. The values of the determination coefficient $r^2$, from 2012-2016 to 2017-2021, in most of the Countries examined decreased. Thus, there were increases in cow's milk, which conclusively could also be attributed to the adoption of digital devices and specifically to the contact sensors on the cow's body adopted to ensure animal welfare, to optimize the use of production factors and to detect animal's physiological activities.

**Keywords:** Innovation, dairy cattle, livestock, animal welfare, economic.

## INTRODUCTION

In recent years, digital technologies have found use on dairy cattle farms to improve economic production efficiency for animals in functional age, but also for improving animal welfare and to reduce anthropic environmental's impact [1]. The use of digital

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equipment, applied on parts of the body of dairy cows has generated favorable feedbacks for precision devices (sensors, biosensors, measuring devices, lameness detection, etc.). These allow observing and synthesizing many information related to the animal's behavior, measuring physiological traits related to milk production by quantity realized and ameliorating the protection of the quality and food safety of the milk itself [2]. Sectorial studies asserted that technological devices favor in herds the improvement of bovine milk production [3]. Therefore, the farmers' decision to exploit innovative technologies worn by cows in production age can be related to reasons of opportunity of various kinds: of environmental, ethical, socio-economic and fresh milk market'qualitypolicy [4]. This is with respect to the different local and supra-local (regional, national, EU, global) territorial systems within which the "network among actors" of active territoriality are developed [5].

The above-mentioned quantitative and qualitative effects on raw milk produced by cows are supported by sectorial studies identifying the existence of a specific correlation between devices (sensors) and milk produced [3] in cases of enterprise restructuring, or of establishment of new farms, in which production increases are the regular prerequisite, even though they are realized in the long term.

From a territorial point of view, total farm restructuring cannot be frequent in time and space (and in any case their effects would be detectable after several years) and therefore the early occurrence of incidence of the corresponding progressive milk production cannot depend on such restructuring interventions. Instead, improvements through the adoption of digital devices (technology capital) placed on animal's body are capable of generating results in a time frame that is likely to be rather shorter than the case of the farm's structural renewal initiatives.

The aim of the present study was to interpret the evolution of cow's milk production in the European Union applying an interpretative logic model based on quantitative data (fresh milk; dairy cows), i.e. statistical units of the near reality, since the technological innovations (sensors) recently has entered in dairy farms with dairy cows [3] in EU Countries.

Thus, the scope of the analysis concerns the interpretation of the statistical information pertaining the evolutionary trends, characteristics, causes and consequences of changes in production's level in Member Countries with the largest dairy cattle herd in the European Union. In particular, the paper focused on Belgium, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland and Spain. The reason behind this selection lies on the extremely ample production situations and different production patterns of these Countries that, for the purposes of the study, represent contexts capable of providing interpretive lumens from the information about the surveyed data analysis, since they depict a complex empirical scenario

The objective of this work, beyond purely statistical purposes, could appear questionable, but at a meso-economic level it seems possible to gather indications for statistical hypotheses that would favor the improvement of the production conditions of the zoo-economic sector under consideration, also benefiting the eligibility for European Union action programs of support in dairy cow sector.

## MATERIALS AND METHODS

The statistical data underlying the study are mainly from Eurostat source. The following data codes: tag00041 (Production of milk on farms - Raw milk, total available on farms) and tag00014 (Number of dairy cows).

The annual values acquired refer to the main Member Countries (MMCs) producing raw milk in the EU. In descending order, considering the production levels, the MMCs are Germany, France, Ireland, Poland, Spain, Belgium, Denmark, Italy, and Netherlands, which, in recent times, together account for more than 80% of raw milk produced in the European Union.
The period of analysis is the decade 2012 - 2021. It is important to point out that the sector under consideration during this period experienced political-economic and health phenomena that influenced market trends. Notably, in the EU there has been the gradual dismantling of milk quotas [6] that lasted until April 2015 (indeed, in some cases until 2016) followed by a visible increase in cattle herds and associated production growth. Subsequently, in recent years, there was a market slowdown due to the consequences caused worldwide by the Covid 19 pandemic. Therefore, for a functional comparison of these events, the decade was divided into two five-year periods 2012-2016 and 2017-2021.
A first stage of the elaborations rests on the intensity of correlation between the two statistical indicators, dairy cattle (independent variable) and raw milk (dependent variable). They are two quantitative variables differing in the statistical unit of measurement, but the application of the coefficient of variation (ratio of standard deviation to the absolute value of the arithmetic mean, for 100 percent) enabled their comparability. In fact, between the dependent variable (y) and the independent variable (x), the relative values of this coefficient were low, that is, falling within the range 0-20% [3].
Thus, Pearson's correlation coefficient r determined the strength of the link between the two aforementioned variables, i.e., direct proportionality, through the following formula.

