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INSTITUTE FOR TEXTILE AND LEATHER
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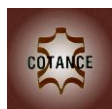
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FOREWORD

ICAMS 2024 is offering the framework for presenting the latest results in research, focusing on the field of Materials Science and Innovative Technologies, which records an impressive dynamic and is recognized as a current national and European priority.

The ICAMS 2024 international event, organised by the National Institute for Research and Development for Textiles and Leather – Division: Leather and Footwear Institute (INCDTP-ICPI) took place online on 30-31 October 2024. ICAMS 2024 brought together different stakeholders and provided a platform for a better understanding of the European innovation ecosystem while raising awareness of the actions needed to enable synergies and drawing lessons for future actions.

The conference provided the opportunity for exchanging ideas and experience with researchers, scientists and experts at international level, and for developing new scientific contributions.

Around 60 participants have virtually attended the event from several academic and research institutions, public and private sectors, as well as Managing Authorities. Participants have presented their experience on research, innovation, policies and the creation of synergies. All these inputs offered insightful elements for discussion in the different participatory sessions throughout the event.

The conference topics included, but were not limited to:

- **Advanced Materials and Nanomaterials**
- **Biomaterials and Biotechnologies**
- **Innovative Systems, Technologies and Quality Management**
- **Ecological Processes for Circular and Neutral Economy**
- **Creative Industries and Cultural Heritage**
- **Education and Digitalization**

We would like to thank all the participants, the International Scientific Committee, and the partners that made this scientific event possible. ICAMS Conference has already become a tradition, contributing to the advancement of Materials Science in research, academic, social and business environments worldwide.

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Dr. Laurenția ALEXANDRESCU

Scientific Secretary, INCDTP – Division: Leather and Footwear Research Institute (ICPI), RO

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OPTIMISATION OF THE CONDUCTIVE MATERIALS DEVELOPMENT FOR SENSORS AND EM SHIELDING

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The paper presents aspects of optimising textile materials for flexible electronics (actuators and sensors) and electromagnetic screens. The optimisation of electroconductive materials for sensors, actuators or materials for electromagnetic shielding has a common denominator: electrical resistance. This electrical resistance must be minimal to increase electrical conductivity for the textile materials used as electroconductive materials for sensors and actuators, respectively, as a screen for the attenuation of electromagnetic radiation. In order to allow the optimal selection of electroconductive fabrics for actuators, sensors or flexible electromagnetic screens for a specific application, certain performance characteristics such as flexibility, low mass, air permeability and low cost are required. For the analysed cases (resistive sensors, actuators depending on the electrical resistance fluctuation such as shape memory alloy, materials for electromagnetic shielding), the optimisation is based on a function to minimise the electric resistance variable to increase the conductivity and effectiveness of the electromagnetic shielding. To select and classify suitable electroconductive fabrics for sensors, actuators, and electromagnetic shields, we used the receiver operating characteristic (ROC) curve to represent the performance of the binary classification model. We also calculated the area under the curve (AUC) for the Rs training set, representing the ROC's integral. The higher AUC values for training (0.8333 and 0.9545) indicated excellent performance of the classifier. We were able to optimize the influential parameters for electromagnetic shielding (SE) to target a value of 50 dB for a fabric with a thickness of 0.46 mm, mass of 87.8795 g/m², air permeability of 9090 l/m²/s, and electrical resistance of 20 Ω.

Keywords: optimisation, conductive, textile.

INTRODUCTION

Researchers meticulously select the most suitable materials and techniques used in electronic textiles to create fabric sensors. The sensing functionality can be ingeniously tailored through intrinsic and extrinsic modifications to textile substrates, depending on the degree of integration into the fabric platform. These sensors accurately measure force, pressure, chemicals, humidity, and temperature variations (Castano & Flatau, 2014).

Manufacturing textile-based sensors involves using a silicone-textile composite resistive strain sensor, which relies on a conductive textile encapsulated into a dual silicone rubber layer. This innovative sensor design allows recording chest wall expansion during respiratory activity and precisely capturing elbow flexion/extension movements (Di Tocco *et al.*, 2022). Moreover, the incorporation of organic conductive polymers such as polypyrrole (PPy) for coating textile materials in order to achieve significant strain sensing capabilities is founded on the creation of a conductive textile exhibiting exceptional sensing performance, including low electrical resistance, high strain sensitivity, and environmental sustainability (Raman & Ravi Sankar, 2022).

The optimisation of electroconductive materials for various applications, such as sensors, actuators, and materials for electromagnetic shielding, is crucial for improving their performance. One of the critical factors in this optimisation process is the electrical resistance

of these materials. It is essential to minimise the electrical resistance to increase the electrical conductivity of textile materials used in sensors, actuators, and electromagnetic shielding applications. This enhanced conductivity improves performance in these devices, making them more effective in their respective roles. In order to select the optimal materials for specific applications, it is essential to consider various performance characteristics such as flexibility, low mass, air permeability, and cost-effectiveness. These attributes are significant in determining the suitability of materials for use in sensors, actuators, and electromagnetic shielding applications. For instance, resistive sensors and actuators that rely on the fluctuation of electrical resistance, such as shape memory alloys, require careful optimisation to ensure optimal performance. Similarly, materials used for electromagnetic shielding must be optimised to minimise electrical resistance and enhance their effectiveness in attenuating electromagnetic radiation.

In electrical material engineering, optimization involves the intricate and detailed process of carefully adjusting the electrical resistance of materials. This adjustment is essential to ensure the materials demonstrate the specific conductivity or semiconductor characteristics required for their intended applications (sensors, actuators or electromagnetic (EM) shields). These properties are crucial for the efficient functioning of electrodes in a wide range of sensors and actuators. Optimization involves fine-tuning the electrical properties of the materials to achieve an optimal balance between resistance and conductivity. This balance is essential for ensuring the reliable and effective performance of the sensors, EM shields and actuators across various real-world applications and scenarios.

EXPERIMENTAL PART

The conductive fabrics analysed were obtained by electroplating polyamide yarns using electrolyte solutions based on silver, copper and aluminium. Considering the electroconductive textiles obtained, we have to make an optimal selection of the materials to be used for sensors, actuators, or electromagnetic shields.

The proposed approach started from the premise that we have to minimize the value of electrical resistance to increase the conductivity of the materials used for sensor electrodes. At the same time, for materials capable of absorbing or reflecting electromagnetic radiation, we have to increase the shielding effectiveness (SE) and as an indirect consequence, the electrical resistance should be reduced and conductivity must have high values.

In the research part, we emphasise the critical importance of minimizing electrical resistance to enhance the materials' conductivity in sensor electrode development. This involves a comprehensive analysis of the material properties (mass (M), air permeability (Pa), thickness (δ), resistance (Rs)) and structure to identify avenues for optimization by reducing the electrical resistance. Additionally, for materials capable of absorbing or reflecting electromagnetic radiation, our focus is directed towards optimizing the shielding effectiveness (SE) through a detailed investigation of the material's electromagnetic properties and the design of the shielding structure. As a result, our approach requires a thorough understanding of both electrical and electromagnetic characteristics to ensure that the electrical resistance is minimized while achieving high conductivity and the shielding effectiveness is maximized. Using data from conductive and semiconductive materials developed and having Pa in the range 38 - 9090 l/m²/s, M in the range 86 - 559 g/m², δ in the range of 0.4 - 1.6 mm, Rs between 20 - 8.5x10⁹, respective SE in the range 0.2 - 47 dB, was developed data analysis, optimization and regression equations creation for Rs and SE. The regression equations for Rs are provided for two distinct cases: electrically conductive materials (1) and dissipative materials (2). The regression equation for SE is presented as (3).

$$y = -2294 + 2868 x_1 + 0.3963 x_3 + 4.591 x_2 + 3.118 x_4 - 879.2 x_1^2 - 0.000020 x_3^2 + 0.001305 x_2^2 - 0.3010 x_4^2 - 0.2837 x_1 * x_3 - 1.744 x_1 * x_2 - 3.550 x_1 * x_4 - 0.000258 x_3 * x_2 + 0.001985 x_3 * x_4 \quad (1)$$

$$y = 8507585 + 2868 x_1 + 0.3963 x_3 + 4.591 x_2 + 3.118 x_4 - 879.2 x_1^2 - 0.000020 x_3^2 + 0.001305 x_2^2 - 0.3010 x_4^2 - 0.2837 x_1 * x_3 - 1.744 x_1 * x_2 - 3.550 x_1 * x_4 - 0.000258 x_3 * x_2 + 0.001985 x_3 * x_4 \quad (2)$$

where Electrical resistance is y; Thickness is x_1 ; Mass is x_2 ; Air permeability is x_3

$$y = -844 - 85 x_1 + 0.1892 x_3 + 4.93 x_2 + 0.000017 x_4 - 1050 x_1^2 - 0.000011 x_3^2 - 0.01594 x_2^2 + 0.1107 x_1 * x_3 + 6.60 x_1 * x_2 - 0.000880 x_3 * x_2 \quad (3)$$

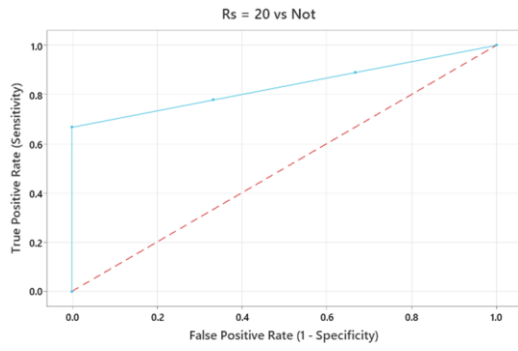
where SE is y; Thickness is x_1 ; Mass is x_2 ; Air permeability is x_3 ; Rs is x_4 .

Table 1 presents the analysis of variance for electrical resistance for sensors.

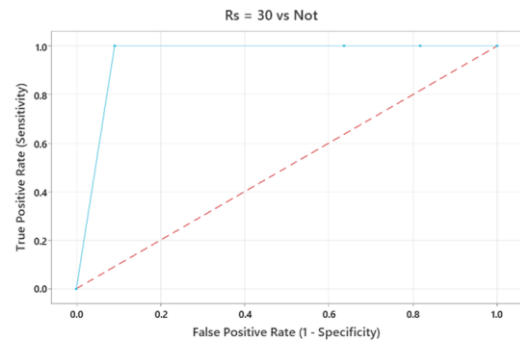
Table 1. Analysis of Variance for Rs depending on Pa, M, SE, δ and categorical variable conductivity (C)

Source	DF	Adj SS	Adj MS
Model	14	1.32769E+14	9.48353E+12
Linear	5	5.66527E+12	1.13305E+12
Thickness	1	257	257
Pa	1	197	197
Mass	1	151	151
SE	1	76	76
Conductivity	1	2.61020E+11	2.61020E+11
Square	4	489	122
Thickness*Thickness	1	202	202
Pa*Pa	1	197	197
Mass*Mass	1	4	4
SE*SE	1	330	330
2-Way Interaction	5	590	118
Thickness*Pa	1	282	282
Thickness*Mass	1	149	149
Thickness*SE	1	9	9
Pa*Mass	1	69	69
Pa*SE	1	79	79
Error	9	0	0
Total	23	1.32769E+14	

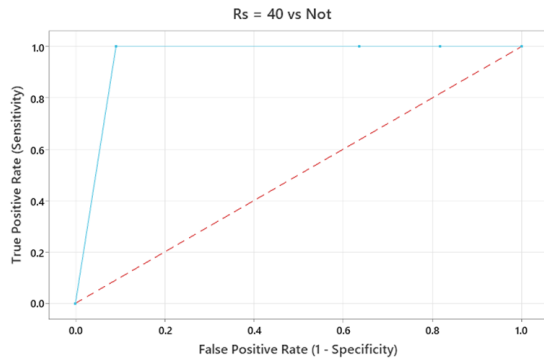
The model's relationship between y and x variables is statistically significant because $p < 0.10$. The regression model explains the 99.59% variation well. The Rs model fits the data well and can be used to predict Rs for specific variables x or find the appropriate x values to obtain a specific value for Rs. Figure 1 presents the ROC (receive operating characteristic) curve, respective in Figure 2 presents the 3D representation of the electrical resistance depending on mass (M), air permeability (Pa), thickness (δ) and shielding effectiveness (SE). The ROC (Receiver Operating Characteristic) curve graphically represents the performance of the binary classification model at various threshold settings. It illustrates the trade-off between true and false positive rates across different threshold values, providing valuable insights into the model's predictive accuracy. The area under the curve (AUC) for the Rs training set is the numerical value indicating the performance of the model and represents the integral of the ROC (Tsang, 2007) and represents the ability of the model ability to classify (Choi *et al.*, 2024) classes. In our cases, the higher AUC values (AUC for training 0.8333 (a), respective 0.9545 (b, c, d)) indicate an excellent performance of the classifier.



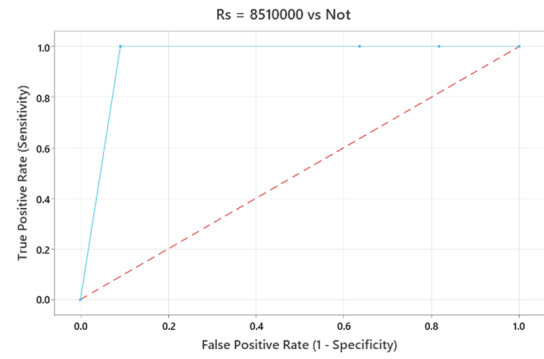
a. Area under the curve: Training = 0.8333



b. Area under the curve: Training = 0.9545

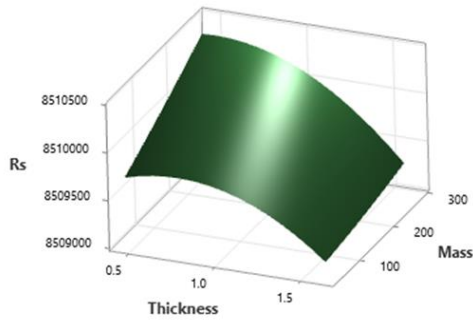


c. Area under the curve: Training = 0.9545

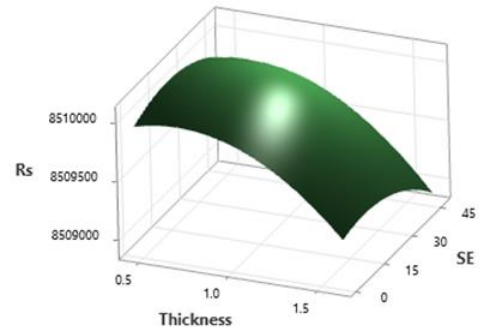


d. Area under the curve: Training = 0.9545

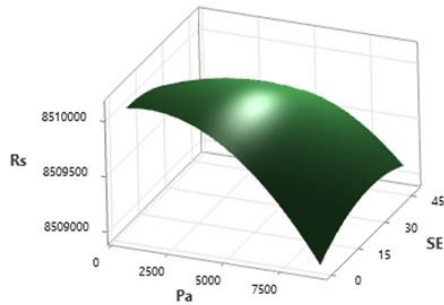
Figure 1. Receiver operating characteristic



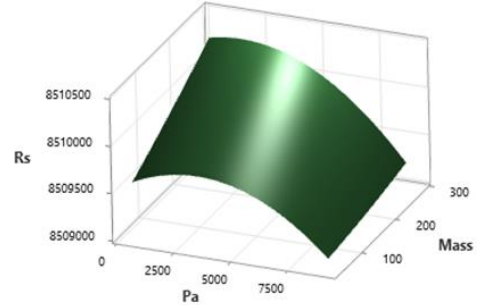
a. $Rs = f(\delta, M)$



b. $Rs = f(\delta, SE)$



c. $Rs = f(Pa, SE)$



d. $Rs = f(Pa, M)$

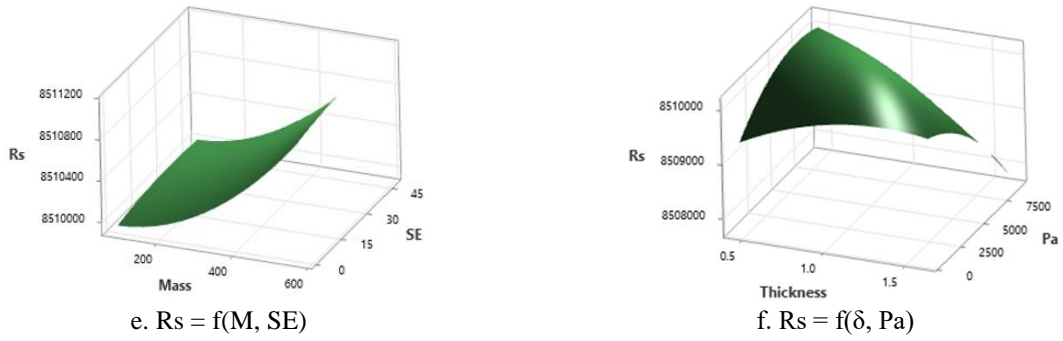


Figure 2. Surface resistance depending on thickness (δ) and mass (a), thickness and SE (b), air permeability (Pa) and SE (c), mass (M) and air permeability (d), mass and SE (e), respective thickness and Pa (f)

Figure 3 presents the optimal tree for electrical resistance starting with root node $R_s=20 \Omega$ at following levels defined by splitting experiments with conditions related to air permeability (Pa) and electromagnetic attenuation effectiveness ((SE). The information within the terminal nodes presents a valuable opportunity to systematically rank all distinct subsets based on their respective class probabilities. This ranking process enables a nuanced understanding of the distribution of probabilities among the various subsets. The method class probability and optimal tree within 1 standard error used for node splitting to minimize misclassification costs. For validation was used the 10-fold cross-validation method (Nagaraj *et al.*, 2021).

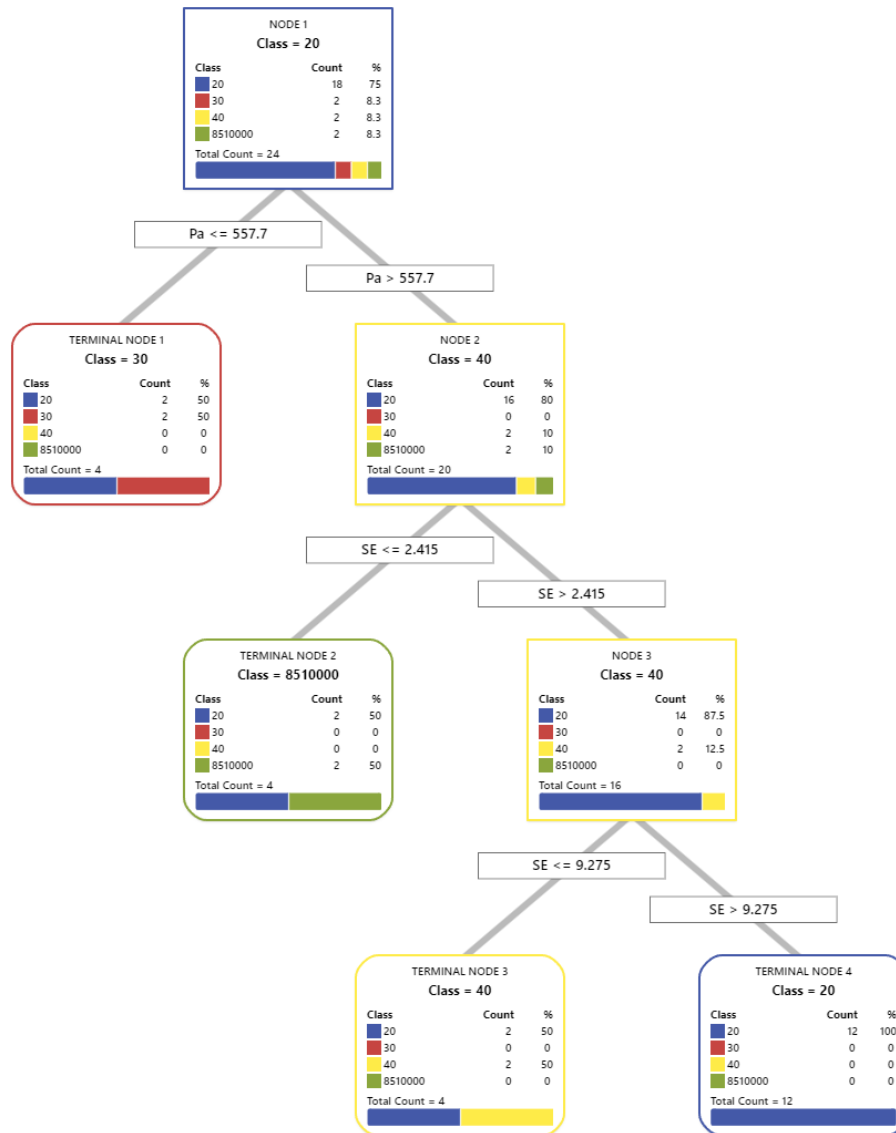


Figure 3. Optimal tree diagram

ANALYSIS AND DISCUSSIONS

The importance of the variables for model improvement is presented in Figure 4 when splits are made on a predictor. We can observe that the importance of SE is 100% and air permeability (Pa) is 86.8%.

Figure 5 presents the optimization of the SE value, targeting 50 dB for shielding effectiveness. It can be observed that this can be achieved if conductive coated materials have estimative values for thickness 0.46 mm, M 87,8795 g/m², Pa 9090 l/m²/s and Rs 20 Ω.