$$r = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum(X_i - \bar{X})^2}\sqrt{\sum(Y_i - \bar{Y})^2}}$$

In addition, the coefficient can be ascending (the two variables tend to increase in parallel), or descending (the two variables vary in the opposite direction) depending on whether the corresponding value r is equal to 1 or -1; instead, when r equals 0 then the two variables are said to be unrelated. Squaring the correlation coefficient r establishes the strength of the linear relationship between the two variables, which is useful in making predictions of the reference phenomenon. The multiplication of the coefficient of determination $r^2$ by 100 gives the percentage of variation in y that can be attributed to the linear relationship between x and y. There is a significant linear association between x and y when the larger the $r^2$ value.
Another official source to document the research refers to data of "EU milk specialised farms" and "Farm economy focus by sector – livestock"; collected and processed in the FADN (Farm Accountancy Data Network). FADN is a European system of sample survey which collects structural and accountancy data relating to farms (EU - AFEB). The variables represent average values per farm. The FADN survey does not include all the farms in the 27 EU countries, only those of a certain minimum size (agridata) representing at least 90% of the production volume of the MMCs concerned.
The FADN variables are updated to 2020, those examined were assembled into the following two groups:
A. Production: 1 Milk yeld; 2. Milk production (t); 3. Milk per ha fodder area t/ha
B. Costitutive information: 1. Machinery; 2. Depreciation.

The first relates to the production level, the second to specific structural magnitudes of firms.

## RESULTS

Concerning the results, in the observed decade, the EU quantity of raw cows' milk realised in EU dairy farms, even in the presence of braking events, never stopped growing, in particular, from about 143 to more than 160 million tonnes. In 2021, 83% was realised by the following main producing countries (MPC), Germany (20.7%), France (16.4%), Poland (9.5%), Netherlands (9.3%), Italy (8.9%), Ireland (5.7%), Spain (5.5%), Denmark (3.6%) and finally Belgium (2.8%).

Regulatory and public situations exerted some influence on the aforementioned evolutionary trend. Thus, up to 2015/2016 (the last years of "soft-landing") acted the last stages of the production quota system "milk quota regime" [7], the termination of which resulted in new favorable production increases in the following years. The Covid 19 pandemic caused milk production rates to slow down with different intensity among the MPCs (Table 1). Livestock units in the MMCs did not remain outside the scope of these phenomena. During the period observed, the dairy cattle herd decreased from 21.4 million in 2012 to 20.2 million in 2021 with some changes in the order of importance of the countries, namely: Germany (19.0%), France (16.4%), Poland (10.1%), Italy (9.1%), Netherlands (7.7%), Ireland (5.0%), Spain (4.2%), Denmark (2.8%) and lastly Belgium (2.6%) (Table 2).

As for milk yields, represented in Figure 1, the angular coefficient indicates a growing evolutionary character generalized in all cases, especially since the "soft-landing" years. Figure 2, on the other hand, proposes for MMCs a comparison of the trends of the raw milk variable (line with orange color in the graph) with the dairy cattle variable (line with blue color).

From the resulting evolutionary trends, it is possible to assume that in the EU, with the end of “soft-landing”, herds would not have been further enhanced when reached the highest breadth. Moreover, it is conceivable that in recent times, cows at the end of their career would be alternated with those included in the end of the “milk quota regime” period having entered, in the meantime, the age of milk production.

As for the degree of statistical dependence between the two variables, the relative levels of average values for the years between the five-year period 2012-2016 and the subsequent 2017-2021 show numerical correlation indices between them that are not perfectly territorially oriented (Table 3), as conversely a natural cause-and-effect relationship between two variables should indicate. In fact, except for Ireland, having almost-perfect systemic relationship of the linear link (+1), the coefficient r appears either concordant (Germany, Denmark and the Netherlands), discordant (Italy, Poland and Belgium), or in mixed form (France and Spain). It is an undefined scenario in which randomness (and not genetic and/or dietary causality) of factors beyond the rules of ordinary management might underlie the co-movements between the two cow - raw milk variables.