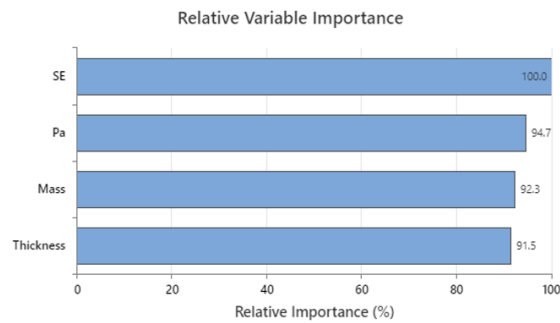


Figure 4. Variable importance measuring model optimization

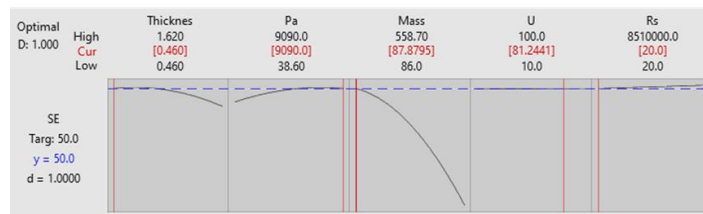


Figure 5. Shielding effectiveness optimization

CONCLUSIONS

Based on the premise that minimizing electrical resistance increases the conductivity of materials used for sensor electrodes, it can be concluded that the optimization of electrical resistance indirectly optimizes the conductivity of materials used in sensors and actuators. This process involves a comprehensive analysis of material properties such as mass (M), air permeability (Pa), thickness (δ), and resistance (Rs), as well as the structure of the materials. By identifying the appropriate path for optimization, which includes reducing the electrical resistance, it is possible to achieve maximum shielding effectiveness and conductivity values. This comprehensive approach ensures that the materials used for sensor electrodes are refined to provide the highest level of performance in terms of electrical conductivity and resistance.

Acknowledgements

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ANTIBACTERIAL COATING FOR SHOE INSOLES WITH TITANIUM DIOXIDE NANOPARTICLES

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To protect the human foot from the attack of bacteria and fungi, it is extremely important to choose an appropriate antimicrobial agent. The main component building skin tissue is collagen. Hydrolyzed collagen is widely used in medicine due to its properties of biodegradability, biocompatibility and non-toxicity. It is characterized by excellent film-forming properties. TiO₂ nanostructures have been widely studied as antimicrobial agents due to their photocatalytic activity under UV light and possess excellent antibacterial properties. The application of nanomaterials to treated leather surfaces resists the growth of microorganisms as well as exhibiting a self-cleaning effect. In this study, TiO₂ nanoparticles were *in situ* synthesized using oxalic acid as a precursor. These particles were deposited on the cross-linked gelatin hydrogel as finishing layer applied to shoe insoles. SEM, UV-Vis, FTIR and antibacterial tests for Gram-positive and Gram-negative bacteria were performed. Microscopic observations prove a distribution of titanium dioxide nanoparticles and enveloped by the collagen film. The presence of chemical and physical bonds between the different components of the biocomposite film has been demonstrated. The antimicrobial activity of the investigated shoe insole samples was evaluated by the reduction of bacterial growth. Shoe insoles coated with gelatin-titanium dioxide nanocomposite show high antibacterial activity against the strains used. Consequently, incorporating metal particles such as TiO₂ into the cross-linked collagen hydrogel and fixing it to the treated leather shoe material is a good alternative for antimicrobial treatment.

Keywords: antibacterial finishing, insole, TiO₂ nanoparticles

INTRODUCTION

Natural leather is the most commonly used material for shoe insoles. Because of its absorbent and breathable properties, leather has unique characteristics, climate-balancing, and good hygienic effects on the shoe. The main component of leather is protein; therefore, it creates conditions for breeding microorganisms, including bacteria and fungi during the storage and use process, which may affect the structure and properties of leather and damage the health of users. Moreover, the human foot is in a continuous condition of increased temperature, pH and humidity. High levels of hydration, humidity and ambient temperatures, lead to an increase in the number of microorganisms, especially *Staphylococcus aureus* (Healy *et al.*, 2010). Commonly used protection chemicals are mostly volatile organic compounds, due to their carcinogenic effect and environmental toxicity, it is prone to become unacceptable (Bai *et al.*, 2022). This has led to the idea of developing antimicrobial material leather for insoles with a more durable and effective treatment without the use of harmful chemicals.

Considerable research has been focused on the treatment of leather with nanometal oxides during the pre-tanning process, where nanomaterials are added to the tanning solution. However, there are still few studies that deal with the nanoprocessing of leather in the final finishing step without adding the nanomaterial to the tanning solution. Several nano-metal oxides (Ag₂O, ZnO, MnO₂, CuO) have great, causing destruction of the cell wall and

considerable binding affinity toward thiol -SH- containing groups, showing significant antimicrobial effects (Muthukrishnan *et al.*, 2019; Wang *et al.*, 2017).

A sole leather loaded with nano-zinc oxide has been developed and the treated leather samples showed significant resistance against fungus and bacteria (Habib *et al.*, 2023). Titanium dioxide nanoparticles (TiO₂-NPs) are already used in various practical applications, such as water and air purification, self-cleaning and self-sterilizing surfaces, and optical and dielectric devices. One of TiO₂ uses is as a pigment because of its brightness, high refractive index, and resistance to discoloration. Because of its interesting photocatalytic properties, TiO₂ has been used in decontamination, purification, and deodorization of air and wastewater but it also has the ability to kill cancer cells, bacteria and viruses under mild UV illumination. TiO₂ proves to be the most suitable safe and broad-spectrum antimicrobial agent (Sunada *et al.*, 2003). Studies have shown an increase in the antimicrobial activity of leather coated with Ag-TiO₂ NPs (Carvalho *et al.*, 2018; Khalid, 2022; Gaidău *et al.*, 2016; Kaygusuz *et al.*, 2016; Marques *et al.*, 2022; Ignat *et al.*, 2020). Others are considering the possibility of use in the leather treatment process with the addition of the TiO₂-SiO₂ nanocomposite (Kaygusuz *et al.*, 2017).

The finishing film of leather can vary greatly, mainly depending on its purpose. The sol-gel method is one of the most widely used techniques for the synthesis of functional coating films for substrate surface modification and the improvement of material properties which are affected by surface conditions (Tan *et al.*, 2021). This technique possesses a number of advantages over conventional film formation techniques, including a relatively low processing temperature, ease of applying homogeneous multicomponent oxide films over large surfaces, and good control of the composition and properties of the final material.

In this study, a finish coating was successfully obtained by modifying leather samples with cross-linked gelatin containing TiO₂ particles, which were synthesized *in situ*.

MATERIALS AND METHODS

Materials

Natural pig leather from a local producer, chrome-tanned, no finish, 0.89 mm thick. The test pieces are 100/50 mm in size, and the average weight of the samples is 2.4 g. TiO₂ from Sigma-Aldrich (Darmstadt, Germany); Glutaraldehyde (25% aqueous solution) from Sigma-Aldrich (Darmstadt, Germany); Gelatin from Merck KGaA (Darmstadt, Germany); and oxalic acid (H₂C₂O₄) from Merck KGaA (Darmstadt, Germany).

Three variants of modification of leather materials were applied: *LT-1* (Le-TiO₂_1), *LT-2* (Le-TiO₂_2), and *LT-3* (Le-TiO₂_3). The finishing films were obtained by immersion in the different solutions in equal concentrations. For all samples, aqueous solutions were used in the following concentrations: 5% gelatin solution, 2.5% GA (glutaraldehyde), 0.1 M TiO₂, and 0.1 M C₂H₂O₄.

LT-1 (Le-TiO₂_1)

The leather samples were immersed in a solution of 0.1 M TiO₂ and a 0.1 M C₂H₂O₄ for 30 min at t=60°C. TiO₂ particles were synthesized *in situ*. After that, the samples removed from the solution and immediately immersed in solution of gelatin and finally the glutaraldehyde solution was added for crosslinking. The leather samples were dried for 2 h at t=60°C. The samples were rinsed with distilled water and left to dry at room temperature.

LT-2 (Le-TiO₂_2)

The leather samples were immersed in a gelatin solution for 2 h at $t=60\text{ }^{\circ}\text{C}$. After that, the samples were immersed in the solution of TiO₂ and oxalic acid for 30 min at $t=60\text{ }^{\circ}\text{C}$. The next stage was adding the glutaraldehyde. Finally, the samples were washed and dried.

LT-3 (Le-TiO₂_3)

The leather samples were submerged into previously prepared solution of gelatin and glutaraldehyde. After crosslinked samples were immersed in solution of TiO₂ for 30 min and heated at $t=60\text{ }^{\circ}\text{C}$. The next stage was immersion in a solution of oxalic acid for 30 min. Then the samples are rinsed with distilled water and dried at $60\text{ }^{\circ}\text{C}$ for 2 h.

Analysis

The surface morphology of the modified materials and the formation of TiO₂ particles were analyzed using a scanning electron microscope (SEM) Philips ESEM XL30 FEG. FTIR analysis was performed on a Fourier transform infrared spectrometer (IRAffinity-1, Shimadzu, Japan), the spectral range of $4000\div 600\text{ cm}^{-1}$. Spectral characteristics are taken using UVA/VIS/NIR spectrophotometer Lambda 750S PerkinElmer, USA, in range of length $\lambda=2500\div 250\text{ nm}$. The leather physical and mechanical tests: Determination of water vapour permeability (ISO 14268:2023) was carried out with apparatus SATRA STM 473. The water vapour absorption indicator is determined by calculation (ISO 17229:2016).

The antimicrobial activity of the tested leather samples was evaluated using the Kirby–Bauer disc diffusion method (Hudzicki, 2009). The test bacterial strains used in this study included *Pseudomonas aeruginosa* 1390 (Gram-), *Bacillus subtilis* 168 (Gram-) and *Escherichia coli* W 1655 (Gram-): sourced from the National Bank of Industrial Microorganisms and Cell Cultures, Bulgaria, along with *Erysipelothrix rhusiopathiae* B40 (Gram+) from the culture collection of The Stephan Angeloff Institute of Microbiology. Bacterial cultures were first grown overnight in Nutrient Broth (Himedia, India) at $37\text{ }^{\circ}\text{C}$, and the cell density was then standardized to McFarland 0.5. Sterile Mueller-Hinton agar plates were inoculated with the standardized bacterial suspensions. Antibiotics known to be effective against the test microorganisms were used as a positive control. The antimicrobial activity was determined after 24 h of incubation by measuring the diameter (mm) of the inhibition zones (ZOI) around each disc.

RESULTS AND DISCUSSION**Morphological Properties of Modified Leather Samples with TiO₂**

The SEM micrographs in Fig. 1 show the surface of a treated and untreated leather samples. It can be seen that the control leather sample (C-control) has the characteristic fibrillar collagen structure. TiO₂ particles appear clearly on the treated surface: LT-1, LT-2, LT-3, indicating the success at their deposition. In addition, the pores are clearly visible on the surface of treated leather, suggesting that the surface coating is thin. Microscopic studies showed that TiO₂ particles were impregnated into the structure of the gelatin hydrogel at LT-1 and LT-2, and were distributed into small spherical particles, while in LT-3 the particles are on the surface of the film. TiO₂-NPs has polyhedral shape with rounded edges. It is worth mentioning that small bright spots can also be identified in LT-1 and LT-2 images, which probably are non-agglomerated. Greater aggregations were observed on the leather surface in LT-3, compared to the other variants studied.

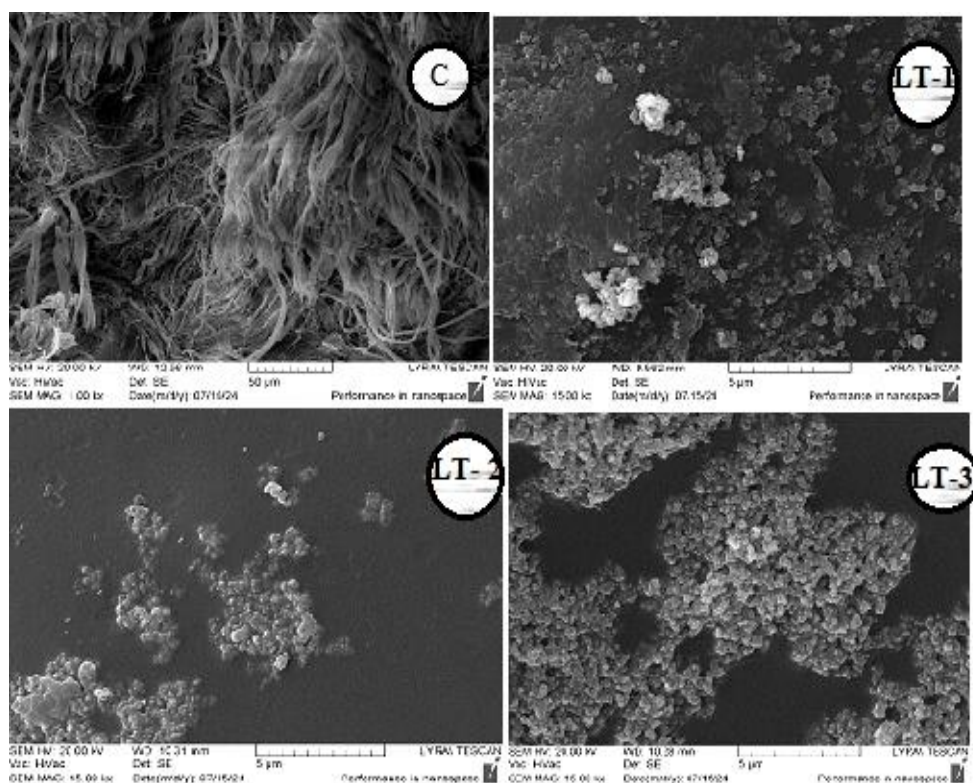


Figure 1. SEM images of the surface of the leather: C – control sample; LT-1, LT-2, LT-3 are modified leather samples

Determination of Water Vapour Permeability and Water Vapour Absorption

Table 1 shows how the various treatment options affect water vapour absorption and permeability. The samples LT-2 have reduced vapour permeability and water vapour adsorption, the samples LT-1 have increased vapour permeability and water vapour absorption as a result of the coating. The LT-3 provided the highest results, consequently these materials have the best absorbent properties and climate-balancing impact for shoe insoles.

Table 1. Vapour permeability and absorption tests of leather samples

Sample	Water vapour absorption W_1 mg/cm^2	Water vapour permeability W_3 $mg/(cm^2.h)$	Vapour permeability coefficient W_2 mg/cm^2
Le (Control)	2.17	17.41	141.45
LT-1	2.89	16.12	131.85
LT-2	1.47	13.20	107.07
LT-3	3.13	18.42	150.49

FTIR and UV-VIS Analysis

Fig. 2 represents the UV-VIS spectra of TiO_2 that showed the maximum absorption peak at 365 nm, which was a primary sign of the successful formation of TiO_2 -NPs (Anandgaonker *et al.*, 2019).

One possible explanation of the modified samples bactericidal effect is that it is due to the presence of Ti particles with a positive charge of titanium ions (Ti⁺) on the surface of the leather, which can cause oxidative stress and destruction of the bacterial cell wall.

CONCLUSIONS

A finish coating was successfully obtained by modifying leather samples with cross-linked gelatin containing TiO₂ particles, which particles were synthesized *in situ*. Three variants of synthesis of TiO₂ NPs were investigated. It was proved that LT-2 shows significant resistance against 4 types of bacteria. Consequently, these finishes can be very effectively used as protective antibacterial coatings for shoe leather materials, protecting the human foot from the effects of microorganisms.

Acknowledgment

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TEXTILE-BASED FOOTWEAR LININGS FUNCTIONALIZED WITH LAUREL OIL MICROPARTICLES: ANTIMICROBIAL PROTECTION

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The aim of this study is to optimize the production of microparticles to be used in antibacterial footwear linings and to characterize their usage and antibacterial properties. In this study, laurel essential oil was encapsulated with a chitosan shell using the spray drying method. The microparticles were applied to different lining materials using the dip-coating method. Scanning electron microscopy was used to characterize the microparticles and treated textile surfaces. The produced microparticles were characterized using scanning electron microscopy and particle size analysis. The finishing solution containing either laurel essential oil or laurel essential oil microparticles was applied to three different lining materials using the dip-coating method. After the finishing process, the lining materials were washed according to standards. The binding capacities of the microcapsules on different types of lining materials were determined using UV-Visible spectrophotometry, and their release properties were examined. Antibacterial tests were conducted to determine the antibacterial capacity of the final product and its effectiveness against washing.

Keywords: microencapsulation, laurel oil, footwear lining

INTRODUCTION

Today, consumers' interest in hygiene and active lifestyles is creating new challenges for the footwear industry. Footwear, with its close contact with the foot, provides an ideal breeding ground for bacteria and fungi due to the combination of moisture, warmth, nutrients from sweat, and oils from insoles (Orlita, 2004). Another important aspect of the modification of footwear components is the antimicrobial activity of the materials. Microbiological cleanliness inside the shoe is one of the factors that reduce the likelihood of dermatoses, ulcers, or other infections. Under actual usage conditions, footwear materials act as a barrier against the flow of moisture and temperature. Therefore, when water accumulates on the skin and lining materials, the temperature rises, and these specific conditions can affect the foot microbiome in the enclosed area around the foot (Serweta *et al.*, 2019).

Microencapsulation, a prevalent technique, allows for controlled release of active substances, safeguarding them from adverse reactions over extended periods (Yalçın, 2020). Encapsulation involves embedding active molecules within a polymer structure, presenting a challenge in selecting suitable components. Spray drying, a widely employed microencapsulation method in the industry, offers cost-effective production, simplicity, and the ability to produce microparticles with desirable properties. It serves as an important commercial process for encapsulating various substances like vitamins, minerals, flavorings, and enzymes, providing economic and effective protection (Naveena, 2020).

Essential oils are known for their antifungal, antibacterial, antioxidant, antiviral and medicinal properties (Snuossi *et al.*, 2016). When used directly, natural oils can stain textile products and cause allergic reactions on the skin. In addition, since the washing resistance is

limited, microencapsulation studies are being carried out to overcome these disadvantages. (Türkoğlu, 2020). Laurel essential oil (*Laurus nobilis* L.) is an important plant of the genus *Laurus*, one of the 40 species in the *Lauraceae* family, and grows naturally in many temperate and warm regions, especially in Mediterranean countries such as Türkiye, Greece, Portugal, Morocco or Mexico (Yilmaz, 2013). Commercial essential laurel oil has a fresh camphor-aromatic odor with a spicy note and is a pale yellow to pale olive green in colour (Alfonso, 2017). Laurel essential oil obtained from *nobilis* leaves has been used in many fields including perfumes, cosmetics, phytotherapy, spices and nutrition (Merghni, 2016). The laurel oil has antibacterial, anti-infectious, analgesic, antiviral, and antiseptic qualities (Nesrine, 2018).

In this study, the spray drying method, known for its high encapsulation efficiency and speed, was used to encapsulate laurel essential oil. The produced microparticles exhibited a highly spherical shape, a rare occurrence with this method, attributed to optimized emulsion preparation processes. The microparticles were characterized using techniques such as scanning electron microscopy (SEM), particle size measurement, and production yield evaluation. The selected optimum microparticle formulation and the non-encapsulated laurel oil solution were applied to three different lining materials using the dip-coating method. After the finishing process, the lining materials were washed according to standards. To determine the binding capacities of the microcapsules on different types of lining materials, quantification and release properties were examined using UV-Visible spectrophotometry. The antibacterial capacity of the final product and its effectiveness against washing were determined through antibacterial tests.

MATERIALS AND METHODS




Materials

In the microencapsulation process, chitosan with a molecular weight of 600,000-800,000 (Acros Organics) was used as the encapsulating polymer at a 1% (w/v) concentration. Laurel oil was donated by Ephesus Spice & Essential Oil, Türkiye. Acetic acid (glacial) was procured from Merck (Germany), while Tween 40 (Fisher Scientific, UK) and Span 20 (Merck Millipore Corporation, Spain) acted as surface-active agents. TANAPUR One, a self-crosslinking polyurethane-based binder, is manufactured by Tanatex Chemicals (Netherlands), and PERIWET ELR, a wetting agent donated by Dr. Petry (Germany). Sodium carboxymethyl cellulose (Sigma-Aldrich, Merck, Germany) served as a thickening agent and dispersant. All other auxiliary materials used in the study are of technical quality.

Textile Materials

Three different footwear lining materials were employed in the research and the properties are presented in Table 1.

Table 1. Characteristics of footwear lining materials

Name	Lining material	Weight (g/m ²)	No of Layers	Lining material properties		
				Outer Layer	Middle Layer	Bottom Layer
Lining 1		350	1	100% raw cotton woven fabric		
Lining 2		400	2	100% PET woven	-	100% cotton nonwoven
Lining 3		400 (L1: 200 L2: 160 L3: 40)	3	70% PA + 30% PET Weft Knitting	100% PET Weft Knitting	100% PET Weft Knitting
				Heat laminated		

Method

Microparticles were obtained by spray drying method in different proportions of chitosan and laurel essential oil. For this purpose, first of all, a 1% chitosan solution was prepared in a 2% acetic acid solution. Then, a determined amount of laurel essential oil was added to the prepared solution and homogenized using IKA T25 digital Ultra Turrax (Germany) (14,000 rpm for 20 minutes). The solutions prepared in different concentrations were sprayed from the 0.5 mm nozzle and fed into the desiccant chamber to form microparticles. It was prepared by using Unopex B 15 Mini Spray Dryer (Turkey). Chitosan: Laurel oil ratios have been studied as 1:1, 2:1 and 1:2 (w/w) ratios. In addition, various surfactant ratios have been tried to ensure emulsion stability, reduce capsule agglomeration, and improve particle size distribution. The optimal formulation was determined as the 1:1 Chitosan: Laurel oil formulation containing 20% surfactant. Spray drying conditions: the inlet temperature was 170 °C, the outlet temperature was 105 °C, and the pump speed was 5 mL/min.