Despite all its limitations, the linear correlation index remains a valid statistical measure of evaluation address, considering the information that its numerical value, raised to square ($r^2$), gives. The values of the coefficient of determination $r^2$ exhibited wide ranges of variation going from 2012-2016 to the five-year period 2017-2021 (Table 3); the more the variation of the relevant numerical index decreases, the greater strength the hypothesis of the presence of effective factors for higher cow productivity assumes.

Table 1 - Raw milk, total available on farms evolution, products obtained (1 000 t)

| Country | 2012 | | 2013 | | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | | 2019 | | 2020 | | 2021 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % | 1 000 t | % |
| Germany | 30.892 | 21,6 | 31.338 | 21,6 | 32.395 | 21,6 | 32.685 | 21,4 | 32.688 | 21,2 | 32.614 | 21,0 | 33.110 | 21,1 | 33.103 | 20,9 | 33.189 | 20,7 | 32.532 | 20,7 |
| France | 25.630 | 17,9 | 25.329 | 17,5 | 26.603 | 17,8 | 26.713 | 17,5 | 26.066 | 16,9 | 26.006 | 16,8 | 26.023 | 16,6 | 26.036 | 16,4 | 26.152 | 16,3 | 25.835 | 16,4 |
| Ireland | 5.399 | 3,8 | 5.601 | 3,9 | 5.821 | 3,9 | 6.604 | 4,3 | 6.872 | 4,5 | 7.499 | 4,8 | 7.831 | 5,0 | 8.245 | 5,2 | 8.561 | 5,3 | 9.040 | 5,7 |
| Poland | 12.685 | 8,9 | 12.735 | 8,8 | 13.002 | 8,7 | 13.253 | 8,7 | 13.252 | 8,6 | 13.702 | 8,8 | 14.179 | 9,0 | 14.511 | 9,2 | 14.831 | 9,3 | 14.890 | 9,5 |
| Spain | 7.538 | 5,3 | 7.631 | 5,3 | 7.854 | 5,2 | 8.105 | 5,3 | 8.218 | 5,3 | 8.301 | 5,3 | 8.418 | 5,4 | 8.560 | 5,4 | 8.686 | 5,4 | 8.703 | 5,5 |
| Belgium | 3.412 | 2,4 | 3.475 | 2,4 | 3.689 | 2,5 | 3.988 | 2,6 | 3.882 | 2,5 | 4.025 | 2,6 | 4.179 | 2,7 | 4.288 | 2,7 | 4.431 | 2,8 | 4.381 | 2,8 |
| Denmark | 4.927 | 3,4 | 5.026 | 3,5 | 5.110 | 3,4 | 5.278 | 3,5 | 5.364 | 3,5 | 5.506 | 3,5 | 5.615 | 3,6 | 5.615 | 3,5 | 5.666 | 3,5 | 5.644 | 3,6 |
| Italy | 12.254 | 8,6 | 12.040 | 8,3 | 12.217 | 8,2 | 12.192 | 8,0 | 12.670 | 8,2 | 12.983 | 8,4 | 13.132 | 8,4 | 13.300 | 8,4 | 13.510 | 8,4 | 13.997 | 8,9 |
| Netherlands | 12.098 | 8,4 | 12.640 | 8,7 | 12.905 | 8,6 | 13.784 | 9,0 | 14.825 | 9,6 | 14.822 | 9,5 | 14.426 | 9,2 | 14.944 | 9,4 | 14.932 | 9,3 | 14.608 | 9,3 |
| Other country | 28.424 | 19,8 | 29.270 | 20,2 | 30.154 | 20,1 | 30.056 | 19,7 | 30.059 | 19,5 | 29.760 | 19,2 | 29.828 | 19,0 | 29.680 | 18,8 | 30.324 | 18,9 | 27.831 | 17,7 |
| Total EU | 143.258 | 100,0 | 145.083 | 100,0 | 149.749 | 100,0 | 152.658 | 100,0 | 153.895 | 100,0 | 155.219 | 100,0 | 156.740 | 100,0 | 158.283 | 100,0 | 160.282 | 100,0 | 157.460 | 100,0 |
| | 100 | | 101 | | 105 | | 107 | | 107 | | 108 | | 109 | | 110 | | 112 | | 110 | |

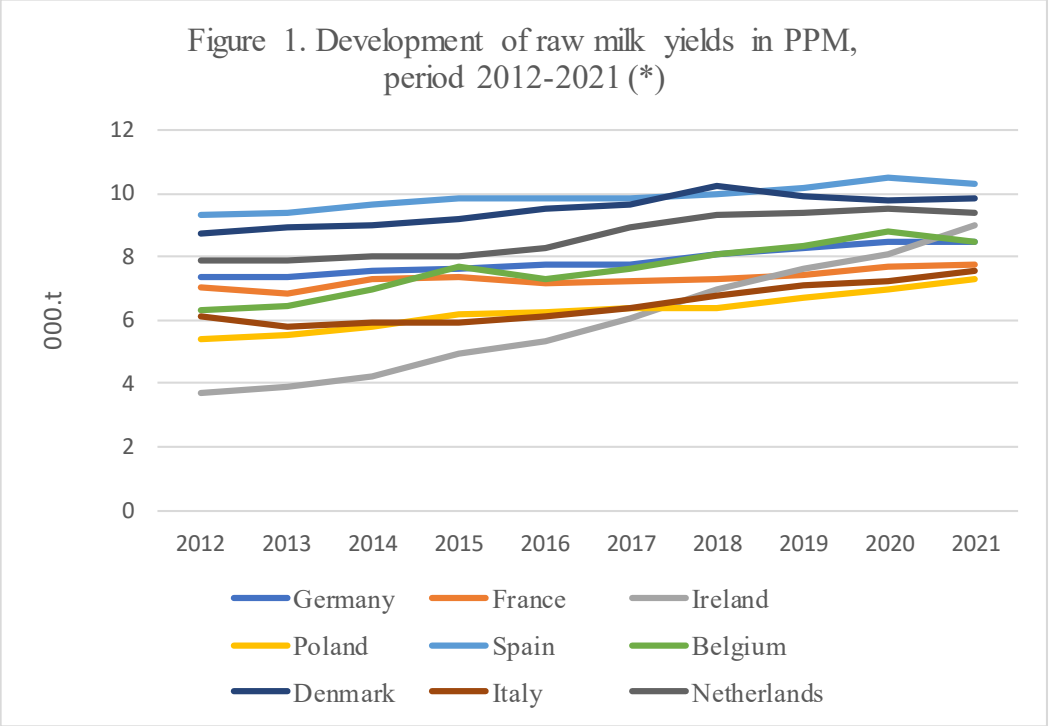
(*) Source: Eurostat

Table 2 -Number of dairy cows evolution (Thousand heads (animals)) (*)