Table 2. Application parameters

	Binder (g/L)	Capsule (g/L)	Laurel oil (g/L)
Solution 1	-	-	10
Solution 2	-	-	20
Solution 3	5	10 L ₅	-
Solution 4	10	20 L ₅	-
Solution 5	5	10 Blank	-

Various methods have been considered to characterize microparticles. To calculate the production efficiency, the amount of microparticles obtained was proportioned as a percentage of the theoretical amount of material added to the process solution. Scanning electron microscope (SEM) images were taken to examine the capsule morphology, which indicates ideal capsule formation. In addition, the size distribution analysis of the microparticles containing and not containing Laurel oil was performed. The optimum microparticle formulation was applied by immersion dip-coating method to different textile structures used as footwear linings. The composition of the process solutions is given in Table

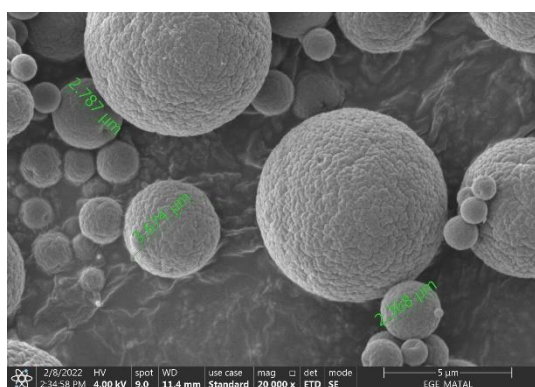
2. 1% CMC and 1% wetting were added to the finishing solutions. After the finishing process, the textile-based footwear linings were dried at 80 °C and the fixation process was applied at 120 °C. The footwear lining materials were washed according to ISO 105-C06:2010 standard 3 times in 30 °C for 30 min at Linitest Washing Machine with IEC Non-Phosphate Reference Detergent (A).

A standard line was plotted using a UV-Vis spectrophotometer to determine the laurel oil trapped in textile materials. Laurel oil was dissolved in various concentrations in n-hexane and the absorbance values of the prepared solutions were determined using a UV-Vis Spectrophotometer at a wavelength of 268 nm. The declaration of the standard line was calculated as $y=(5.62 \times 10^{-2})x+(8.96 \times 10^{-3})$, R^2 value was calculated as 0.99. In order to determine the adhesion capacities of laurel oil in different types of linings, the unwashed and three consecutive washed lining fabrics were extracted using n-hexane. After the solutions were filtered with a 0.45 µm membrane filter, measurements were made with a UV-Vis Spectrophotometer.

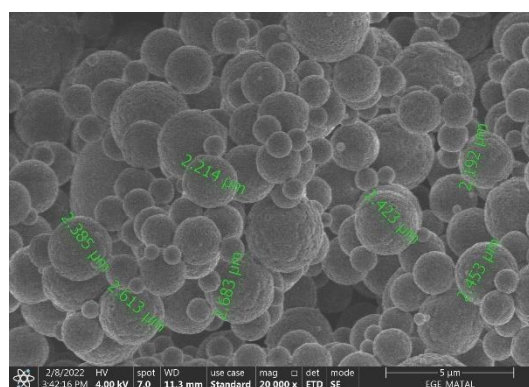
The antibacterial properties of footwear lining materials were evaluated by agar disk diffusional method. The antibacterial and antifungal properties of textile-based lining materials containing laurel oil have been determined for *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 90028. The textile samples were placed in petri dishes and incubated at 37 °C for 24 hours. Antimicrobial activity was determined by measuring the zone diameters formed around the samples after incubation.

RESULTS

The production efficiency of different microparticles obtained by spraying method ranges from 10 to 42. Among these formulations, especially the formulation containing 1:1 ratio of Laurel essential oil with chitosan and 20% surfactant exhibited significant advantages such as a particle size ranges from 5 to 100 µm, more homogeneous particle distribution and higher production efficiency compared to other microparticles. SEM images of microparticles are given in Figure 1. When the images were examined, it was seen that the shapes of the chitosan microparticles containing laurel oil were spherical, and the capsule wall was close to smooth.



Chitosan Microparticles



Chitosan Microparticles with Laurel Oil

Figure 1. SEM images of microparticles

Color measurements were made using the CIELAB color space and significant color changes were detected in the footwear linings according to the ΔE values. The L^* (lightness) values of all linings have generally decreased. It was determined that the total color change

(ΔE) of only laurel oil applied footwear lining materials was higher than capsule applied footwear lining materials. While the b^* value increased in the direction of yellowness, textile linings containing microparticles usually exhibited the orange color of chitosan. There was no significant effect of capsule color on the L^* . Lining 3, containing 20 g/L laurel oil (Solution 2), showed the greatest loss of lightness value. Redness (a^*) was towards greenness, while the b^* value increased in all solutions to the yellowness direction.

Table 3. Values of research lining materials

Sample	CIE Lab Values					ΔE
	L^*	a^*	b^*	C^*	H^*	
Lining 1 -	82.061	2.126	13.134	13.305	80.808	
Lining 1 Solution 1	78.354	2.836	15.404	15.663	79.569	4.404
Lining 1 Solution 2	79.558	2.620	14.125	14.366	79.493	2.737
Lining 1 Solution 3	82.076	2.081	11.276	11.466	79.543	1.859
Lining 1 Solution 4	81.688	1.756	13.072	13.189	82.349	0.529
Lining 1 Solution 5	79.941	2.273	14.177	14.359	80.892	2.367
Lining 2 -	29.708	2.849	8.012	8.503	70.425	
Lining 2 Solution 1	27.370	2.635	7.882	8.311	71.514	2.351
Lining 2 Solution 2	27.293	2.529	8.056	8.444	72.574	2.437
Lining 2 Solution 3	28.331	2.192	6.932	7.270	72.454	1.869
Lining 2 Solution 4	27.491	2.489	7.486	7.889	71.611	2.307
Lining 2 Solution 5	26.177	2.314	7.428	7.780	72.695	3.619
Lining 3 -	52.432	-2.385	1.913	3.058	51.263	
Lining 3 Solution 1	51.048	-2.246	2.148	3.107	46.275	1.411
Lining 3 Solution 2	50.268	-2.162	2.576	3.363	40.001	2.274
Lining 3 Solution 3	51.786	-1.885	3.069	3.602	31.560	1.416
Lining 3 Solution 4	52.052	-1.981	3.964	4.431	26.550	2.125
Lining 3 Solution 5	50.345	-1.966	2.044	2.836	43.898	2.133

Table 4. The loading efficiency of laurel oil in different types of fabrics

	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5
Lining 1	4.47 ± 0.10	3.66 ± 1.01	4.81 ± 0.21	3.11 ± 0.18	0.0 ± 0.0
Lining 1 - 3 Washes	0.958 ± 0.014	0.786 ± 0.005	0.514 ± 0.010	0.739 ± 0.010	0.0 ± 0.0
Lining 2	6.29 ± 0.67	3.50 ± 0.39	4.98 ± 0.58	3.95 ± 0.03	0.0 ± 0.0
Lining 2 - 3 Washes	2.417 ± 0.010	2.102 ± 0.005	1.237 ± 0.015	1.614 ± 0.010	0.0 ± 0.0
Lining 3	4.03 ± 0.29	5.97 ± 0.52	6.33 ± 1.01	5.12 ± 1.61	0.0 ± 0.0
Lining 3 - 3 Washes	1.142 ± 0.010	0.970 ± 0.000	0.902 ± 0.014	2.565 ± 0.005	0.0 ± 0.0

The loading efficiency of laurel oil in linings with Solution 3 is higher than in linings treated with other solutions. After three washes, the essential oil ratio has decreased, but there is still essential oil content in the fabric. All the linings can retain the essential oil in their structure. No significant difference was observed between fabrics treated with essential oil and those treated with microcapsules.

The antimicrobial test results obtained by the disk diffusion method showed that the application of laurel oil on the medium exhibited antibacterial activity against all test

organisms, with the largest inhibition zones observed on *C. albicans*, confirming the oil's strong antifungal properties. These findings highlight the potential of laurel oil as an effective antimicrobial agent in various applications. However, no inhibitory effect on the growth of microorganisms was observed for chitosan capsules containing laurel oil and chitosan capsules without laurel oil against standard bacterial and yeast strains.

CONCLUSIONS

This study aims to develop various textile-based footwear linings using laurel oil-containing microparticles. In the production of capsules, chitosan polymer with antibacterial properties was used as shell material. Spray drying has been selected as a suitable method for industry and has been preferred in capsule production. Laurel oil has been selected for the control of bad odor of microbial origin that may occur in footwear due to its antimicrobial properties and pleasant smell. Laurel oil is strongly antibacterial on all test organisms, and especially it has shown significant antifungal effects on *Candida albicans*. The products developed are suitable for industrial production and laurel oil has the potential to be used as an effective antimicrobial agent.

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REVIEW OF TANNINS CURRENTLY USED IN THE LEATHER INDUSTRY. PART 1: HYDROLYSABLE TANNINS

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Tannins are water-soluble phenolic substances obtained from plants by various extraction methods. Tannins can be found in almost every plant. However, tannin content varies from plant to plant. Even different parts of the same plant may contain different amounts of tannin. Tannins can be found in various parts of plants such as leaves, roots, fruits, wood, galls and bark. Tannins have many different kinds of uses in the food, cosmetics, painting, pharmaceutical, wood adhesive, and other industries due to their antibacterial, antioxidant, and other qualities. On the other hand, they have long been used in leather production due to their ability to chemically react with the leather protein collagen. Dicotyledonous plants are generally used in leather tanning due to their rich tannin content. According to a common classification, tannins are divided into three classes: Hydrolyzable, Condensed and Complex tannins. Of these, hydrolyzable tannins are hydrolyzed in the presence of various chemicals (dilute bases, dilute acids and enzymes) and are divided into 2 subclasses as gallotannins and ellagic tannins. This review includes the general chemical structures of hydrolyzable tannins. In addition, hydrolyzable plant tannins used in leather production (sumac, chestnut, myrobalan, valonia and tara) and the properties they impart to the leather are explained.

Keywords: leather, tannins, hydrolysable tannins

INTRODUCTION

Tannins are phenolic compounds with the molecular formula C_6H_5OH that are present in many different parts of higher plants, including the bark, leaf, stem, fruit, root, and wood (Redwood, 2020). Tannins participate in the defense mechanisms of plants, protecting them from insects and microorganisms. Since tannins are typically found in plant growth regions like secondary phloem, xylem, and the layer in root tissue between the cortex and epidermis, it is also believed that they can help regulate the growth of these tissues (Pizzi *et al.*, 2024).

Tannins can chemically interact with proteins to form complexes (Pizzi, 2019). They are derived from plants using a variety of extraction techniques or are directly ground into powder for have been used for centuries in the tanning and retanning of leather. Besides, tannins can be used as dyes because they can give color to the leather. In addition, they can eliminate free formaldehyde that occurs on the leather due to release by resins and/or other chemicals (Bayramoğlu, 2013; Bayramoğlu *et al.*, 2008; Çolak *et al.*, 2004; Mirzamuratova *et al.*, 2024a). On the other hand, tannins are a group of secondary metabolite polyphenols known as natural antioxidants and exhibit UV protective properties and so they can be inhibitors of lipid peroxidation and prevent Cr(III) from converting to Cr(VI) (Mirzamuratova *et al.*, 2024b; Ismayati *et al.*, 2024).

These properties of tannins show that they are highly functional materials and make them industrially attractive. In this study, the general chemical structures of hydrolysable tannins and hydrolysable vegetable tannins commonly used in the leather industry are mentioned.

Chemical Structure of Tannins

According to a generally accepted classification, tannins are divided into three parts as “hydrolysable”, “condensed” and “complex”. Hydrolysable tannins are also classified as two

subunits as “gallotannins” and “ellagic tannins”. Gallotannins are polyesters of glucose and gallic acid that are commonly found in nature and when they are hydrolyzed, gallic acid is released (Molnar *et al.*, 2024; Okuda & Ito, 2011; Porter, 1992; Hemingway & Karchesy, 2012; Seigler, 1998). Ellagic tannins are characterized by a glucose center esterified with at least one unit of hexahydroxydiphenyl acid, formed by the oxidative bonding of two units of gallic acid (Mavlyanov *et al.*, 2001; Auad *et al.*, 2020; Schofield *et al.*, 2001; Porter, 1989; Mueller, 2001; Ekambaram *et al.*, 2016; Romani *et al.*, 2006).

Hydrolysable Tannins

Hydrolysable tannins are a class of compounds with polyols as core and phenolic carboxylic acids connected by ester bonds, C₆–C₁ type polyphenols. Therefore, they are susceptible to hydrolysis of ester bonds in the presence of dilute bases, dilute acids or enzymes (Guo *et al.*, 2024). The molecular weights of hydrolysable tannins range from simple glycogaline (MW of 332 Da) to pentameric ellagitannins, and their MWs are above 5000 Daltons (Da) (Molnar *et al.*, 2024).

Gallotannins

It is composed of a gallic acid and a polyol (in most cases D-glucose) whose hydroxyl functions can be replaced by one or more gallic groups. Gallotannins are found in many plants as TGG (2,3,4,6-tetra-O-galloyl-D glucopyranose) and β -PGG (1,2,3,4,6-penta-O-galloyl- β -D glucopyranose). They serve as basic compounds for the biosynthesis of hydrolysable phenolic compounds (Ben Aziz *et al.*, 2024).

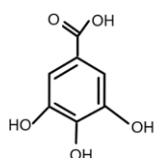


Figure 1. Gallic acid molecule

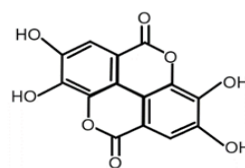


Figure 2. Ellagic acid molecule

Ellagic Tannins

Ellagic tannins are biosynthetic products of gallotannins. The most important acyl group, Hexahydroxydiphenic (HHDP) group, is produced by oxidative coupling between two galloyl groups. Some other ellagic tannin acyl groups are also produced by oxidative coupling of galloyl and HHDP groups (Liu, 2024).

Because there are so many phenolic hydroxyl groups in close proximity to one another, they are very astringent; in this sense, gallotannins are more astringent than ellagitannins. The acidity of the carboxylic acid groups in the hydrolysable tannins-for example, the pH values of valonia, sumac and chestnut solutions are 3.2, 3.7-4.2, and 2.6-2.8, respectively-contributes to their reactivity. As a result, the less acidic tannins act as self-buffers (Covington, 2009).

Leathers tanned using these tannins shrink at a temperature between 75 and 80 °C. Given that this is a defining characteristic of hydrolyzed tannin (Covington, 2009).

Hydrolysable Tannins Widely Used in Leather Production

The interaction between tannins and proteins is widely used in leather production. Tannic acid (TA), which is abundant in hydroxyl groups that can oxidize to quinones, improves film properties by establishing covalent bonds (C=N or C-N) with amino acid residues of proteins (Choi & Kim, 2020). In contrast, under acidic or neutral pH conditions,

reversible non-covalent bonds can be formed between proteins and polyphenols, including hydrogen bonds (Figure 3), hydrophobic interactions, and electrostatic interactions. In particular, oxidized TA has been reported to improve the mechanical properties of collagen films (Zhang *et al.*, 2010; Zhao *et al.*, 2024).

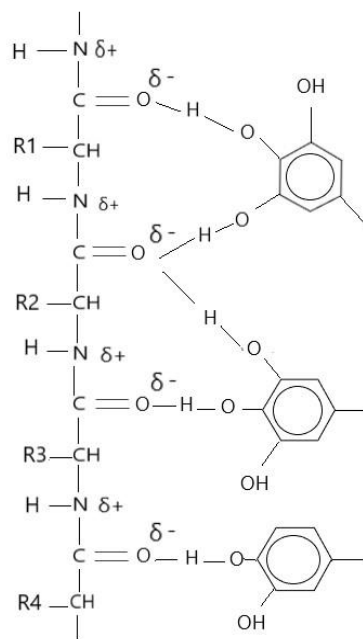


Figure 3. H bonds between the polypeptide chain of collagen and polyphenols (Covington, 2009)

Tannins form hydrogen and covalent bonds with functional groups (e.g. COOH, $-\text{NH}_2$) of the leather protein collagen. However, these bonds only occur if the tannin molecules are large enough to bind to neighboring collagen chains and contain enough phenol to form cross-links in several places. The vegetable tanning process consists of two stages: penetration and fixation. Penetration refers to the diffusion of tannins into the leather, while fixation ensures that the penetrated tannins bind to the collagen, which forms the stable material. In this process, different components such as temperature, pH, mechanical effects and particle size come into play to obtain the desired products (Tasnim *et al.*, 2024; Mia *et al.*, 2024). Sumac, chestnut, myrobalan, valonia and tara are hydrolysable tannins that are generally used commercially in terms of tanning efficiency, quality, performance and extraction efficiency.

Table 1. Hydrolysable tannins commonly used in leather production and their properties (Redwood, 2020)

Plant	Source of tannin	Tannin content (%)	Non-tans content (%)
Sumac (<i>Rhus coriaria</i> / <i>Rhus cotinus</i>)	Leaves	22-35	14-15
Chestnut (<i>Castanea sativa</i>)	Wood	5-15	16-17
Myrobalan (<i>Terminalia chebula</i>)	Nuts	25-48	14-17
Valonia (<i>Quercus Aegilops</i>)	Fruit	4-10	1-2
Tara (<i>Caesalpinia spinosa</i>)	Pods	30-35	1-2

Sumac

Sumac tannins are produced from the leaves of shrubs belonging to the *Rhus coriaria* genus, which is native to Southern Europe and the Mediterranean (Türkiye, KKTC (Turkish Republic of Northern Cyprus), Greece, Bulgaria, Croatia, Montenegro, Italy). Sumac tannins belong to the gallotannin family of hydrolyzed tannins. They are highly sensitive to heat compared to other tanning agents and break down into gallic acid when exposed to heat. Sumac leaves contain approximately 30% tannin. They also contain gallic acid, glucose, chlorophyll and inorganic salts. Leather tanned with sumac tannin is light and soft. It has a pleasant touch and a smooth texture. This type of leather can be dyed with both anionic and basic dyes. It has high light, oxidation and sweat resistance (Shabbir, 2012; Falcão & Araújo, 2011; Plavan *et al.*, 2010).

Chestnut

Castanea sativa belongs to the *Fagaceae* family and is among the most common chestnut species (De Vasconcelos *et al.*, 2007). The main functional units in the wood, bark and fruit of the chestnut tree (*Castanea sativa* L.) are hydrolyzed tannins such as gallic acid and ellagic acid (Buyse *et al.*, 2021). In addition to these main units, subunits such as castalin, vescalagin, castalagin, vescalagin, kurigalin, 5-O-galloylhamamelose, (3',5' dimethoxy-4'-hydroxyphenol)-1-O- β -D-(6-O-galloyl) glucose, chestanin and acutissimin A have been recorded in chestnut extract. Chestnut extract is commercially used in animal feed, leather production and the food industry (wine and alcoholic beverages) (Comandini *et al.*, 2014).

The standard extract has a pH of 3.5; inorganic salts and hydroxides are added to create a "sweetened" extract with a pH of 4.5. Softer leather is produced by the less astringent nature of the sweetened extract. It can be used alone or in conjunction with syntans to retain chrome leather and tanning sole leather. These combinations result in leather that is lighter in color and has superior tanning qualities (Krisper *et al.*, 1992). Sweetened chestnut penetrates the leather better. Leathers tanned with sweetened chestnut are more yellow than those tanned with normal extract. On the other hand, leathers produced with normal chestnut extract are firmer, fuller, harder, and more resistant to water and abrasion (Yahia *et al.*, 2019).

Myrobalan

Myrobalan is the unripe fruit of the *Terminalia chebula* tree. It is in the hydrolyzed tannins class. Its structure contains compounds that are esters of glucose such as phenol carboxylic acid and 1,2,3,4,6-pentagalloyl glucose. It contains around 35-40% tannin. Its tanning effect is light, it is generally used as an additive in vegetable tanning. Leathers produced with Myrobalan are light in color (Sivakumar *et al.*, 2018).

Valonia

Oak, which belongs to the *Fagaceae* family, is common in temperate climates in some parts of Europe, Asia and North Africa. Its fruits are called acorns and grow in groups of 2-5 on a stem. Known as a high-calorie (339 kcal/100 g) nutrient, acorn seeds are rich in vitamin C, magnesium and calcium. Other active substances found in acorns are quercitanic acid, ellagic and gallic acid, tannin, quercin, fluoroglycine, pectic substances, resins, calcium oxalate, pentadigalloylglucose, cyclogallifolic acid and carbohydrates. These substances provide astringent, antiseptic, anti-inflammatory and antioxidant properties to acorns (Simion *et al.*, 2023).

Oak trees of the *Quercus* species, which have different varieties, are generally used in furniture making and construction materials. Tannins can be obtained from wood, bark, gall

and acorn. Depending on the species, there is 50-70% tannin in galls, 10-20% in shells, and approximately 10% in acorns (Baytop 1999; Bayramoğlu, 2012). The tanning material obtained from acorns is called 'Valex'. The acorns of the *Quercus aegilops* tree, also known as the Turkish oak, which grows abundantly in Türkiye, Greece and neighboring countries, are usually collected in August and dried in domes. It is widely used in leather production in Austria, Germany and France, allowing faster, harder, tighter, heavier and waterproof leather production. The main components of valex are ellagitannins, which are castalagin, vescalagin and pentagalloylglucose. This material is often used mixed with ground oak bark in the production of shoe soles to increase quality and durability (Falcão & Araújo, 2018).

Tara

Tara (*Caesalpinia spinosa* (Molina) Kuntze), which is widespread in South America and Africa, is found in the form of a small legume tree or thorny shrub. The world's largest producer and exporter (about 80% of production) is Peru, and production takes place in different semi-arid regions of this country. It contains gallotannic tannins, and is grown for leather production and food use. Pre-Inca civilizations, whose origins are based on the Andean Region, used the fruits of the tara tree to produce dyes for textiles and ceramics, tanning agents for leather, and medicine. Due to the light color and light resistance of the leathers tanned with tara, it is widely used especially in automobile upholstery (Ibieta & Penarrieta, 2021; Castel *et al.*, 2013).