| Country | 2012 | | 2013 | | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | | 2019 | | 2020 | | 2021 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n. | % | n. | % | n. | % | n. | % | n. | % | n. | % | n. | % | n. | % | n. | % | n. | % |
| Germany | 4.190 | 19,6 | 4.268 | 19,7 | 4.296 | 19,9 | 4.285 | 19,8 | 4.218 | 19,5 | 4.199 | 19,6 | 4.101 | 19,5 | 4.012 | 19,3 | 3.921 | 19,1 | 3.833 | 19,0 |
| France | 3.644 | 17,0 | 3.698 | 17,1 | 3.661 | 16,9 | 3.637 | 16,8 | 3.637 | 16,8 | 3.597 | 16,8 | 3.554 | 16,9 | 3.491 | 16,8 | 3.406 | 16,6 | 3.322 | 16,4 |
| Ireland | 1.456 | 6,8 | 1.426 | 6,6 | 1.369 | 6,3 | 1.343 | 6,2 | 1.295 | 6,0 | 1.240 | 5,8 | 1.128 | 5,4 | 1.082 | 5,2 | 1.060 | 5,2 | 1.007 | 5,0 |
| Poland | 2.346 | 11,0 | 2.299 | 10,6 | 2.248 | 10,4 | 2.134 | 9,9 | 2.130 | 9,8 | 2.153 | 10,1 | 2.214 | 10,5 | 2.167 | 10,4 | 2.126 | 10,4 | 2.035 | 10,1 |
| Spain | 811 | 3,8 | 813 | 3,8 | 817 | 3,8 | 823 | 3,8 | 834 | 3,9 | 844 | 3,9 | 845 | 4,0 | 844 | 4,1 | 827 | 4,0 | 845 | 4,2 |
| Belgium | 538 | 2,5 | 538 | 2,5 | 529 | 2,4 | 519 | 2,4 | 531 | 2,5 | 529 | 2,5 | 519 | 2,5 | 516 | 2,5 | 504 | 2,5 | 518 | 2,6 |
| Denmark | 565 | 2,6 | 563 | 2,6 | 570 | 2,6 | 575 | 2,7 | 565 | 2,6 | 570 | 2,7 | 547 | 2,6 | 567 | 2,7 | 579 | 2,8 | 573 | 2,8 |
| Italy | 2.009 | 9,4 | 2.075 | 9,6 | 2.069 | 9,6 | 2.057 | 9,5 | 2.060 | 9,5 | 2.040 | 9,5 | 1.939 | 9,2 | 1.876 | 9,0 | 1.871 | 9,1 | 1.844 | 9,1 |
| Netherlands | 1.541 | 7,2 | 1.597 | 7,4 | 1.610 | 7,4 | 1.717 | 7,9 | 1.794 | 8,3 | 1.665 | 7,8 | 1.552 | 7,4 | 1.590 | 7,7 | 1.569 | 7,6 | 1.554 | 7,7 |
| Other country | 4.307 | 20,1 | 4.376 | 20,2 | 4.469 | 20,7 | 4.561 | 21,1 | 4.569 | 21,1 | 4.573 | 21,4 | 4.630 | 22,0 | 4.621 | 22,3 | 4.651 | 22,7 | 4.673 | 23,1 |
| Total EU | 21.407 | 100,0 | 21.652 | 100,0 | 21.638 | 100,0 | 21.652 | 100,0 | 21.634 | 100,0 | 21.409 | 100,0 | 21.029 | 100,0 | 20.766 | 100,0 | 20.514 | 100,0 | 20.204 | 100,0 |
| | 100 | | 101 | | 101 | | 101 | | 101 | | 100 | | 98 | | 97 | | 96 | | 94 | |

(*) Source: Eurostat

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(*) Our elaborations from Tables 1 and 2.

Table 3 - Indices measuring the link between the variability of x and y data.

| Country | Coefficient of correlation | | | Coefficient of determination | |
|---|---|---|---|---|---|
| | r | | | $r^2$ | |
| | 2012-2016 | 2017-2021 | Shapes | 2012-2016 | 2017-2021 |
| Germany | 0,4913 | 0,0276 | moderate concordance to minor concordance | 0,2414 | 0,0008 |
| France | -0,5709 | 0,3535 | from medium high discordance to medium concordance | 0,3259 | 0,1249 |
| Ireland | 0,9981 | 0,9975 | linear | 0,9963 | 0,9949 |
| Poland | -0,9858 | -0,6109 | from high to low discordance | 0,9718 | 0,3732 |
| Spain | 0,2426 | -0,9759 | from low concordance to almost perfect discordance | 0,0589 | 0,9524 |
| Belgium | -0,9096 | -0,8793 | from high to low discordance | 0,8274 | 0,7731 |
| Denmark | 0,4132 | 0,1562 | moderate concordance to minor concordance | 0,1707 | 0,0244 |
| Italy | -0,0995 | -0,8209 | from low discordance to high discordance | 0,0099 | 0,6739 |
| Netherlands | 0,9957 | 0,4520 | from high concordance to low concordance | 0,9914 | 0,2043 |

(*)Our elaborations

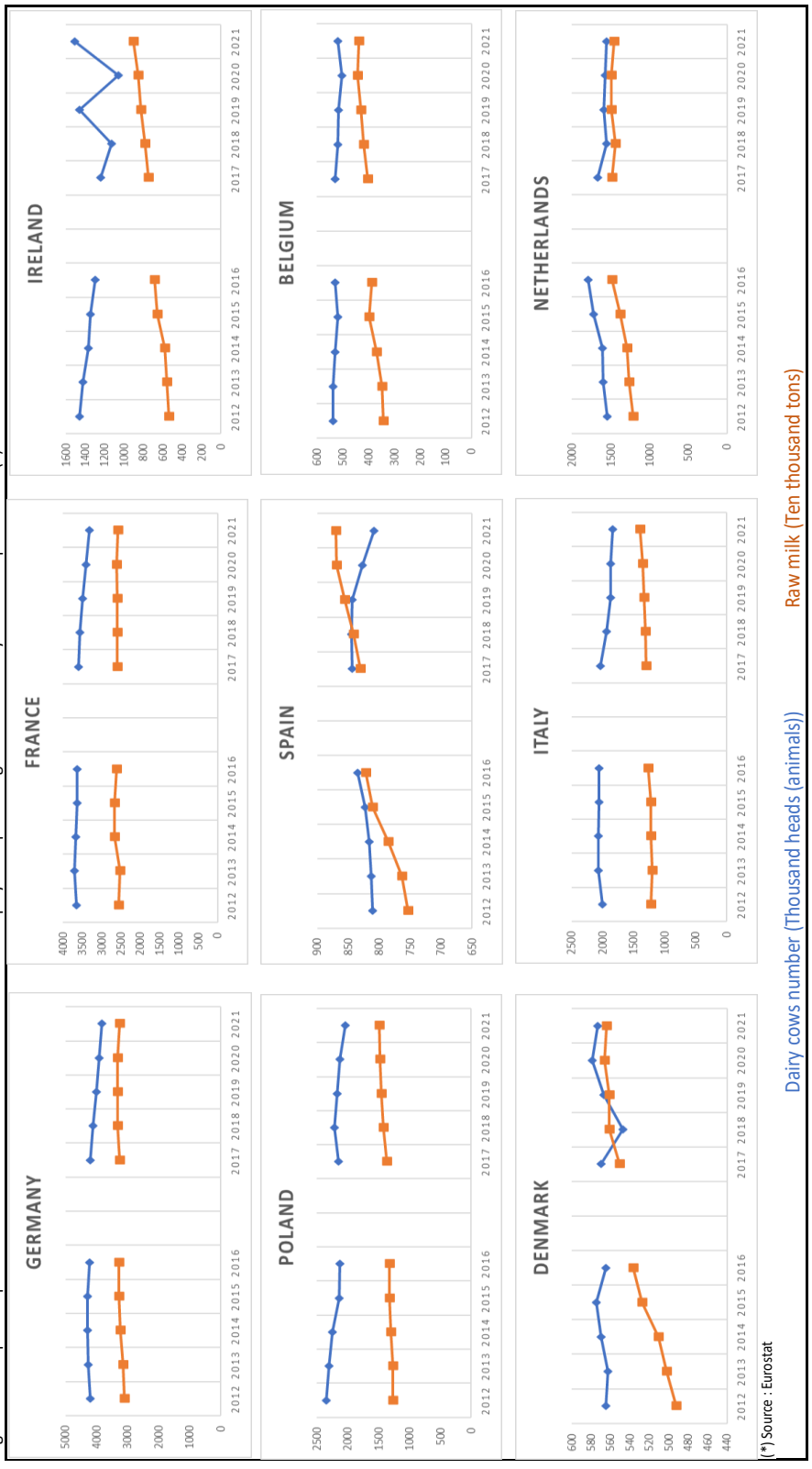



Figure 2 - Temporal representation of the bovine-milk-collected relationship by main producing member country of the European Union (*)

In this context, the role played by digital contact tools ("sensors"), applied to the cow's body, emerges as likely in concurring improvement of animal welfare and consequently in increasing production levels and thus unit yields of raw milk realized by the animal.

Certain economic management indicators of cow milk production may provide further information that could support this hypothesis (Table 4). Thus, "Milk production" and "Milk yeld" showed generalised increases in cow's milk, while "Milk per ha fodder area" had, in the MMCs, a certain stability in realised levels despite a cow herd size that has contracted somewhat in recent years (2017-2020).

Table 4 - Development indicators linked to raw cow's milk production levels for two four-year periods d 2013-2016 and 2017-2020(*)