What distinguishes tara tannin from other commercial tannins is that it is not an extract but a finely ground powder obtained from tara pods. Therefore, it has a high amount of insoluble matter (Gaidău *et al.*, 2014). The tannin content varies between 30-35%. Tara gives fullness and softness to the leather. The resistance of the leathers tanned with tara to tear strength is higher than those obtained with other vegetable tannins (Aravindhan *et al.*, 2015).

CONCLUSION

Various plant tannins are used in leather production. These tannins are divided into 3 classes: hydrolyzed, condensed and complex. Hydrolyzed tannins are also divided into 2 subclasses: gallotannin and ellagic tannins. They are susceptible to hydrolysis of ester bonds in the presence of dilute bases, dilute acids or enzymes. They have the ability to react with proteins. Thus, they have been used for leather tanning for many years. For this purpose, the most commonly used hydrolyzable tannins in the market are sumac, chestnut, myrobalan and tara. Tannin from sumac leaves gives the leather smoothness. In addition to chestnut extract, there are also chemically treated chestnuts called sweetened chestnuts on the market. The leather is better penetrated by sweetened chestnut. When using sweetened chestnut extract instead of regular extract, leathers get a deeper yellow color. However, leather made using regular chestnut extract is harder, fuller, firmer, and more resilient to abrasion and water. Myrobalan is often used in combination with other tanning agents. Valonia makes it possible to produce leather that is waterproof, heavier, tighter, faster, and harder. Tara is applied directly, without the need for other tanning agents, by powdering the pods. Leathers tanned with tara are light colored and have high light fastness. Therefore, tara is popular in the creation of light-colored leather and also suitable for pastel shades. In general, when vegetable tanning agents are used alone, desired quality leathers cannot be obtained. The reasons for this are; they give color to the leather, have a low shrinkage temperature and are hydrophilic. Therefore, they are open to research in terms of improving these properties.

In recent years, sustainable production has gained importance in the leather industry. Vegetable tanning agents are natural products and are very easy to break down in nature.

Leather processed with vegetable tanning agents has a special interest for ecological leather production. In addition, plant tannin production provides employment opportunities for many people. Tannins can be used in different places and for different purposes in leather processing. The advantages of vegetable tanning agents for leather can also be presented as a separate article.

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REVIEW OF TANNINS CURRENTLY USED IN THE LEATHER INDUSTRY. PART 2: CONDENSED TANNINS

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Tannins are macromolecules found in different proportions in the structures of various plants and are thought to be their defense mechanisms. They have been used in the tanning and retanning processes in leather production, and their use for this purpose dates back to very old times. They are used together or with other tanning agents in the production of shoemaking, saddlery, stout leather, etc. They have become more popular recently, because they can provide the opportunity to produce metal-free leather. Therefore, they can be categorized as green, environmentally friendly materials. In addition, industrial production of tannins is also common due to the relative practicality of production and easy availability of the raw material. They are divided into three main classes—hydrolyzable, condensed, and complex (different combinations of hydrolyzed and condensed tannin monomers). This study includes condensed tannins, which are secondary metabolites in plants consisting of various mixtures of oligomeric and polymeric substances called flavan-3-ols. The chemical structures of condensed tannins and condensed tannins such as mimosa, quebracho, gambir and mangrove used in leather production and the properties they impart to the leather are explained.

Keywords: leather, tannins, condensed tannins

INTRODUCTION

The term “tannin” was first used to refer to the compounds found in vegetable extracts that turn animal hide/skin into leather. These compounds are found in plant extracts as polyphenols with different molecular sizes and complexities. All of the chemical characteristics of tannin are also present in many nonpolyphenolic substances found in plants; however, no research has been done on their ability to leather hides (Harborne, 1967; Chung *et al.*, 1998).

Vegetable tannins are water-soluble phenolic compounds with a molecular weight of 500–3000 Da, according to Swain and Bate-Smith. These polyphenols have the ability to form cross-linkages with proteins and other macromolecules because they have a high number of hydroxyl or other functional groups (1 to 2 per 100 Da). Phenolic compounds with low molecular weights (less than 500 Da) and high molecular weights (more than 3000 Da) cannot be used as tanning agents. Additionally, proteins, gelatin, and alkaloids can combine with vegetable tannins to form precipitation (Swain & Bate-Smith, 1962; Chung *et al.*, 1998).

In this review, the structures of condensed tannins and the most commonly used condensed tannins in leather production such as mimosa, quebracho, mangrove and gambir are explained.

Chemical Structure of Condensed Tannins

Condensed tannins, also referred to as “proanthocyanidins,” are made up of oligomers or polymers of flavan-3-ol (Figure 1), a subclass of flavonoids, at its fundamental structure (Mavlyanov *et al.*, 2001; Auad *et al.*, 2020; Schofield *et al.*, 2001; Porter, 1989; Mueller, 2001; Ekambaram *et al.*, 2016; Romani *et al.*, 2006).

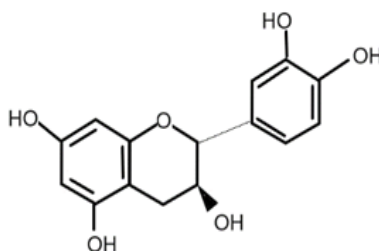


Figure 1. Chemical structure of flavan-3-ol molecule

Condensed tannins are secondary metabolites in plants. They have subunits such as catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin gallate (GCG) and epigallocatechin gallate (EGCG). The chemical structures of these molecules are as shown in Figure 2. Studies have shown that its biological activity is related to the presence of galloyl and gallic moieties in flavan-3-ol units. EGCG contains both galloyl and gallic moieties in flavan-3-ol units, which makes EGCG the strongest ability to inhibit bacteria among condensed tannins (Huang *et al.*, 2024)

Compared to hydrolysable tannins, condensed tannins have a broader molecular weight range of 500–2000 Da. Additionally, according to Hoque *et al.* (2024), they can react with aldehydes to create polymeric materials.

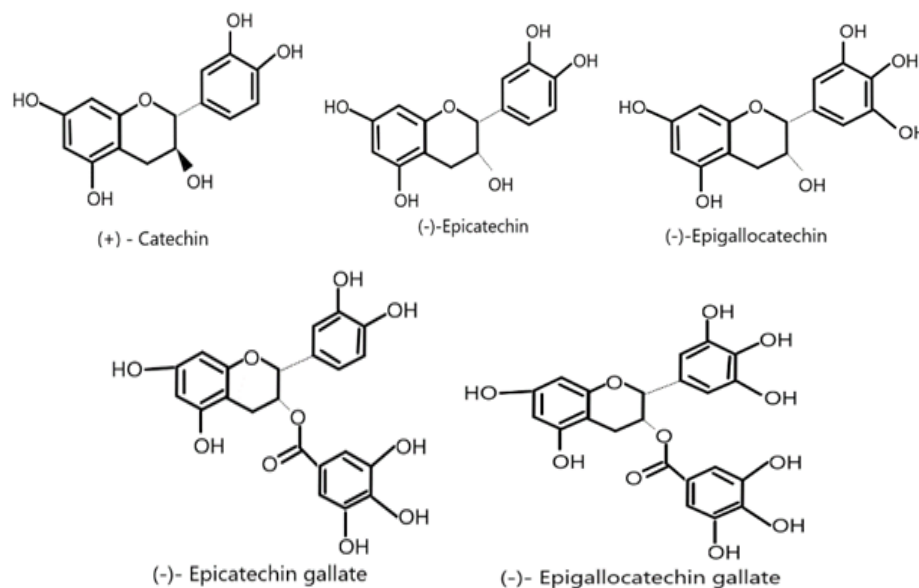


Figure 2. Condensed tannin subunits

Condensed tannins give a greenish black stain when they chemically react with iron, while hydrolyzed tannins give a bluish black stain. Condensed tannins are more astringent than hydrolysable tannins and their structures are more complex (Redwood, 2020).

Condensed tanning agents are not decomposed by enzymes and when heated they give completely pyrocatechin. Under the influence of oxidizing agents or when treated with strong acids they form high molecular weight and insoluble phlobaphenes by condensation. Their precipitation with formaldehyde, hydrochloric acid or bromine water is characteristic (Sarı, 1993).

Reddish-brown leathers with condensed tannins have a tendency to darken in the presence of light. Additionally, these leathers have a propensity to absorb air pollutants, the

most harmful of which is sulfur dioxide (SO₂), which encourages the hydrolysis of collagen acid (Falcão & Araújo, 2011).

The majority of the bonds formed by both hydrolysable and condensed tanning agents with the leather are based on H bridges. The phenolic OH groups of the tanning agents act as H atom donors (Sari, 1993).

The leather that has been tanned using condensed tannin(s) has a shrinkage temperature between 80 and 85°C. Similar to hydrolysable tannins, the Ts is characteristic, meaning that an observed shrinkage temperature greater than 80°C is highly suggestive of the use of condensed tannins. It is interesting to note how different tanning processes with different tannin types produce different results. This variation must arise from a different type of reaction, i.e., the shrinkage temperature can be measured depending on how covalency contributes to similar reactions (Covington, 2009).

Condensed Tannins Widely Used in Leather Production

Tannins attach themselves to the functional groups (COOH, -NH₂) of the leather protein collagen through hydrogen and covalent bonds. But these connections only happen when the tannin molecules are big enough to attach to nearby collagen chains and have enough phenol in them to create multiple cross-links. There are two steps in the vegetable tanning process: penetration and fixation. The diffusion of tannins into the leather is referred to as penetration, and the binding of the penetrated tannins to the collagen, which creates the stable material, is known as fixation. To produce the desired products, this process involves a number of variables, including temperature, pH, mechanical effects, and particle size (Tasnim *et al.*, 2024; Mia *et al.*, 2024). In terms of tanning efficiency, quality, performance, and extraction efficiency, condensed tannins from mimosa, quebracho, gambir and mangrove (Table 1) are typically utilized in commercial applications.

Table 1. Condensed tannins commonly used in leather production and their properties (Redwood, 2020)

Plant	Source of tannin	Tannin content (%)	Non-tans content (%)
Mimosa (<i>Acacia decurrens/mearnsii</i>)	Bark	22-48	7-8
Quebracho (<i>Quebrachia lorentzii</i> , <i>Schinopsis balansae/lorentzii</i>)	Wood	14-26	1-2
Gambir (<i>Uncaria gambir</i>)	Leaves	20-50	1-2
Mangrove (<i>Avicennia germinans</i>)	Bark, Leaves, Fruit	16-50	9-15

Mimosa

Certain acacia tree cultivars are marketed as mimosa extract because of their high tannin content. Among these, the invasive *Acacia mearnsii*, or Black Mimosa, is grown in Australia, America, and South Africa. It is a significant plantation for the wood chips industry and tannin production. It is used commercially to obtain tannin for leather tanning owing to its affinity for binding proteins (Xiong *et al.*, 2016; Featherstone, 2024).

However, 68% of the *Acacia mearnsii* bark extract by weight consists of proanthocyanidins (PAC) and is an important industrial source for the production of this substance. In addition, proanthocyanidin oligomers in this extract contain 5-deoxy extender units that make interflavanyl bonds resistant to acid-catalyzed hydrolysis (Mia *et al.*, 2024). It is also valued for its health-related applications, including antioxidant and antitumor effects, hair growth promotion, antihypertension and antiallergic properties (Xiong *et al.*, 2016).

Tekmeledhin *et al.* used commercial mimosa extract as a control group in their study investigating the usability of *Cassia singueana* bark extract in leather tanning. In this study, the pH of the mimosa extract was measured as 5.3. In addition, some properties of the leathers tanned with mimosa in the findings of this study are as indicated in the Table 2 (Tekmeledhin *et al.*, 2023).

Table 2. Some properties of mimosa tanned leathers (Tekmeledhin *et al.*, 2023)

	Values	Standard values	References
Tensile strength (N/mm ²)	14,8	20	IULTCS/IUP 6 (2006)
Elongation at break (%)	38,7	>%40	IULTCS/IUP 6 (2006)
Tearing strength (N/mm)	22,5	20	IULTCS/IUP 8(2016)
Shrinkage temperature (°C)	80	> 75	IULTCS/IUP 16 (2015)

Quebracho

The best species of Quebracho, which is a tree native to South America, in terms of tannin are found in the Gran Chaco region in northern Argentina and in Uruguay. Both the bark and heartwood contain tannin, and the average tannin content was determined as 14-26% in the heartwood; 22-45% in the bark. However, Quebracho extract is usually produced from the wood part. The parts left over from the construction of construction materials and railway sleepers are used for the production of tannin extract. Quebracho wood, which contains high amounts of condensed tannin, meets approximately one third of the world's total tanning material demand (Yahia *et al.*, 2019; Erkan and Deniz, 2016). The most important species in terms of tannin are *Quebracho colorado* (*Schinopsis balansae*) and *Quebracho maco* (*Schinopsis lorentzii*). Quebracho extract is soluble in hot water and can be used in the final stage of leather production in this form. When sulfite is used in the production of Quebracho, sulfite Quebracho is obtained. Sulphite Quebracho is soluble in cold water. Sulphite Quebracho has a higher penetration rate into the skin than raw Quebracho extract, less sludge formation rate and the color of the leathers produced is lighter (Yahia *et al.*, 2019).

Yahia *et al.* used Quebracho as a control group in their study investigating the effects of the combination of Quebracho and Oxazolidine on leather tanning and obtained some properties of the leathers tanned with Quebracho as shown in the Table (Yahia *et al.*, 2019).

Table 3. Some properties of quebracho tanned leathers (Yahia *et al.*, 2019)

	Values	Standard values	References
Tensile strength (N/mm ²)	215±3	200	IULTCS/IUP 6 (2006)
Elongation at break (%)	55±1.5	40-65	IULTCS/IUP 6 (2006)
Tearing strength (N/mm)	50±0.8	30	IULTCS/IUP 8(2016)
Shrinkage temperature (°C)	84±0.5	>75	IULTCS/IUP 16 (2015)

Gambir

Gambir (*Uncaria gambir*) is an annual plant widely used for industrial and pharmaceutical purposes and grows in tropical regions (Bancin, 2021). Gambir (*Uncaria gambier*) plants can be obtained by boiling the leaves and branches and then pressing to

extract the gum. Depending on the percentage of tannin content, gambir has various types and is included in the class of condensed tannins (Griyanitasari *et al.*, 2020). Leathers tanned with gambir are soft, smooth and light colored (Andini *et al.*, 2023).

Mangrove

It is found in forests in Southeast Asian countries. The leaves, fruits and bark of mangrove (*Rhizophora mucronata*), which is among the condensed tanning materials, are used to obtain tannin. The bark contains approximately 26% tannin (Pancapalaga and Nitiharjo, 2019).

Dewi *et al.* investigated the effects of the combination of chromium and mangrove on leather tanning. When they used 8% mangrove alone in tanning, they obtained leathers with a shrinkage temperature of $55.23\text{ }^{\circ}\text{C} \pm 0.49$. When they used 4% basic chromium sulfate and 4% mangrove together, they measured the shrinkage temperature as $83.03\text{ }^{\circ}\text{C} \pm 1.86$ (Dewi *et al.*, 2021). As can be understood from this, the use of mangrove alone is insufficient for the leather tanning process.

CONCLUSION

The tannins found in various plants are used to make leather. Three classes of tannins are identified: hydrolyzed, condensed, and complex. They are capable of interacting with proteins. As a result, they have long been employed in the tanning of leather. The condensed tannins that are most frequently utilized for this purpose on the market are mimosa, quebracho mangrove, and gambir. They are used in the tanning and retanning processes and in the production of leathers such as shoemaking, saddlery and stout leather. Among all vegetable tanning agents, mimosa and quebracho stand out commercially in terms of the properties they provide to the leather. However, vegetable tanning agents have some disadvantages such as being hydrophilic, giving color to the leather and having a low shrinkage temperature. Therefore, they are usually used together with each other or other tanning agents.

One of the main issues of sustainable leather production is the rapid biodegradation of the finished product when it is thrown away after its useful life. The most important microorganisms of recycling in nature are fungi. It has been observed that various fungi grow easily on vegetable tanned leather. In studies conducted by inoculating fungi onto vegetable tanned leather and chrome tanned leather, it was observed that fungi developed later in chrome leather. While many fungi grow later and slower on the leather due to the oligodynamic effect of chromium, they are not compatible with fungi. However, studies generally show that molds can easily grow on the leather (Bayramoğlu *et al.*, 2017, Bayramoğlu & Maltaş, 2011). While fungus formation during production is a disadvantage in vegetable tanned leather, it is an advantage to ensure rapid fungus formation and decomposition of the leather into its minerals at the end of its life. For sustainable leather production, leather that has expired and is thrown into the environment must quickly decompose and mineralize without harming the nature. In this context, the mineralization of waste leather by fungi in leather produced with vegetable tanning agents is extremely important for sustainable leather production.

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SHORT REVIEW ON KERATIN SYNTHESIS AND ITS APPLICATION AS A BIO REINFORCEMENT AND FLAME RETARDANT AGENT IN POLYMER COMPOSITES

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Recently, attention to the environment has led to the development of a circular economy by stimulating some initiatives to obtain functional materials based on natural resources with specific characteristics. Agriculture, the food industry and the leather processing industry produce large amounts of waste, mainly in the form of fibers and fibrous materials, among them keratin fibers, which represent a good candidate for the development of material biosource. Due to their chemical composition, keratin fibers are physically resistant and most importantly, flame retardant. However, over five million tonnes of keratin-rich waste (including wool, hair, feathers, hooves and horns) are generated each year by slaughterhouses, textile industries and tanneries. The thermal degradation and flammability behavior of polymer composites from natural fibers recovered from waste is studied to obtain biocomposites, due to their wide field of application in industry, agriculture, medical field, pharmaceuticals, cosmetics. Different research studies have suggested that the thermal stability of biocomposites can be improved by adding micro/nanoparticles, additives and crosslinking compounds. This approach could be considered as an effective way of ecologically recycling keratin fiber waste and obtaining functional materials characterized by increased fire safety and low environmental impact. Keratin waste recovery and processing through different optimal methods can transform a material with low economic value (remnants of tannery hair and sheep wool) into a product with applications in various fields: leather processing, cosmetics, agriculture, pharmaceutical, medical, industry.

Keywords: Keratin, biopolymer, fire-proofing

INTRODUCTION

Wool waste from sheep farming is a source of keratin (Maurizii *et al.*, 2024; Chereji and Munteanu, 2024). The annual production of this wool waste is around 200 thousand tons in Europe alone (Chereji and Munteanu, 2024). Part of this waste is used to produce fertilizers for agriculture and animal feed. The recovery of wool waste consists of biorefining processes for the extraction of keratin and its use in compositions of added-value materials (Ossai *et al.*, 2022) with applications in the textile industry (Zhu *et al.*, 2023), air and water purification (Posati *et al.*, 2020), controlled delivery of active substances (Khorshid *et al.*, 2023), regeneration of biological tissues (Hu *et al.*, 2012; Ranjit *et al.*, 2022), composites of materials with specific mechanical properties or flame retardants (Mengistu *et al.*, 2024; Abba *et al.*, 2025). Keratin belongs to the family of fibrous structural proteins known as scleroproteins and is the most common structural protein found in animal horns, claws, nails, hair and feathers. Its unique characteristic is that it contains more cysteine than other fibrous proteins such as collagen, elastin and myofibrillar protein (Khorshid *et al.*, 2023; Hu *et al.*, 2012). The mechanical structure of keratin is due to the high concentration of hydrogen bonds, hydrophobic groups and disulfide cross-links. It contains a high percentage of cysteine

(7–13 %) which stabilizes keratin. The recovery of keratin waste can transform a material with low economic value (remnants of tannery hair and sheep wool) into a product with applications in various fields: leather processing, cosmetics, agriculture, pharmaceutical, medical, industry (Mengistu *et al.*, 2024; Abba *et al.*, 2025; Wang *et al.*, 2016).

The purpose of this review is to highlight the main sources of keratin, extraction methods, characterization techniques and possible industrial applications. A special attention was paid to the study of those compositions with high synergy, based on keratin and other flame-retardant additives, for the development of textile coatings with multifunctional properties, and also of composite materials used in the electrical insulators industry, etc.

Material Sources of Keratin

Keratinous materials are formed by keratinized cells organized specifically and filled with proteins, mainly fibrous. They make up the hard appendages in animals, for example epidermis, wool, hair, horns, nails in mammals, as well as feathers, claws, beaks in birds and reptiles, turtle shells having a variety of functions, such as protection against external environment, armor against predators (Sarma, 2022). A thorough understanding of the relationships between the units that make up keratin materials and their functional properties provides useful knowledge in the design of new materials (Mengistu *et al.*, 2024; Wang *et al.*, 2016).

Methods of Obtaining Keratin

To solubilize keratin from various sources, numerous extraction methods have been devised. Reduction, oxidation, microwave irradiation, alkaline extraction, sulfitolysis, and ionic liquids, microbiological, enzymatic are procedures for the solubilization and isolation of keratin from keratin-rich materials (Maurizii *et al.*, 2024; Mengistu *et al.*, 2024; Sarma 2022; Olvera-Valdez *et al.*, 2024; Wang *et al.*, 2016; Abba *et al.*, 2025; Balaji *et al.*, 2012; Duverger *et al.*, 2015; Palmer *et al.*, 2008). The purpose of these keratin production processes is to break the disulfide and peptide bonds in the protein chains and their fragmentation (Table 1).

Table 1. Keratin production processes

Keratin production process	Action	Applications
Alkaline hydrolysis	Obtaining an advanced fragmentation of protein chains.	Pharmaceuticals, cosmetics
Acid hydrolysis	It creates extremely severe conditions that can be harmful certain amino acids.	Composites with industrial applications
Reduction processes	Preserves keratin microstructure by reducing (with mercaptoethanol) disulfide bridges in protein fibers without appreciably cleaving peptide bonds.	Composites with industrial applications
Enzymatic hydrolysis	A very slow approach unsuitable for industrial applications, produces the lowest degree of species splitting.	Composites with miscellaneous applications, agriculture, animal feed
Sulphytolysis	Breaking disulfide bonds by treating with sulfite, bisulfite.	Fertilizers in agriculture
Ionic liquids	It accelerates the dissolution of natural polymers. The most used ionic liquids are soluble salts of imidazole derivatives.	Industrial applications in different polymer compositions

Keratin production process	Action	Applications
Microbiological degradation	The action of some microorganisms such as <i>Actinomyces</i> (Vasileva-Tonkova <i>et al.</i> , 2009; Syed <i>et al.</i> , 2009), <i>Bacillus</i> sp. (Suntornsuk & Suntornsuk 2003; Gessesse <i>et al.</i> , 2003), <i>Serratia</i> sp. (Khardenavis <i>et al.</i> , 2009), <i>Kocuria rosea</i> , led to the obtaining of keratin hydrolysates by ecological methods.	Soil improvement products and protein supplements in animal feed
Microwave	Microwave at 60°C, 400 W, 40 kHz for 10 min. Reduces extraction time from hours to 10 min.	In water treatment processes
Mw		

Keratin Characterization Techniques

Various procedures are used to evaluate and characterize extracted keratin to confirm protein and amino acid content. Investigation on crosslinking mechanism can be performed using attenuated total FTIR (ATR-FTIR) and XRD but also the presence of specific amino acids. The infrared absorption spectrum of keratin shows the characteristic absorption bands attributed mainly to the amide bond (-CONH-): the broad band centered at 3200 cm⁻¹ is associated with stretching vibrations for O-H, the band from approximately 1600-1700 cm⁻¹ is mainly associated with stretching vibrations for C=O (amide I), while the amide II band, which falls at about 1550 cm⁻¹, is associated with bending vibrations for N-H and stretching vibrations for C-N. Around 1200-1300 cm⁻¹ is the band corresponding to amide III, which results from the combination of C-N stretching and N-H bending vibrations, with the contribution of C-C stretching vibrations and C-O bending vibrations. All these peaks are visible in the keratin spectrum, suggesting the presence of a α -helix and a β -sheet structure. Following hydrolysis, the amide bands move to longer wavelengths, which indicates conformational changes in the protein chains (Fraser & Parry 2011; Zhao *et al.*, 2015; Khosa *et al.*, 2011). Circular dichroism (CD) spectroscopy can be utilized to analyze the secondary protein structure of keratins (Lin *et al.*, 2024). Rheological characterization with a rotational rheometer can investigate crosslinking properties by storage modulus, G' and loss modulus, G'', the morphology can be analyzed with Scanning Electron Microscopy (SEM), also texture analyzer (Maurizii *et al.*, 2024; Lin *et al.*, 2024). The sodium dodecyl sulfate and polyacrylamide gel electrophoresis analysis (SDS-PAGE) shows the molecular masses of the obtained hydrolysates and highlights the electrophoretic model of keratin specific to the presence of the primary group of proteins associated with keratin, the proteins from the intermediate filaments but also bands corresponding to proteins with high molecular weight at the level of 50 kDa, attributed to the proteins of the intermediate filaments with low sulfur content, which are mainly characteristic of the α -helical keratin chain (Lv *et al.*, 2016; Olvera-Valdez *et al.*, 2024).

Mechanical testing can be done by determining Young's modulus, stress and tensile strength. Micro-computed tomography (Micro-CT) evaluates the 3-D structures of keratin as films. After scanning, pore size, porosity and connectivity are analyzed. Investigation by confocal laser scanning microscopy is done at different excitation wavelengths. The obtained images highlight the network structure of keratin through red fluorescence (Lv *et al.*, 2016). Field emission scanning electron microscopy (FE-SEM), with energy dispersive spectroscopy (FESEM-EDS) is performed on network structures to investigate the surface morphology. The demineralization of the samples is evaluated by an energy dispersive spectroscopy unit attached to the SEM. In the SEM results of keratin, the characteristic three-dimensional network structure is highlighted, which is consistent with the confocal laser scanning image

(Pon-On *et al.*, 2016). Thermogravimetric analysis (TG) can be used to confirm the thermal stability and composition of the synthesized materials (Olvera-Valdez *et al.*, 2024). The antimicrobial and antifungal activity of wet keratin films can be investigated by the diffusion method in the presence of microorganisms of interest.

This method is performed in petri dishes on solid agar culture medium. Wet keratin films are placed in Petri dishes with agar cultured with the microorganism to be tested. The test samples are then incubated at 37°C for 24 hours, and the antimicrobial activity of the wet keratin films is determined by measuring the diameter of the zone of inhibition (Pon-On *et al.*, 2016).

Composition and Structure of Keratin

Sheep wool contains up to 95% by weight of pure keratin with molecular mass ranging from 45 to 60 kDa and 11–28 kDa. Keratin consists of intermediate filaments, structured in different models: the α model, the β model and the amorphous model (Table 2). The α -keratin proteins are organized as spirals with an α -helix conformation of the polypeptide chains stabilized by hydrogen bonds. β -keratin is like a folded sheet, made up of laterally assembled strands, which can be parallel or antiparallel (more stable), and the chains are held together by intermolecular hydrogen bonds. Keratinized materials present a complex hierarchical structure consisting of polypeptide chains and filamentary matrix structures on a nanometric scale. The primary structure of keratin is a chain of amino acids, with various different sequences. Keratin is rich in cysteine (17.5%), serine (11.7%), glutamic acid (11.1%), threonine (6.9%), glycine (6.5%), arginine (5.6 %) (Gupta *et al.*, 2012; Mengistu *et al.*, 2024).

Table 2. Keratin sources and distribution of α - and β - keratin

Keratin type	Keratin Resources
α -keratin	Wool, hair, nails, hooves, horns, stratum corneum (Mengistu <i>et al.</i> , 2024; Olvera-Valdez <i>et al.</i> , 2024).
β -keratin	Bird feathers, beaks and claws, reptilian claws and scales (Wang <i>et al.</i> , 2016; Olvera-Valdez <i>et al.</i> , 2024).
α and β -keratin	Reptile epidermis, Pangolin scales (Wang <i>et al.</i> , 2016).

Properties of Keratin

Keratin is one of the hardest biological materials, with a high modulus of elasticity, although it contains only polymeric compounds and rarely minerals, it is a complex mixture of proteins and enzymes taken from epithelia. They are insoluble in dilute acids, alkaline solutions, water and organic solvents and are resistant to degradation by the pepsin and trypsin proteases. Keratins are insoluble in aqueous salt solutions, but are soluble in solutions with denaturing agents such as urea (Sarma, 2022). Keratin has specific mechanical properties, biodegradation, biocompatibility, bioactivity through the ability to promote cell growth, the ability to bind toxic substances (heavy metals, formaldehyde) and transport hydrophilic and lipophilic active ingredients (Wang *et al.*, 2016).

Processing Keratin Extracts – Additivation and Crosslinking Methods

Keratin-based products can be processed by various chemical and physical cross-linking methods with the potential to increase mechanical resistance. The formation of new interchain disulfide bonds can be favored by oxidative methods or by acid treatment, by thermal or dehydration methods. The inclusion of some solvents in the solubilized keratin can favor increased chain mobility. Also, various chemical agents can be used, such as aldehydes

(formaldehyde, glutaraldehyde), or nanoparticles can be incorporated as reinforcing agents (montmorillonite) in keratin-based materials (Table 3) (Mengistu *et al.*, 2024; Wang *et al.*, 2016).

Table 3. Compounds and methods of keratin crosslinking

Crosslinking compounds	Remarks
Carbonyl compounds such as formaldehyde and glutaraldehyde	It reacts through condensation reactions with the amide groups in the structure of glutamine and asparagine. Different cross-linking densities of the formed networks are obtained with the improvement of the superior mechanical properties (Young's modulus, resistance to elongation) of keratin cross-linked with formaldehyde and glutaraldehyde, compared to non-cross-linked keratin
Montmorillonite, comes from the class of clay materials	Nanostructured material is obtained, used as a filling agent
Acrylic monomers of the 1,3-diglycerolate diacrylate type	“Click-chemistry”-type chemical treatment that gives keratin fibers a superior resistance to contraction

Industrial Applications of Keratin

Keratin is a protein with specific characteristics that contains amino acids with active amino ($-NH_2$) and carboxylic ($-COOH$) functional groups. In addition to these groups, it also has a high content of cysteine in the amino acid sequence, with thiol ($-SH$) groups, which are responsible for intra- and intermolecular sulfur-sulfur (disulfide) bonds. Keratin is a biopolymer with numerous functional properties, applicable for the creation of biomaterials with controlled characteristics used in various fields. For the textile industry, it is important to develop filaments with a high keratin content for the renewal of raw wool (Cao *et al.*, 2020) and for the pharmaceutical and cosmetic industry for the creation of protein scaffolds that mimic the cellular matrix capable of promoting the biological reconstruction of tissues and skin regeneration (Mengistu *et al.*, 2024; Ye *et al.*, 2020; Liu *et al.*, 2023). Potential uses of keratin compounds may be in agriculture as organic fertilizers, and animal feed or in the production of adhesives, processing of leather products, polymer composites for household or industrial products, eliminating common chemical compounds used in cosmetics, pharmaceuticals or biomedical materials (Mengistu *et al.*, 2023).

Keratin Flame Retardant

Proteins in keratin fibers contain 50% carbon, 21%–24% oxygen, 12%–21% nitrogen, 6%–7% hydrogen, 2%–5% sulfur, and other elements. Due to their chemical composition, keratin fibers are physically resistant and have flame retardant properties (Alaffi *et al.*, 2020). Only a few articles can be found on the use of keratin fibers as a raw material for the preparation of composites with flame retardant properties. Perez-Chavez *et al.* (2022) used keratin fiber in a biodegradable cornstarch copolyester material to make a composite with flame retardant properties avoiding the use of toxic flame retardants. Keratin fibers have also been used as a filler in polyurethane foams to reduce flammability and improve mechanical and thermal properties (Wrzesniewska-Tosik *et al.*, 2020). The use of deoxyribonucleic acid (DNA) in combination with keratin as green intumescent additives has been studied in polymer blends based on polyethylene (PE) / ethylene vinyl acetate (EVA) with applications as an insulator in the electrical wire and cable industry. The flame retardancy mechanism of keratin is based on the formation of a carbonaceous intumescent layer with the role of

protecting the polymer matrix during combustion (Albite-Ortega *et al.*, 2019). Another approach focused on the extraction of keratin from bird feathers and wool by the hydrothermal method, without additional pretreatments, in order to use it as an innovative multifunctional layer with new properties (UV protection, flame retardancy and antioxidant activity) for polyester fabrics (Zemljich *et al.*, 2024). Another research study describes a chemical method of modifying keratin with melamine (a nitrogen-rich compound) and sodium pyrophosphate (a phosphorus-rich compound) for use as a flame-retardant additive for cotton fabrics. The limiting oxygen index (LOI) of cotton after the application of modified keratin increased by ~ 66.7% compared to that obtained on untreated cotton (Patankar *et al.*, 2021). The use of keratin and tannin derived from renewable biomass (rich in polyphenols) as a reinforcing agent in the polyamide 66 (PA66) matrix was studied, in order to improve the fire-retardant and anti-drip performance. The obtained results showed that the peak of heat release rate (PHRR) and total heat release (THR) of the fireproof PA66 composite decreased by 75.3% and 38.4%, compared to PA66. Following the determination of the LOI, the fire-retardant PA66 composite reached 29.65%, which places it in the V0 combustibility class (Jiang *et al.*, 2023).

Future Prospects

Keratin is one of the most durable biological materials. The specific characteristics can be the basis for the development of new bio-inspired models. Biomaterials based on keratin, such as sponges, hydrogels, patches, films, fibers, various biocomposites can be created for biomedical, pharmaceutical and cosmetic applications. Due to its resistance to acids, bases and solvents as well as its flame-retardant nature, keratin can be recommended for polymer compositions for use in anti-shock, fire-resistant materials or with specific mechanical properties for different industrial sectors or for household use. The main methods of disposal of waste from poultry farms, sheep farms, the leather processing industry are waste storage, burning or dumping in the open air at the landfill, all of which pollute the environment. Recycling and efficient use of these resources is important from an ecological and financial point of view (Mengistu *et al.*, 2024; Abba *et al.*, 2025).

CONCLUSION

The application of knowledge from the area of keratinous materials to the design of new structures is a vast field of research, in which carbon nanotubes, graphene and other synthetic fibers can be combined with biopolymer matrices, offering many possibilities for the development of biomaterials with applicability in different industrial sectors, in medicine or in agriculture. Keratin waste recycling and processing through optimal methods can generate functional materials characterized by excellent fire safety and low environmental impact.

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ENZYMES IN WET-BLUE NEUTRALIZATION: EFFECT ON PROCESSES AND LEATHER PROPERTIES

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Enzymes are biocatalysts that have wide applications in many industries. Traditionally, they are employed in leather industry for bating to remove non-collagenous proteins and open-up the fibres for better penetration of chemical substances. Due to growing environmental concerns, more studies are done to use enzymes in other leather processing steps as auxiliaries in soaking, liming-unhairing of hides/skins or for bating of chromed leather (wet-blue). However, enzyme application in post-tanning is still a new field; there is limited information if enzymes can be employed in leather finishing. The aim of this research was to investigate neutralization with enzyme preparations and evaluate the influence on further wet-finishing operations and its effect on the qualities of the semi- and finished product. In the study two enzyme preparations were used: Novo Bate WB and Vilzim PRO N. After enzymatic process the shrinkage temperature and the amount of removed collagen were evaluated; after wet finishing the dye and tannin exhaustion, the penetration of the dye, the matter soluble in dichloromethane were determined. Using enzymes led to greater collagen removal compared to conventional neutralization; however, shrinkage temperature after the neutralization as well as after wet finishing did not show any significant differences. Moreover, only dye penetration through grain side was markedly better when using enzyme preparations. The obtained results showed that some characteristics improved while others got worse or remained similar during further processing. Because of this, it is crucial to analyze and optimize usage of each new enzyme preparation to produce leather with desired qualities.

Keywords: neutralization, enzyme, chromed leather.

INTRODUCTION

Leather is pliable and strong material produced by tanning cattle hide or other animals' rawhides. Even though leather is by-product of meat industry, during manufacturing high amount of solid and liquid waste is produced (Kolomaznik *et al.*, 2008). Tannery wastewater is highly polluted in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, sulphate, sulphide, chromium and other chemicals used in the processing (Dandira *et al.*, 2012). Due to growing environmental concerns researchers study new technologies to reduce negative impact of leather industry and employment of enzymes could be one of the solutions.

With advancements in development, purification and improvement, enzymes have wide applications in many industries. Conventionally, in leather industry these biocatalysts are used in bating process to remove non-collagenous proteins and open-up the fibres for better penetration of chemical substances (Gallego-Molina *et al.*, 2013; Zhang *et al.*, 2021). Studies show that enzymes can be used in all beam house processes and have even entered the tan house providing advantages for high-quality leather (Khambhaty, 2020). Ferments in beam house operations can be use as auxiliaries in soaking (Valeika *et al.*, 2019), liming-unhairing of hides/skins (Sujitha *et al.*, 2018), degreasing (Palop *et al.*, 2000).

However, enzyme application in post-tanning is still a new field; there is limited information about enzymes usage in leather finishing. Recent studies showed new possibilities of enzyme use in wet blue rebating; after enzymatic rebating chrome and/or other

chemicals exhaustion increases (Biškauskaitė *et al.*, 2023; Jayakumar *et al.*, 2019). Also, ferments were studied in leather dyeing. Song *et al.* (2017) findings suggest better fastness properties against rubbing and better dyed absorption in treated leather. Nevertheless, there are no information about enzyme use in neutralization process. Effective neutralization adding enzyme preparations (EP) would allow to exclude additional enzymatic bating processes after chroming to obtain best quality of leather. The aim of this research was to investigate the neutralization with enzyme preparations and evaluate the influence on further wet-finishing operations and its effect on the qualities of the semi- and finished product.

MATERIALS AND METHODS

Materials

Bovine chromed leather (wet blue) was purchased from tannery “TDL Oda”, Lithuania. Samples for experiments were cut into 6x8 cm series of samples in such a way that all leather parts would be presented in each experiment.

The chemicals used for the analysis were of analytical grade. Analytical and technical grade materials were used for the technological processes.

The EP Novo Bate WB (acid protease, optimal pH 4.5 – 7.0, Novozymes, Denmark) and Vilzim PRO N (neutral protease, optimal pH 6.5 – 7.5, Baltijos enzimai, Lithuania) were used for the neutralization process study.

Enzymatic Neutralization

To study enzymatic neutralization process samples were treated as follows:

- 1) NaHCO_3 1.5%; H_2O 150%, drum ran for 30 min;
- 2) NaCOOH 2%, drum ran for 10 min;
- 3) EP 1% or 5%, drum ran for 85 min.

For conventional process (control) neutralization was performed without EP. All percentages are based on wet blue leather.

Analysis Methods

The amount of collagen proteins removed was estimated from the amount of hydroxyproline in the neutralization solution using a photo colorimetric method (Zaides *et al.*, 1964) using GENESYS-8 (Spectronic Instruments, UK).

For non-collagen proteins removal determination Lowry method was used (Lowry *et al.*, 1951).

The shrinkage temperature of wet blue after neutralization and wet-finishing was determined as described in the literature using special equipment and replacing the distilled water with glycerol (Golovteeva *et al.*, 1982).

Matter soluble in dichloromethane and pH of aqueous leather extract were determined according to standards (Standard ISO 4048, 2018; Standard ISO 4045, 2018).

For pH measurements pH-meter WTW 526 (WTW Xylem brand, UK) was used.

Dye exhaustion was estimated by colorimetric method by measuring the absorbance of the dye solution at a 495 nm wavelength. Dye consumptions were calculated using the calibration curve.

Tannin exhaustion was measured by gravimetric analysis.

The penetration of dye through the hide was evaluated using a special optical microscope with scale (magnification 15 times) MPB-2 (Izyum Instrument Making Plant, Izyum, Ukraine).

RESULTS AND DISCUSSION

Neutralization is performed after chroming to increase wet blue pH, in order to avoid the surface fixation of negatively charged post-tanning chemicals on the positively charged chromium cross linked matrix (Saravanabhavan *et al.*, 2006). Neutralization helps to achieve a better and more uniform distribution of retanning, dyestuff and fat liquors substances (Sundar *et al.*, 2001). To study enzymes influence in the neutralization process, four experimental variants were tested and after the process they were compared to conventional method. Firstly, pH values of neutralization solution and aqueous leather extract were measured (Table 1). The results show slightly higher pH of the neutralization solution when using Novo Bate WB; lowest pH value was obtained with Vilzim PRO N. However, only sample processed with lower concentration of Vilzim PRO N resulted in higher aqueous leather extract pH, that may suggest better neutralization process, values of other measured samples were alike. Furthermore, differences between determined shrinkage temperature were negligible, indicating that EP did not have effect on shrinkage temperature.

Table 1. pH and shrinkage temperature after neutralisation

	pH of neutralization solution	pH of aqueous leather extract	Shrinkage temperature, °C
Control	6.11±0.21	4.33±0.15	118.00±0.50
1% Novo Bate WB	6.36±0.15	4.43±0.15	118.17±0.47
5% Novo Bate WB	6.53±0.30	4.30±0.17	118.27±0.63
1% Vilzim PRO N	5.81±0.20	5.23±0.20	117.93±1.18
5% Vilzim PRO N	5.80±0.20	4.35±0.15	119.00±0.50

Secondly, after neutralization amount of removed non-collagenous and collagenous proteins were evaluated (Fig. 1). Even though, shrinkage temperature after process was not affected, using EP led to higher protein removal compared to conventional method. The amount of removed proteins increased with increasing EP concentrations. Results show that EP acted differently on proteins; highest effect on collagenous proteins had Vilzim PRO N, while on non-collagenous proteins Novo Bate WB. Similar shrinkage temperature, despite that addition of enzymes leads to higher removal of collagen proteins, presumably be due to EP act on telopeptide regions in collagen structure which do not have critical role in stabilizing reactions or preparative processes (Covington, 2009).

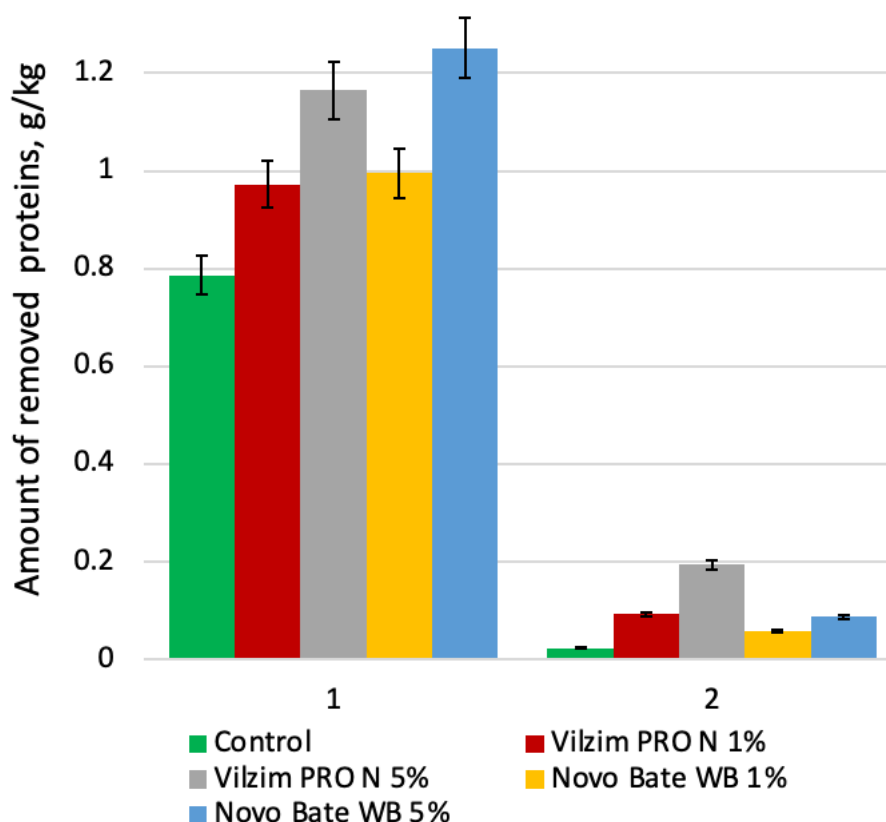


Figure 1. Influence of enzyme on the amount of removed non-collagenous (1) and collagenous (2) proteins during neutralization

After neutralization wet-finishing processes such as re-tanning, dyeing and fat-liquoring were performed. To determine the effectiveness of them and the quality of leather, tannin and dye exhaustion, matter soluble in dichloromethane and shrinkage temperature were evaluated (Table 2). Study findings indicated that during re-tanning only using 5% of Novo Bate notably improved tannins exhaustion, other experimental samples resulted in lower tannin consumption than conventional process or just slightly better. Furthermore, dye exhaustion after enzymatic neutralization did not show improved process; control process was the most effective. Shrinkage temperature after wet-finishing as well as after the neutralization were similar between the samples, only using 1% of Vilzim Pro N temperature increased slightly.