| Countries | Milk production (t) | | | | Milk yeld (kg/cow) | | | | Milk per ha fodder area (t/ha) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2017 | 2018 | 2019 | 2020 | 2017 | 2018 | 2019 | 2020 | 2017 | 2018 | 2019 | 2020 |
| Germany | 578,7 | 587,2 | 604 | 608,5 | 7921 | 7987 | 8233 | 8273 | 9,3 | 9,5 | 9,4 | 9,4 |
| France | 453,3 | 453,3 | 461,7 | 477,6 | 7098 | 7059 | 7101 | 7223 | 5,7 | 5,7 | 5,7 | 5,8 |
| Ireland | 468,5 | 478,5 | 454,6 | 466,9 | 5824 | 5851 | 5921 | 5938 | 7,4 | 7,2 | 7,2 | 7,2 |
| Poland | 116,2 | 112,5 | 123,3 | 127,3 | 6065 | 6108 | 6334 | 6440 | 8,4 | 8,2 | 7,8 | 7,5 |
| Spain | 502,8 | 516,3 | 544,9 | 607,2 | 7946 | 8329 | 8355 | 8746 | 18,67 | 16,7 | 16,9 | 17,3 |
| Belgium | 566,3 | 609,8 | 602,8 | 605,3 | 7725 | 7946 | 7953 | 8118 | 11,2 | 11 | 11,2 | 10,9 |
| Denmark | 1823 | 1824 | 1915,9 | 1936,3 | 9729 | 9805 | 9988 | 9967 | 15,2 | 14,3 | 14 | 14,6 |
| Italy | 347,1 | 350,1 | 389,7 | 391,3 | 6581 | 6681 | 7187 | 7191 | 13,9 | 14,1 | 13,5 | 13,4 |
| Netherlands | 904,9 | 910,2 | 888,7 | 920,5 | 8728 | 8901 | 8816 | 8863 | 15,5 | 15,6 | 15,8 | 16,1 |
| | Index number | | | | Index number | | | | Index number | | | |
| Germany | 100 | 101 | 104 | 105 | 100 | 101 | 104 | 104 | 100 | 102 | 101 | 101 |
| France | 100 | 100 | 102 | 105 | 100 | 99 | 100 | 102 | 100 | 100 | 100 | 102 |
| Ireland | 100 | 102 | 97 | 100 | 100 | 100 | 102 | 102 | 100 | 97 | 97 | 97 |
| Poland | 100 | 97 | 106 | 110 | 100 | 101 | 104 | 106 | 100 | 98 | 93 | 89 |
| Spain | 100 | 103 | 108 | 121 | 100 | 105 | 105 | 110 | 100 | 89 | 91 | 93 |
| Belgium | 100 | 108 | 106 | 107 | 100 | 103 | 103 | 105 | 100 | 98 | 100 | 97 |
| Denmark | 100 | 100 | 105 | 106 | 100 | 101 | 103 | 102 | 100 | 94 | 92 | 96 |
| Italy | 100 | 101 | 112 | 113 | 100 | 102 | 109 | 109 | 100 | 101 | 97 | 96 |
| Netherlands | 100 | 101 | 98 | 102 | 100 | 102 | 101 | 102 | 100 | 101 | 102 | 104 |

(*) FADN

In addition, among the cost items referring to farm structures, 'Machinery' put in evidence values in increase that refer to mobile investments. Therefore, it may be assumed that this item also includes digitisation tools, i.e. sensors used on cows, also because the cost item 'Depreciaton', on the other hand, indicates decreasing values and thus reductions among fixed factor investments (Table 5).

Table 5 - Development indicators linked to specific bovine fixed capital over the period 2017-2020 (*)

| Countries | Machinery | | | | Depreciaton | | | |
|---|---|---|---|---|---|---|---|---|
| | 2017 | 2018 | 2019 | 2020 | 2017 | 2018 | 2019 | 2020 |
| | Thousand euro | | | | Euro AWU | | | |
| Germany | 133 | 139 | 147 | 152 | 18 | 18 | 17 | 17 |
| France | 91 | 93 | 97 | 105 | 23 | 22 | 22 | 22 |
| Ireland | 69 | 72 | 73 | 79 | 10 | 9 | 9 | 8 |
| Poland | 45 | 41 | 43 | 42 | 4 | 4 | 4 | 4 |
| Spain | 23 | 23 | 25 | 27 | 4 | 5 | 4 | 5 |
| Belgium | 89 | 93 | 97 | 103 | 19 | 18 | 18 | 18 |
| Denmark | 318 | 315 | 333 | 333 | 24 | 24 | 25 | 26 |
| Italy | 40 | 43 | 46 | 54 | 5 | 6 | 6 | 6 |
| Netherlands | 140 | 146 | 153 | 161 | 28 | 28 | 28 | 27 |
| | Index number | | | | Index number | | | |
| Germany | 100 | 104 | 111 | 114 | 100 | 97 | 94 | 92 |
| France | 100 | 102 | 107 | 115 | 100 | 97 | 95 | 95 |
| Ireland | 100 | 105 | 105 | 114 | 100 | 93 | 95 | 88 |
| Poland | 100 | 91 | 95 | 94 | 100 | 98 | 95 | 95 |
| Spain | 100 | 99 | 109 | 116 | 100 | 105 | 100 | 104 |
| Belgium | 100 | 104 | 110 | 116 | 100 | 96 | 94 | 94 |
| Denmark | 100 | 99 | 105 | 105 | 100 | 100 | 102 | 105 |
| Italy | 100 | 107 | 115 | 133 | 100 | 120 | 120 | 120 |
| Netherlands | 100 | 104 | 110 | 115 | 100 | 98 | 99 | 97 |