Table 2. Influence of enzymatic treatment in wet-finishing processes

	Tannin exhaustion, %	Dye exhaustion, %	Matter soluble in dichloromethane, %	Shrinkage temperature, °C
Control	58.20±1.75	84.37±1.31	5.46±0.10	120.33±0.66
1% Novo Bate WB	58.29±1.61	79.81±0.89	5.69±0.11	119.75±0.40
5% Novo Bate WB	65.24±2.02	82.35±1.15	5.45±0.17	119.00±0.50
1% Vilzim PRO N	54.91±1.14	83.05±1.50	5.47±0.21	121.57±0.47
5% Vilzim PRO N	47.96±1.21	83.22±0.97	6.27±0.18	120.00±0.50

Even though dye exhaustion did not show improvement while using enzymes, dye penetration enhances with EP (Fig. 2). From results it was observed that using EP led to significantly better dye diffusion through grain layer compared to control samples. Usually,

greater penetration of dyes is achieved on the flesh side, due to well-known structural differences between the two sides; flesh side has more open structure fiber that helps dye penetrate better (Haroun, 2015). Using these EP led to better dye diffusion on grain side almost in all cases; wet blue affected by neutralization with enzymes behaved differently during dyeing than control sample. Overall, total dye exhaustion was better with EP; using 1% Novo Bate WB led to best dye diffusion.

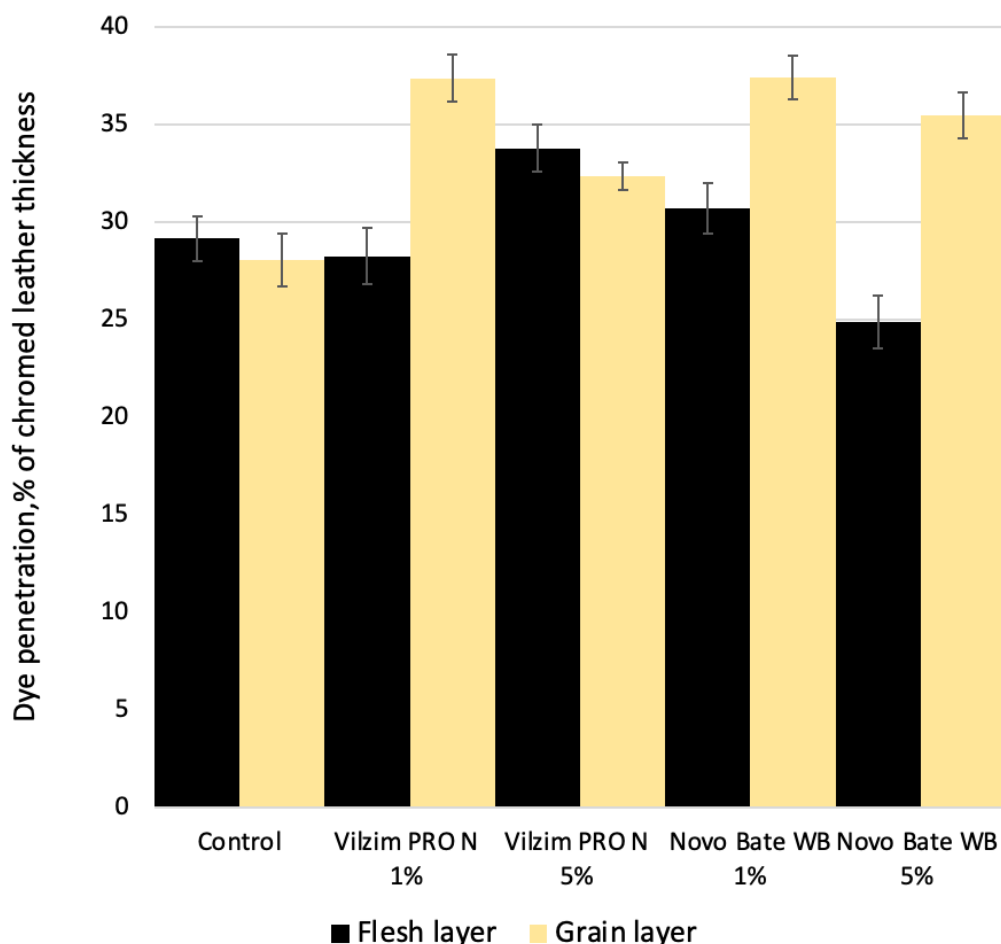


Figure 2. Depth of dye penetration into the chromed leather

CONCLUSIONS

Results obtained during the research show that with the use of enzymes more collagenous and non-collagenous proteins are removed from wet blue. However, shrinkage temperature after neutralization and wet-finishing did not show any significant differences between the samples. Also, dye exhaustion values indicated better dye process of conventional neutralization. Nevertheless, enzymes in neutralization process led to better dye penetration through grain side. Finding show that some characteristics may improve while others may worsen or remain constant after enzymatic processing. Because of this, it is crucial to analyse and optimise the use of each new enzyme preparation to produce leather with desired qualities. However, from an economical point of view enzymes in neutralization do not have many advantages.

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RESEARCH ON BUILDING MANAGEMENT MODEL AND ENTERPRISE RESOURCE PLANNING FOR FOOTWEAR BUSINESSES IN VIETNAM

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Enterprise resource planning (ERP) is a fundamental tool in the digital transformation of enterprises. Currently, on the market, many companies are providing ERP solutions. ERP solutions can be packaged, customized, or can be open source. Packaged ERPs are often suitable for large enterprises. Small and medium shoe businesses need an ERP system appropriate for their financial resources, human resources, technology, management level, and management model. The article presents the method and results of building a suitable ERP and standardized management model for Vietnam's small and medium footwear enterprises (SMFE). In this study, the methods used were document review; a survey of SMFE and some footwear enterprises that have used ERP; software development methods. The built management model and ERP includes five subsystems: Supply chain management; Financial resource management, Human resource management, Customer relationship management, and Production management. The Production management subsystem includes five modules: Warehouse Management, Equipment management, Design-technology management, Production deployment, and Quality management. The results of ERP applications in Vietnamese shoe enterprise have proven that it is suitable and beneficial to enterprises.

Keywords: Digital transformation, Footwear enterprise, Enterprise resource planning.

INTRODUCTION

The digital transformation of companies, industries, and society is progressing at an accelerating pace (Baalmans *et al.*, 2023). Digital transformation refers to the integration, use, and exploitation of digital technologies to create major changes in value creation, appropriation, and distribution. The recent COVID-19 pandemic has shown the important role of digital transformation for enterprises, and the socio-economic sectors of a country (Hai *et al.*, 2021; Anh *et al.*, 2023). The Government of Vietnam considers digital transformation to be an important strategy for the country's socio-economic development (Decision No. 749/QĐ-TTg, 2020). The goal is that by 2030, Vietnam will become a digital country, in which, digital transformation for enterprises plays a particularly important role.

Enterprise digital transformation includes the application of digital technology to the production process of enterprises, business processes, business model innovation, and decision-making support. The three main components of enterprise digital transformation include the application of digital technology, organizational change, and value chain change. Digital transformation activities including: 1) Digitization of management and business data of enterprises; 2) Applying digital technology to automate and optimize business processes, management, production, reporting processes, standardize production processes and corporate governance processes; 3) Coordinate work in the enterprise to transform all business activities to create new value for the enterprise (Anh *et al.*, 2023; Annual Report on Vietnamese Enterprises' Digital Transformation, 2023; Phuc & Huong, 2024). Therefore, ERP systems are an important tool for enterprises to successfully digitally transform (Bui *et al.*, 2020). ERP is applied in many footwear enterprises in the world and brings benefits to them (Silva *et al.*, 2016; Study on Industry 4.0 applied to the footwear industry in Europe). Many ERP systems

have been developed for footwear enterprises in different countries (The Zymmetry Group, 2006; The Answer Company, 2015; SAP, 2009).

Vietnam is the 2nd largest exporter of footwear in the world (World Footwear, 2023). The leather and footwear industry is a major industry in Vietnam and has the potential to develop strongly in the coming period (Decision No. 1643/QD-TTg, 2022). The number of enterprises in Vietnam's footwear industry is quite large (nearly 2000 enterprises), of which over 80% are small and medium enterprises. Most of them are domestic enterprises (LEFASO, 2023). Digital transformation of enterprises with the application of ERP systems in the management of SMFE in Vietnam faces many obstacles, especially when applying foreign packaging ERP systems (Phuc & Huong, 2024; Bui *et al.*, 2020; LEFASO, 2023). Some of the main reasons are limitations in financial resources, human resource qualifications, technology and management models (Phuc & Huong, 2024; Bui *et al.*, 2020). In this study, we presented the results of building a standardized management model and an ERP system in accordance with the conditions of SMFE in Vietnam. They are built based on actual surveys of footwear enterprises and enterprises that have been using ERP. This is an important basis for the successful digital transformation of Vietnam's SMFE.

RESEARCH METHODOLOGY

The overall diagram of the research contents and main research methods is shown in Fig. 1.

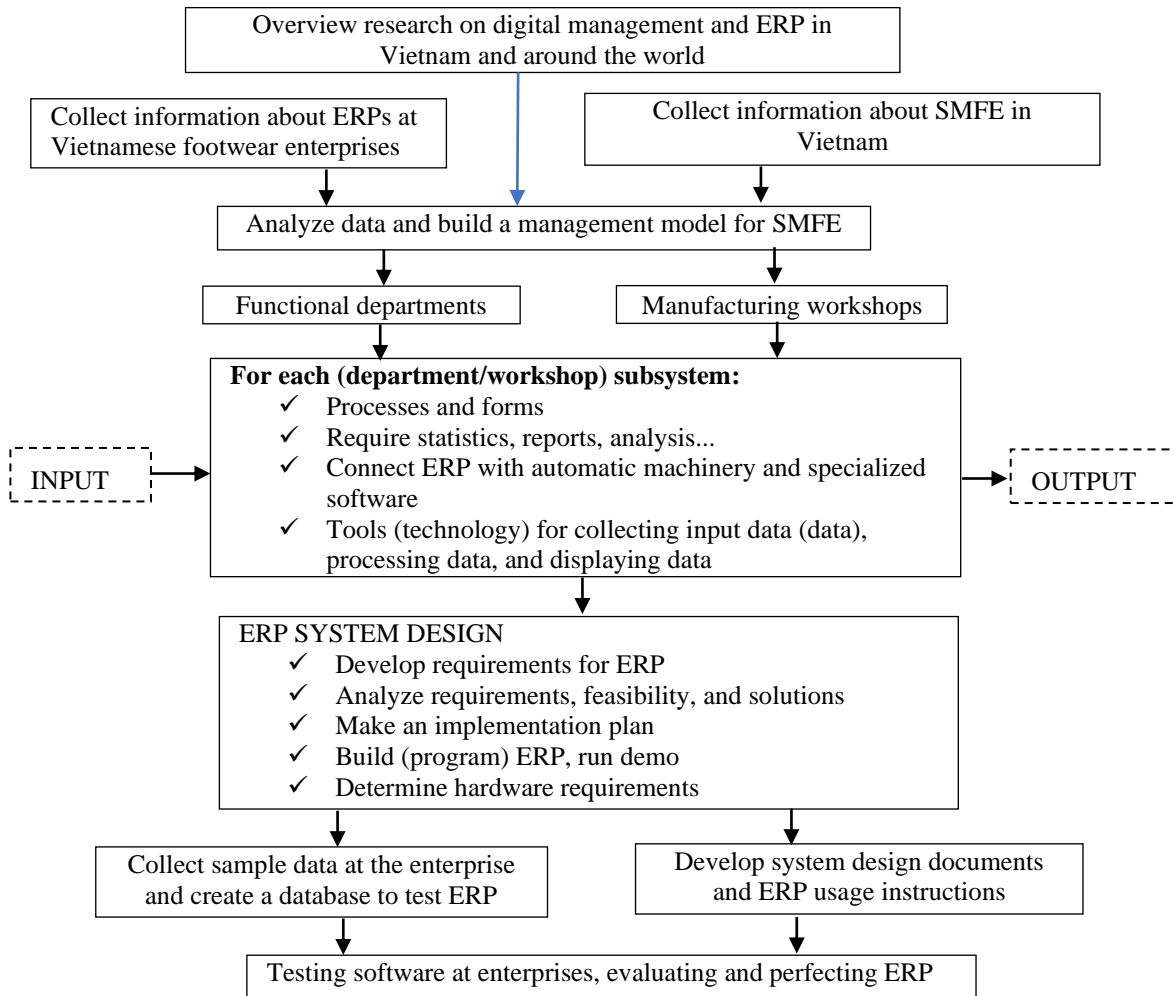


Figure 1. Overall diagram of the content and research methods

The basis for building the Vietnamese footwear enterprise management model and ERP for them is the information collected from these enterprises. The information that needs to be collected includes labor; enterprise management model; software usage; automated machinery and equipment in enterprises, equipment management; desired benefits in digital transformation; difficulties in digital transformation; suggestions and recommendations.

The basis for building ERP is a standardized enterprise management model. We build it in three steps: 1) determine the organizational model of the SMFE; 2) build a management organization structure suitable for the SMFE; 3) standardize each module. For each subsystem/module, we have identified and set up: 1) content or function; 2) requirements; 3) structure; 4) data linkage with other subsystems and modules; and 5) reporting, analysis and evaluation forms. From here, we build processes and forms for each subsystem/module.

ERP is a system that consists of software and hardware. Therefore, the method of building a system includes building software, identifying hardware equipment to collect, process and display data. The construction of ERP software is carried out according to the general and basic software construction method by following steps (Aptech): 1) determine requirements and solutions; 2) software design; 3) software programming; 4) testing and bug fixing. In order to complete the ERP system as well as promote it to footwear enterprises, we organized an ERP demonstration workshop for SMFE.

RESULTS AND DISCUSSIONS

Information Obtained from Vietnamese SMFE

SMFE has a labor scale of 500 to 2000 employees. These are private enterprises with domestic investment. A compact scale is also a favorable condition for the digital transformation of enterprises.

SMFE are organized according to a functional structure. The organization of these enterprise is compact, usually with a board of directors, below are functional departments and workshops (Fig. 2). The compact production organization and management apparatus is also a favorable condition for enterprise digital transformation.

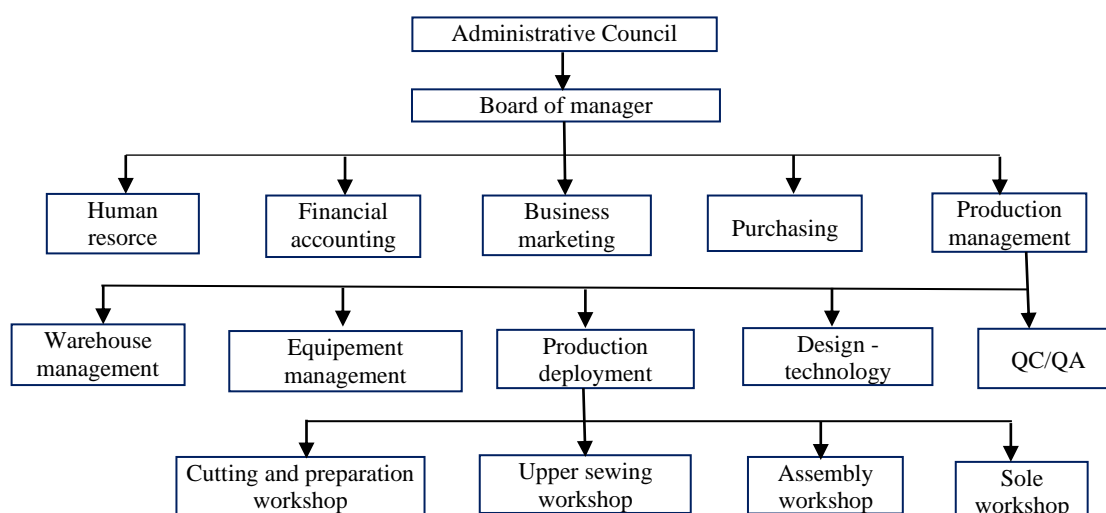


Figure 2. Diagram of the organizational model of the management apparatus of SMFE

SMFE usually produces a type of footwear such as sports shoes, canvas shoes, leather shoes, sandals... according to a certain sole assembly technology. In which, sole cementing technology is often used for sports shoes, leather shoes, and vulcanized rubber sole technology for canvas shoes. These are usually shoes with a long production technology

process with many stages, using a lot of manual labor, and the level of automation is not high.

About 70% of Vietnamese SMFE manufacturing products by CMT (Cut-Make-Trim) and FOB (Free One Board) methods for foreign brands. Some enterprises are more deeply involved in the development of products.

Machinery and equipment at SMFE are outdated compared to large and FDI enterprises. Automation equipment has not received much attention and investment. This not only affects labor productivity and product quality, but also hinders the application of Lean manufacturing and digital transformation. The flowchart of the footwear production process at SMFE is shown in Figure 3.

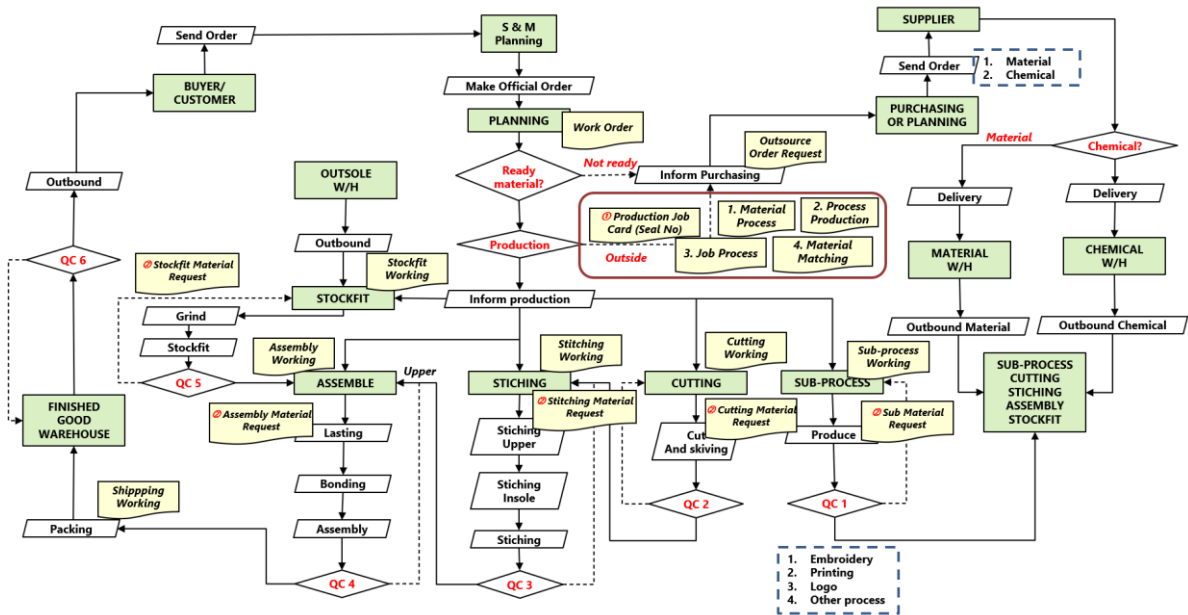


Figure 3. Flowchart of the footwear production process at Vietnamese footwear enterprises

SMFE are using the traditional method of organizing production with long lines. The enterprise organizes production according to separate workshops: Cutting and preparing semi-finished products; sewing uppers; producing soles and preparing footwear components; assembling, finishing shoe packaging; auxiliary parts or workshops (Fig. 3). Thus, the production cycle is long. This causes difficulty in quality management and enterprise digital transformation.

In surveyed enterprises, university-educated workers accounted for 5.7%, intermediate vocational workers accounted for 7.6%, and unskilled workers accounted for 86.7%. The low rate of trained workers leads to poor production management and limited application of advanced technology. This leads to low labor productivity and unstable product quality. This is also a major barrier for businesses when implementing digital transformation.

SMFE use individual software according to functions such as footwear design software, accounting software... About 10% of the surveyed enterprises use production and equipment management software.

Compared to large enterprises, the financial ability and access to finance of SMFE are much more difficult. Especially the difficulty in accessing bank loans.

Identifying Technology Tools for the Management of SMFE Applying ERP

Based on analyzing the types of technological equipment that can be used in the digital management of enterprises; the results of the actual survey of enterprises producing footwear; and the results of the survey of enterprises that have applied the ERP system; we found that there was no possibility to connect data of automated machinery and specialized software in

SMFE with ERP software. According to the characteristics of SMFE Vietnam, we selected the equipment according to the following criteria: 1) they are suitable for the ERP system; 2) they are not too expensive; 3) their technology is not complicated, as follows:

- Magnetic card or fingerprint reader timekeeping.
- Barcode system for statistics: Barcode printer, barcode scanner.
- The screen displays data and controls.
- Dashboard screen.
- Tablets and smartphones for data display and control (mobile).
- The workstation computers are ordinary PCs with a powerful enough configuration
- Investing in a physical server or renting a cloud server.

The Management Model Suitable for SMFE

The management model needs to meet the following requirements: 1) ensure representativeness, in accordance with the characteristics of Vietnamese SMFE; 2) be built based on the current organizational model of SMFE for convenient application; 3) be suitable and create favorable conditions for enterprise digital transformation. The enterprise management model consists of five subsystems with main functions (Fig. 4). The Production management subsystem consists of five modules (Fig. 5).

For each subsystem/module in the model, we have analyzed and established the following contents: 1) the content or function of the subsystem/module; 2) requirements for subsystem/modules; 3) structure of the subsystem/module; 4) link data to other subsystems/modules (Fig. 6); and 5) processes, forms, reports, analysis, and evaluation.

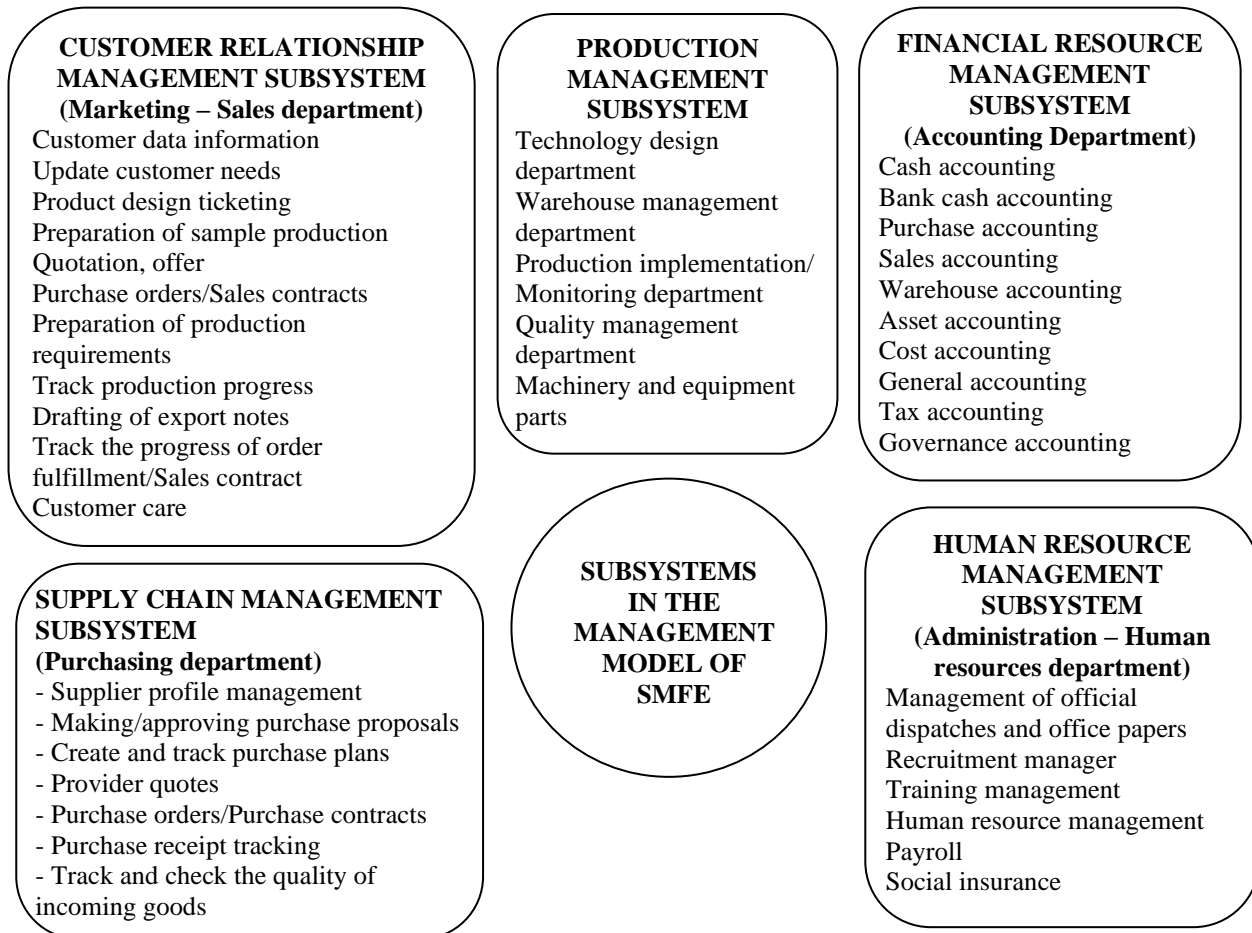


Figure 4. The management model of SMFE

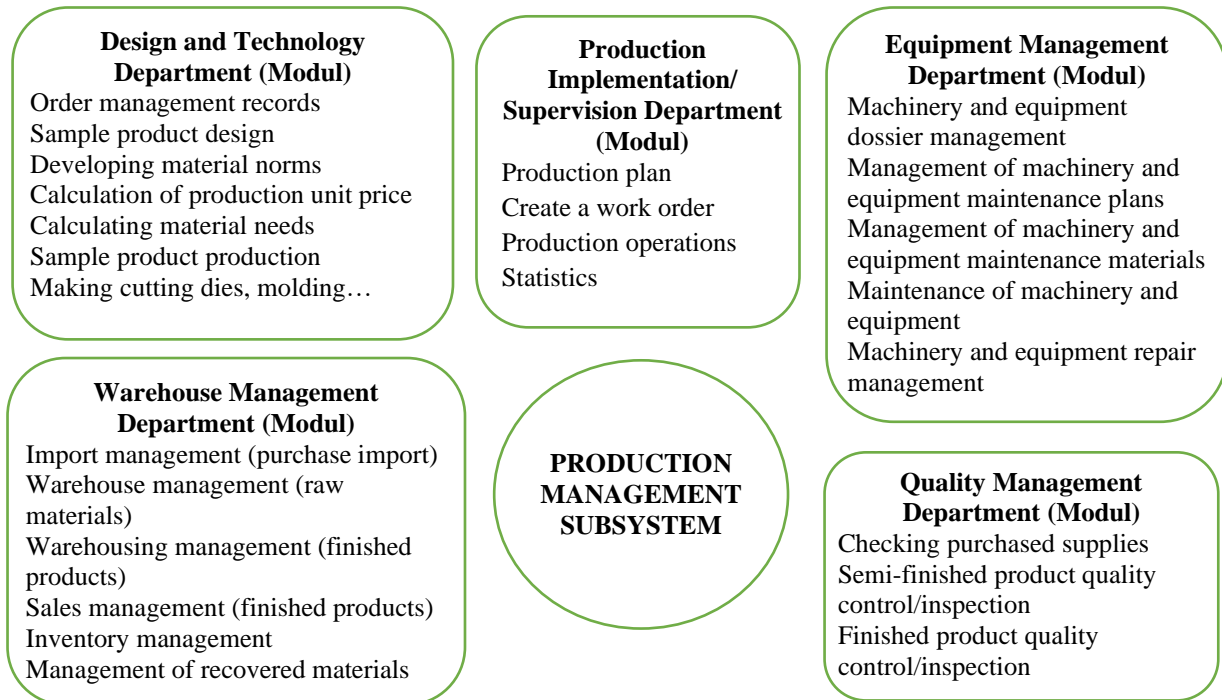


Figure 5. Production management subsystem structure in the governance model of SMFE



Figure 6. Modules and data links between modules in the enterprise

Results of Building an ERP System for SMFE

Requirements for SMFE: 1) ERP is suitable for the characteristics of Vietnamese SMFE; 2) integrating tightly between modules; 3) analyzing management operations; and 4) usability requirements: openness; correctness; usability; efficiency; compatibility; evolution; ease of testing, debugging and maintenance; reusability; and security (Aptech).

Solutions for ERP software: After analyzing the requirements, feasibility, to build ERP software for SMFE, we used:

- SQL SERVER database management system;
- Windows Server 2012 server operating system for PM ERP;
- Desktop Application Programming Language: VB.NET;
- Web Application Programming Language: C#;
- Desktop Application Programming Model – 3 layers (3-layer model);
- Web Application Programming Model – ASP. Net MVC.

Analyzing and designing systems, and technical aspects of ERP software: The overall structure and requirements of the modules/modules in the ERP system are shown in Figures 4 and 5. We perform the software programming for subsystems/modules in the ERP system for SMFE as follows: 1) building detailed functions for the software of each subsystem/module; 2) setting up UseCase for programming/coding the software. These tasks are performed on the basis of the functions, tasks, processes, forms/reports of the subsystems/modules in the built enterprise management model.

Building software programming and running ERP software demo: According to the results of analysis, software system design and setting up Usecases, we have designed the interface, programmed the software, and run a demo for each subsystem/module. The results show that, after fixing the errors, basically the ERP system can operate and perform the functions of each subsystem/module according to the set requirements. However, this is a large software with a high level of complexity, so there are still quite a few errors that need to be fixed to be perfected.

Applying ERP Software Testing to Footwear Enterprises

We set up a database from information collected at a shoe company that tested ERP. The company selected for ERP testing is Dong Hung Shoe Joint Stock Company in Ho Chi Minh City. The data is standardized according to processes, forms and reports according to the established management model.

We have developed a very detailed user manual, suitable for the labor level of Vietnamese SMFE. This document is trained for users. We supported the business to implement 3 shoe orders within 6 months. The results showed that the ERP system achieved the expected results, supporting the business well in implementing digital transformation.

Efficiency of ERP application at footwear enterprises: Although the time of applying ERP at shoe enterprises is not long, it has been positively evaluated by the leaders and employees of the enterprise. This ERP has brought good results to the enterprise, specifically: enhancing operational efficiency for businesses; integrating information; improving the competitiveness of enterprises; increasing transparency; enhancing customer satisfaction, efficiency, inventory management and customer care; changing the enterprise culture.

CONCLUSIONS

We have built an standardize management model suitable for Vietnamese SMFE. This is an important step in the digital transformation process of enterprises. This is the basis for us to build ERP. Subsystems/modules are integrated together on a software system that allows: 1) receiving information between different departments; 2) synchronizing all information, reducing data update and processing at multiple locations; and 3) allowing the establishment of professional rotation processes between departments. The results of the trial operation of the ERP system at the footwear enterprise show that: 1) The ERP is built in accordance with the characteristics of the SMFE in Vietnam; 2) ERP meets the management requirements of

enterprises, is highly appreciated by enterprises; 3) This ERP is ready to be replicated in Vietnamese footwear enterprises.

Acknowledgment

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COLLAGEN EXTRACTED FROM PERCH SKIN: RHEOLOGICAL CHARACTERIZATION AND *IN VIVO* ANIMAL STUDIES

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To recover as much collagen as feasible to meet market demand, more efficient and sustainable sources should be exploited instead of mammalian collagen. One of the most effective potential sources of high-quality collagen is fish wastes, which represent over 75% of a fish's weight and is produced in huge quantities all over the world, during fish processing. This waste includes skin, bones, scales, viscera, ligaments and fins. Consequently, collagen extracted from fish wastes is environmentally friendly, sustainable, and especially profitable when using fish industry by-products as possible sources of the material. Furthermore, different socio-political groups consider collagen extracted from marine resources as religiously acceptable, making it more valuable in all industry sectors. The purpose of this paper was to analyse the rheological behaviour of collagen gel extracted from perch (*Perca fluviatilis*) skin and to evaluate the therapeutic effect of spongy matrix (obtained through freeze-drying process of the gel extracted from perch skin) in the healing process of an induced burn in an animal model (Wistar rats).

Keywords: perch skin collagen, rheological analysis, *in vivo* animal analysis.

INTRODUCTION

Studies on the extraction of collagen from fish waste were initiated over two decades ago. One of the most prevalent proteins in mammals is collagen, which is present in the extracellular matrix of connective tissues such as blood vessels, skin, bones, tendons, ligaments, cartilage, and intervertebral discs (Nimni & Harkness, 2018). Collagens play regulatory roles (i.e., through mechano-chemical transduction processes) during tissue growth and repair in addition to their involvement in the preservation and strength of tissue architecture (Mahmood *et al.*, 2022). Collagens are inherently bioactive, biocompatible, and biodegradable due to their nature. Collagens considered as the most widely needed and used biomaterials across a wide range of industries, including the food, pharmaceutical, medical, cosmetic, and nutraceutical sectors. Collagens can be found in injectable solutions, thin substrates, porous sponges, nanofibrous matrices, and micro- and nano-spheres.

Despite the fact that fish collagen normally has a lower molecular weight and denaturation temperature than mammalian collagen, recent studies have shown that the molecular structure and biochemical properties of collagen derived from fish and mammalian sources are very similar (El Blidi *et al.*, 2021; Xu *et al.*, 2021). Multiple ways of extracting fish collagen have been established based on the type of tissue and fish species that are being exploited. Currently,

in the literature, there are not so many studies, as we know, regarding marine collagen extracted from perch (*Perca fluviatilis*) skin used for biomaterials synthesis, such as wound dressings.

In this respect, understanding the flow properties of perch collagen gels represents a key factor, due to the influence of these properties upon manufacturing technology, quality control, stability during storage, type of use, and therapeutic activity (Ghica *et al.*, 2012).

The objective of this paper is represented by the rheological evaluation of collagen extracted from perch (*Perca fluviatilis*) skin, and pre-clinical tests of corresponding collagen spongy matrix (*in vivo* animal analysis) used for biomaterials synthesis, such as wound dressings.

MATERIALS AND METHODS

Materials

The fresh perch skin was bought from a local fishing company. The fresh skin was washed with cold distilled water in the first step. After washing, several treatments (acidic and alkaline) followed in order to extract collagen gel. The acid used for acidic treatment was ascorbic acid (Scharlau, Sentmenat, Spain).

Collagen gel extracted from perch skin through various treatments was rheologically analysed. The extracted perch gel was poured in a glass Petri dish and freeze-dried for 48 hours using the Martin Christ 24 Delta LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) (Albu *et al.*, 2010) to obtain the collagen spongy matrix. The collagen sponge resulted from lyophilization was subsequently *in vivo* animal tested. The animals were obtained from the biobase of the “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania.

Methods

Rheological Evaluation of Perch Collagen Gel

The rheological determination of the perch collagen gel was carried out with the RM100 PLUS rheometer (Lamy Rheology Instruments, France), using the MS-DIN11 coaxial cylinder measurement system. The temperature of the rheological analysis was kept constant at $24 \pm 0.1^\circ\text{C}$ (conditioning temperature of semisolid formulations), using a circulating water bath (Julabo Corio CD). During the experiment, the gel must be kept at a constant temperature because the rheological properties are strongly dependent on temperature. The experimental program was set-up from the RheoTex software. To determine the flow properties of the collagen gel, the limits for the shear rate were chosen between 0.4 and 78 s^{-1} , corresponding to rotational speeds in the range of 0.3-60 rpm.

Evaluation of the Spongy Matrix Therapeutic Effect in Healing Process on Animal Model

The *in vivo* study of therapeutic effect of the collagen sponge, extracted from perch skin, in the healing process of an induced burn to an animal model (Wistar rats), was carried out in accordance with Directive 2010/63/EU (Council of the European Union) and Law no. 43 (Romanian Parliament - April 11, 2014).

This study was performed on 12 Wistar male rats, weighing about $180 \pm 10\text{ g}$, kept under regular laboratory conditions (they received water *ad libitum* and food twice a day). The experiment was carried out in “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania with the agreement of the Ethics Committee no. 21226/ 26.07.2023. The animals were distributed into two groups of 6 animals each: group 1 – collagen perch matrix (COLL_A) and

group 2 – control (the wounds were covered with sterile gauze). The *in vivo* experiment was carried out as previously described (Ghica *et al.*, 2017; Udeanu *et al.*, 2018; Marin *et al.*, 2018). Shortly, in order to properly perform the experiment, the animals were anesthetized with ethyl ether, and the hair in the dorsal area was removed. On this shaved surface, the experimental lesion was induced using a metallic accessory with a diameter of 1 cm, previously heated in a physiological solution, at the point of boiling. The metallic accessory was applied to the skin for 10-15 seconds. Afterwards, the collagen spongiuous matrix obtained from perch gel and the sterile gauze were applied on the experimentally induced injuries, being fixed with a silk bandage. The evolution monitoring of healing process was observed with a digital camera and the diameter of the lesion was measured at different intervals of time for a period of 16 days.

RESULTS AND DISCUSSION

Rheological Evaluation of Perch Collagen Gel

The results of the rheological study obtained for collagen gel from perch skin (GEL_COLL_A), tested at a temperature of 24°C, led to flow profile plotted as viscosity versus shear rate as shown in Figure 1.

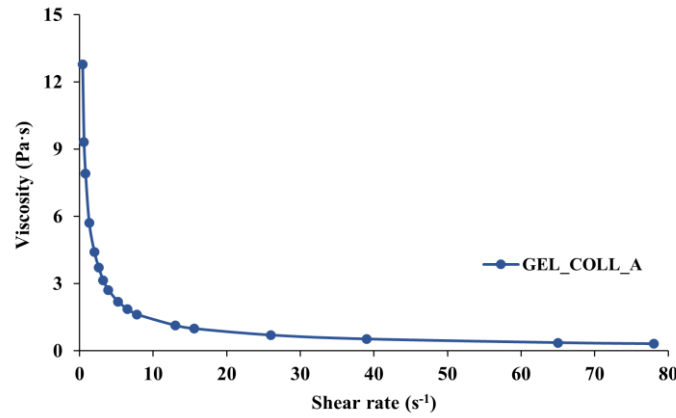


Figure 1. Flow profile – viscosity versus shear rate of GEL_COLL_A, recorded at 24°C

According to the rheogram illustrated in Figure 1, it can be seen that the viscosity of the tested gel decreases when the shear rate increases (Popa *et al.*, 2021), suggesting a non-Newtonian pseudoplastic (shear-thinning) behaviour at 24°C. This rheological feature can be explained by the deformation or orientation of the gel molecular network that are induced by the shear succeeding the flow direction. This effect can become more detectable when the shear rate increases, inducing a decline of the internal friction considering the less interaction between the molecules (Tudoroiu *et al.*, 2023).

The mathematical model used to quantify the shear-thinning behaviour of the perch collagen gel was the Power Law model that describes two principal parameters: m - consistency index factor, and n - flow behaviour index. The constitutive equation of Power Law model is presented by Equation (1) as follows:

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1)$$

The m parameter can be attributed to a Newtonian material viscosity for a shear rate value of $1 \cdot s^{-1}$. On the other side, n parameter indicates the pseudoplasticity degree of a semisolid formulation. Its values range between 0 and 1, and it is dimensionless; accordingly, a material is more pseudoplastic when its n value is lower than 1 (Tudoroiu *et al.*, 2023). Both rheological

parameters are evaluated through the linearization of the previous equation by double logarithm. The Power Law model parameters values for the perch collagen gel tested at 24°C are listed in Table 1.

Table 1. Values of Power Law model parameters for the perch collagen gel, analyzed at 24°C

Gel	m	n	R ²
GEL_COLL_A	6.795	0.68	0.9990

According to Table 1, the determination coefficient R² value higher than 0.99 illustrates that the previously described mathematical model is adequate to properly fit the experimental data. Considering that the pseudoplasticity degree is greatly influenced by the value of the flow index, it can be noticed that the GEL_COLL_A sample has a n parameter value closer of 0.68, indicating a moderate pseudoplasticity character. The pseudoplastic behaviour represents a fundamental requirement for a semisolid formulation that is addressed for topical use, because this property allows a proper flow of the gel when it is expelled from the conditioning container, and when it is administered on skin surface.

An important rheological feature of certain dispersed pharmaceutical formulations is the dependence of the shear stress - shear rate relation on shearing time, but also on their earlier exposure to a shear stress. Accordingly, the formulation viscosity suffers modifications with the shear rate, but if the pharmaceutical system is maintained at rest long enough, its viscosity can restore to the initial value, the modification being reversible. Therefore, this behaviour named thixotropy represents the progressive decrease of a pharmaceutical system viscosity and consequently of its shear stress as a consequence of the agitation caused to the system through shear. After a more or less extended rest period, the pharmaceutical system recovers the rheological characteristics and restores its initial structure, which was impaired during the continuous shear measurements. The thixotropy is a characteristic of dispersed formulations with plastic and pseudoplastic flow (Ghica *et al.*, 2012).

To study this type of flow, the rheogram shear stress as a function of shear rate is plotted, which corresponds to the ascending, S_{asc} (forward) curve for increasing shear rate values, and to descending, S_{desc} (backward) curve for decreasing shear rate values. For the same shear rate, the points on the backward curve are correlated with smaller shear stress values compared to those on the forward curve. Thus, the thixotropy hysteresis loop is obtained.

The rheological profiles corresponding to the ascending and descending curves were built in order to assess the thixotropic behaviour of the perch collagen gel, tested at 24°C (Figure 2).