(*) FADN

This set of information may lead to attribute the aforementioned increases in cow's milk registered to the adoption of digital devices and specifically to contact sensors on the cow's body, used to ensure the animal's welfare, to optimize the use of production factors and to detect the animal's physiological activities.

## DISCUSSION

Innovations offer a continuous and relevant contribution to the modernisation and development of dairy farms through appropriate digital technology for the competitiveness and sustainability of production.
Their adoption aims to improve the achievable income conditions resulting from related increases in levels of raw milk collected, in a context of safeguarding environmental sustainability. Essentially, digitisation provides tools for managing the complexity of the EU's most diverse dairy farming systems.
In particular, digital devices make it possible to track the production progress of individual cows [8] to computerise feed management [9], the automation of phases and/or operations of herd management (milking machines, automation of stations, control devices), to control the ration composition and fodder sizes for cows that have different needs. In addition, all this has undoubtedly positive effects on the implementation of higher levels of animal welfare.
It is now a fact that the scientific community believes that dairy cows, reared in full respect of animal welfare, produce more and better quality milk [10] and have a lower impact in terms of $CO_2$ emissions per litre of milk produced [11]. In fact, limiting the incidence of disease would reduce the intensity of emissions as healthier animals are more productive and thus create fewer emissions per unit of product [12].
However, there is no doubt that multiple factors influence the characteristics of raw cow's milk related to the individual animal or the environment in which it lives [13]. In particular, strictly subjective factors of the animal such as breed [14], genetics [15] and the cow's age give rise to the natural composition of the milk and quantity produced, but many of the mechanisms of cow breeding management underlie these effects. Indeed, environmental factors of the habitual environment in which the animal lives together create the opportunity for greater expression of the cows' natural behaviour, thus favouring better organoleptic conditions, and optimisation of the levels of the raw milk produced. These circumstances explain the general interest of the European Union (particularly in the MPCs) in the use of digital instruments worn by cows. In this context, among the strategies of the European Commission take shape with the Green Deal for a better planning of the digitalization of activities in dairy farms there is the planned Revision of the animal welfare legislation foreseen among the Green Deal animal policies' objectives, namely "Evaluation ("Fitness Check") of the EU Legislation on the welfare of farmed animals".

## CONCLUSIONS

The analysis conducted in the present paper, although it involved not easy problems of simplification in view of the many variations in the spatial realities of dairy herds, suggested the hypothesis that the use of "digital sensors" worn by dairy cows could be a co-responsible factor in production variations resulting from Eurostat data in the decade examined. The question underlying the present research is whether the improvement in economic production efficiency related to accidental causes or systematic causes to determine whether to accept or reject the hypothesis set forth. That is, to make the

technological device (in the case of this work, “contact sensors”) assume or not assume, in the natural association between the two variables dairy cow (independent variable) and raw milk (dependent variable) the function of a “third variable” (explanatory variable).
The question obviously remains open. The most methodologically relevant aspect is the a priori choice of the delimitation criteria and the prioritization of the variable(s) involved with respect to the objective.
However, under the 'assumption that official statistics provide a contribution to the interpretation of developmental differentials as a result of the increased level of technology in livestock farms, the present survey allowed to support the propositional considerations arising precisely from the interpretive analysis. In particular, the analysis represents a contribution of knowledge on the use of technological factors with advanced performance and functionality to increase the efficiency and productivity of dairy cows in EU producing Countries in a context that guarantees the animal welfare and a reduction of environmental impact causes. All this in a context in which the new agricultural policy lines emerging in the National Strategic Plans (Reg. (EU) 2021/2115) envisage a reinforcement of the environmental and sustainable compatibility of agricultural practices and cattle breeding.

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