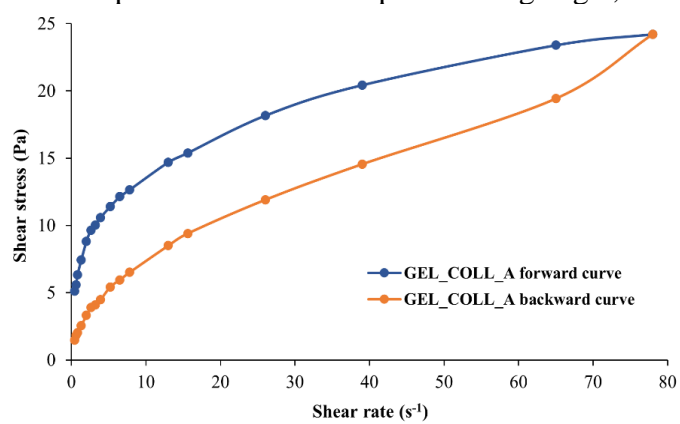


Figure 2. Ascending and descending shear rate versus shear stress curves of perch collagen gel, recorded at 24°C

Figure 2 shows that the perch collagen gel exhibits a thixotropic behaviour at 24°C, because the descending curve is placed below the ascending one, suggesting that the shear stress corresponding to the backward curve is lower at the same shear rate value.

The thixotropy phenomenon can be described by two main descriptors:

- thixotropy area (S_{thix}) represents the area between the ascending and descending curve;
- thixotropy index ($T_{hyst\%}$) defines the thixotropy relative area, which can be determined as percentage of the by the stirring at maximum rotational speed depending on the ascending area. Consequently, a system is more thixotropic if the thixotropy rheoimpaired area index value is greater than 5% (Tudoroiu *et al.*, 2022).

The corresponding values of the thixotropic descriptors for GEL_COLL_A sample, analyzed at 24°C, are shown in Table 2.

Table 2. Values of thixotropic descriptors for perch collagen gel, tested at 24°C

Gel	$S_{asc} (Pa \cdot s^{-1})$	$S_{desc} (Pa \cdot s^{-1})$	$S_{thix} (Pa \cdot s^{-1})$	$T_{hyst\%} (\%)$
GEL_COLL_A	1490.685	1104.387	386.298	25.92

According to Table 2, the value of the thixotropy index is much higher than 5% for GEL_COLL_A sample, which means that the tested semisolid system presents a strong degree of thixotropy at 24°C.

Evaluation of the Spongy Matrix Therapeutic Effect in Healing Process on Animal Model

In Figure 3 are illustrated the macroscopic images regarding the evolution of the healing process, recorded for each group for 16 days.

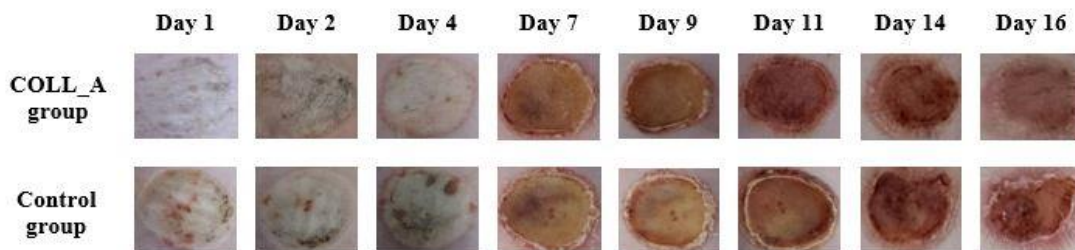


Figure 3. Evolution of the healing process of Wistar rats' lesions without treatment (Control group), and after treatment with spongy matrix from perch gel, at different time intervals

As it can be noticed in Figure 3, on the first day when the burn was induced, the injury can be described as a white eschar, with epidermal and dermal tissue destruction, and a hyperemic area that highlights the presence of an inflammatory process (Marin *et al.*, 2018). According to the above images, the application of collagen spongy matrix, based on collagen extracted from perch skin, led to an acceleration of the healing process compared to the control group.

On the second day of investigation, it was found that the application of collagen spongy matrix conducted to the absorption of the exudate from the wounds. Consequently, on 4th day of animal observation, the initiation of the healing process at the edges of the lesions can be observed. Thereby, seven days after the induction of the burns, the complete formation of the scab for both groups can be noticed.

The evaluation of the healing process was carried out by measuring the dimensions of the wound and was calculated according to Equation (2):

$$\text{Healing process (\%)} = \frac{W_i - W_t}{W_i} \times 100 \quad (2)$$

where W (wound size) generally represents the average of the largest and smallest lesion size, W_i - initial size of the wound, and W_t - wound size at different intervals of time. The healing process was calculated according to Equation (2) and its evolution is shown in Figure 4.

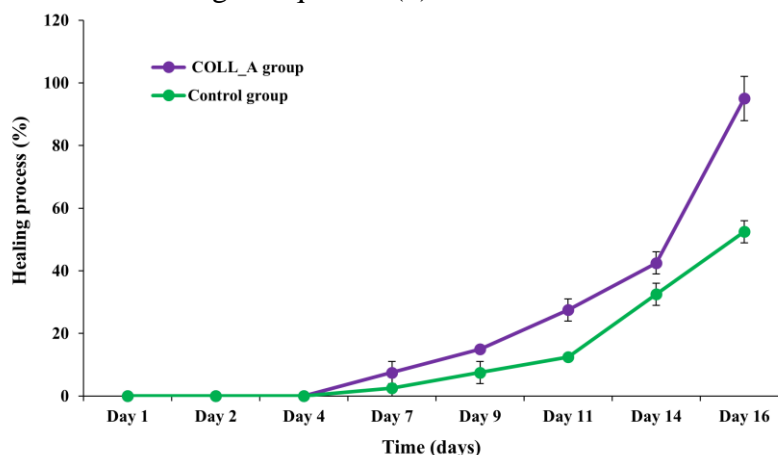


Figure 4. Evolution of the wound healing process after treatment with perch collagen spongiuous matrix and for untreated animals (control group)

According to Figure 4, after 11 days of observation, the lesions size reduced by 27.5% for the animals treated with perch collagen spongiuous matrix (COLL_A group) compared to the control group, where the wounds size reduced by only 12.5%. On 14th day of observation, the wounds size decreased by more than 40% for the COLL_A sample, while for the control group, the lesions dimension decrease was by 32.5%. On the last day of observation, the injuries size reduced by 95% for animals treated with the COLL_A sample, the animals being almost healed compared to the control group where the reduction of the animals' injuries was only by 52.5%.

CONCLUSIONS

Concluding, the rheological analysis of collagen gel extracted from perch (*Perca fluviatilis*) skin evaluated the flow properties by investigating the pseudoplastic behaviour through the analysis of the viscosity - shear rate dependence and by analysing the thixotropic character using the specific descriptors (thixotropy area and thixotropic index). Therefore, perch collagen gel exhibited a non-Newtonian pseudoplastic and thixotropic behaviour at working temperature of 24°C, which are fundamental features for a semisolid system in terms of improving its conditioning and spreadability on epidermal surface.

In vivo animal studies demonstrated that the animals treated with collagen spongiuous matrix from perch skin presented a faster healing, without any secondary effect compared to the control group. Thus, this perch collagen matrix can represent a potential scaffold to treat burns, with valuable effects on the cutaneous tissue's restoration.

Acknowledgement

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MORPHOLOGICAL CHARACTERIZATION OF CHEMICALLY CARBON-CONDUCTIVIZED COTTON

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Solutions of green electric energy production are ever more intensively studied, developed and implemented in different activity areas, like textile industry, as a result of climatic changes. Such energy sources are intermittent by their nature; thus, the energy storage systems are necessary to guarantee the availability of supply at interruptions. The electric charge storage structures, like supercapacitors and batteries, are suitable for e-textiles with storage functionality due to the possibility of obtaining in flexible form. In this work, cotton material was used as substrate for a conductive carbonic deposition to obtain electrodes usable inside a further developed supercapacitor. The treatment was applied on a textile material using a carbonic conductive paste synthesized by a chemical route which has used pristine graphite and activated carbon as raw materials. The morphology of electrodes influences the ion mobility, and so the charging and decaying performance. The size of pores is a very important characteristic in such meaning, and its statistics were found based on the measurements performed by scanning electron microscopy (SEM). The chemical analysis was performed by X-ray energy-dispersive spectrometry (X-EDS), both in spectral and mapping mode, revealing the distribution of elements on surface of the finishing product applied on cotton substrate.

Keywords: energy storage, electrode porosity, conductive textiles

INTRODUCTION

The circular economy concept, which involves the reusing of the materials from goods after their lifetime, is also applicable in electricity production by harvesting the environment renewable energy (e.g. solar, wind, etc.) This approach consists of the so-called green technical solutions which aim to stop the growing of climatic changes and of ecosphere pollution (Ahmad *et al.*, 2023). These energy sources have variable or interruptible features, a fact which leads to the needing the electricity storage systems. Especially at low power consumption, the most effective way to storage is electrochemically, using batteries or supercapacitors.

There are applications where such systems, both for harvesting and storage, must possess flexibility besides efficiency, e.g. wearable technologies. The textile materials are suitable to compose these structures by virtue of the rigidity lacking (Islam *et al.*, 2022).

These storage devices consist of an electrolyte medium placed between two electrodes (cathode and anode), alternatively connected to the direct current generator (G) and to the energy consumer (X), during on the charge or the discharge cycle, as shown in Figure 1.

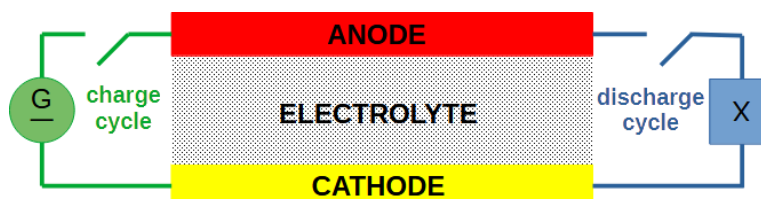


Figure 1. The sketch of electro-chemical storage device

The electrodes require a porous morphology to allow inside a good impregnation of electrolyte, which increases the area of the electrolyte-electrode interface, thus a high storage capacity is available (Salleh *et al.*, 2023). Also, this favors enough ion mobility which enhances the charge-discharge power. The structure of textile materials is very indicated to be involved in manufacturing such electrodes. The conductive finishing based on carbon compounds (graphite, graphene, nanotubes, carbon nanofibers, activated carbon) has shown an enhanced electrical performance (Paleo *et al.*, 2018; Maher *et al.*, 2021; Rădulescu *et al.*, 2023). The activated carbon presents the most usability from such compounds (Maher *et al.*, 2021).

In this work, textile electrodes were fabricated using cotton material as substrate for a conductive carbonic paste previously prepared. The electrode morphology was investigated by SEM followed by the statistical analysis of measurements. The elemental composition of the conductive treatment was performed by X-EDS on an electrode sample, both in spectral and mapping mode.

MATERIALS AND CHARACTERIZATION METHODS

Preparation and Functionalizing

In a one-liter Berzelius beaker (Figure 2), the following reagents were mixed using a glass rod: 45 g graphite powder, 45 g activated carbon powder, 10 g chitosan and 750 mL of 1% aqueous acetic acid solution. Firstly, the acidic solution and chitosan were mixed until the polymer dissolved. Then, the remaining ingredients were added to the obtained solution.

A cotton woven were cut in round substrates which were scoured before functionalizing, having the specific mass of 326 g/m², the thickness of 0.73 mm, and the warp and weft yarn densities of 340 yarns/10 cm and of 220 yarns/10 cm (Rădulescu *et al.*, 2023).

The obtained paste was applied two times on a cotton substrate using a spatula, on both sides, followed by heating at 105°C for 30 min to dry and fix the conductive treatment.



Figure 2. The prepared conductive carbonic paste

Morphological Characterization

The investigation of porous structure is essential to evaluate the suitability of a material for electrode use. While the materials show a variation in pore sizes, a statistical analysis of these measurements is necessary.

The characterization of porous morphology was performed by SEM using FEI Quanta 200 equipment. The image acquisition was made at 15 kV electron acceleration voltage, in top and transversal sample view modes.

The pore sizes were measured using an image processing tool (Scandium software) applied on the images from Figure 3.

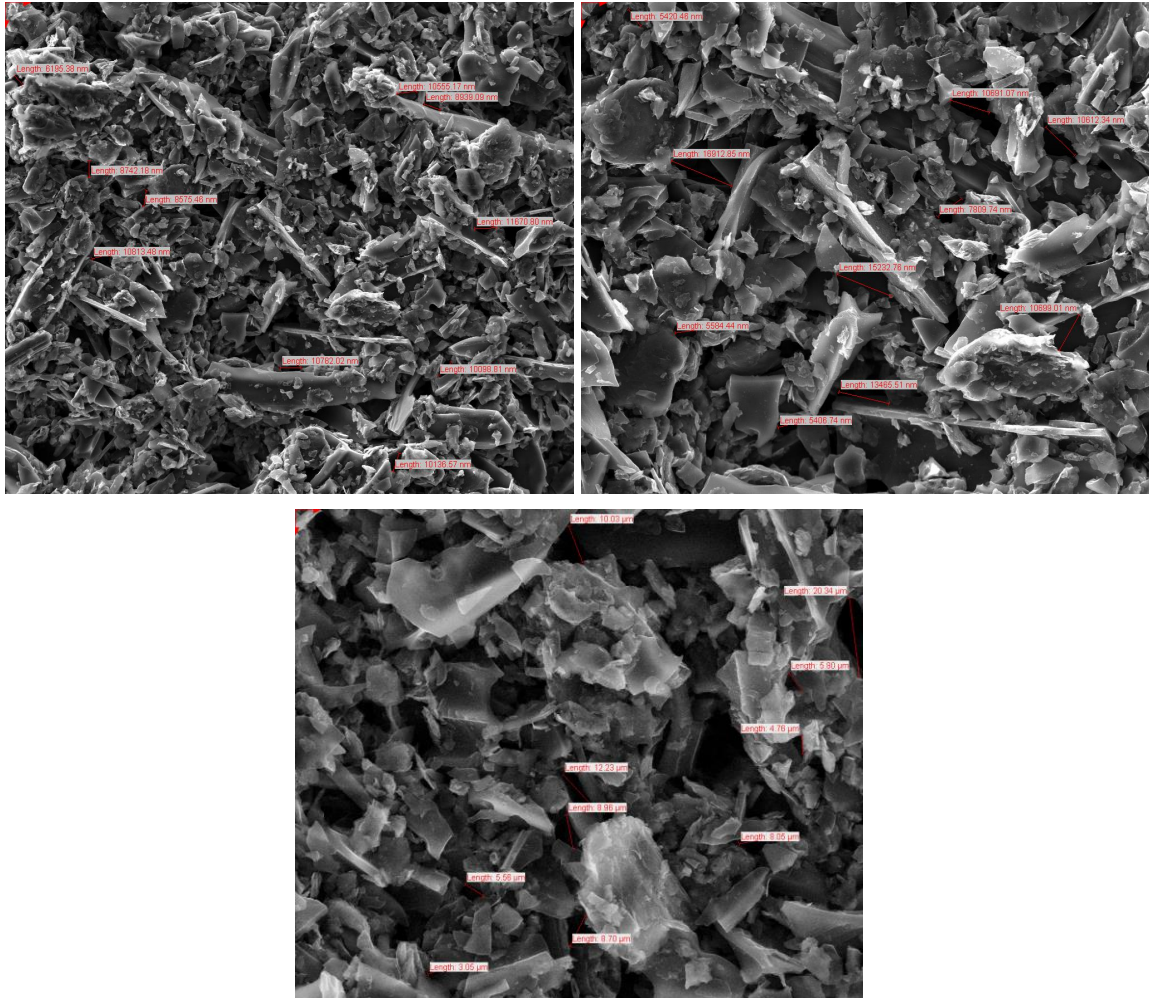


Figure 3. SEM images with porous morphology (pore size measurements)

The porous morphology on electrode surface is revealed in the acquired SEM images, generated by the granular structure of the dried applied paste. The statistical parameters found on the set of the 30 size values from the preceding images are 9.5 μm for average and 3.7 μm for standard deviation (STD), with the corresponding histogram from Figure 4.

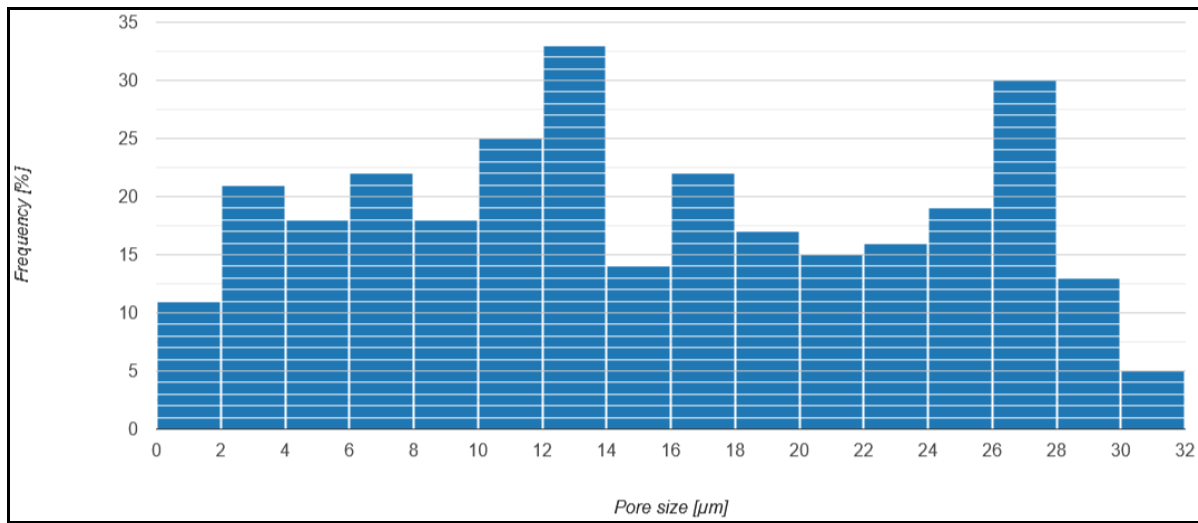


Figure 4. The histogram of pore sizes

The impregnation percentage of carbonic paste inside the textile substrate was evaluated by SEM in transversal section mode measuring the thicknesses of the layers formed after the conductive functionalizing (Figure 5).

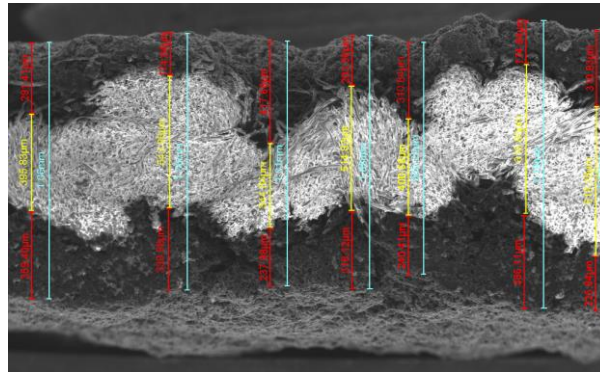


Figure 5. SEM image with layers thicknesses measurements (transversal view)

The transversal structure consists of three layers with variable thickness: one top and one bottom layer formed by impregnation of cotton substrate, and one middle layer of the substrate portion remained unimpregnated. In Table 1, the thickness values measured are shown. The total thickness was separately measured, not by the addition of the layers' thicknesses.

Table 1. Layers thicknesses

	Top layer thickness [μm]	Middle layer thickness [μm]	Bottom layer thickness [μm]	Total thickness [μm]
Average [μm]	269	491	301	1071
STD [μm]	90	111	66	83

Figure 6 shows the SEM images from which the cotton fibre dimensions were measured. In transversal section were found the values of 12 μm, 10 μm and 10 μm for width, and 22, 18 and 16 μm for length. In longitudinal view, the width and length are 8 μm and 16 μm, respectively.

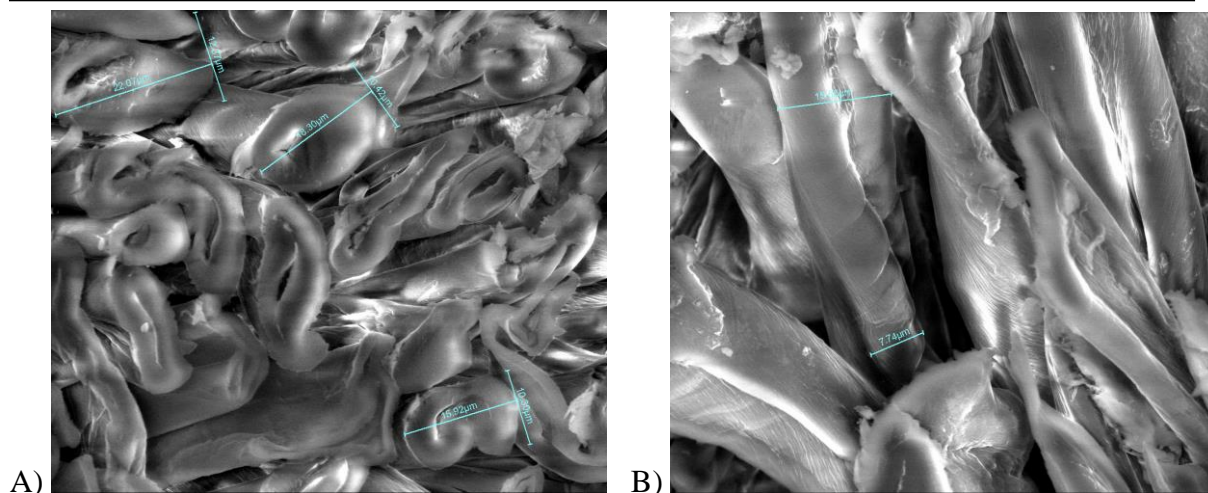


Figure 6. Cotton fibres SEM images: A) transversal section; B) longitudinal

Chemical Analysis

The elemental composition of the compounds used in the preparation of the applied treatment was determined by X-EDS in spectral mode (Figure 7). The concentration of elements, in percentage, agrees with the atomic composition of the ingredients used and of the cotton substrate: 90.23% carbon, 7.34% oxygen, and traces under the detection limit of the nitrogen, aluminum and silicon which were measured to verify the impurities missing.

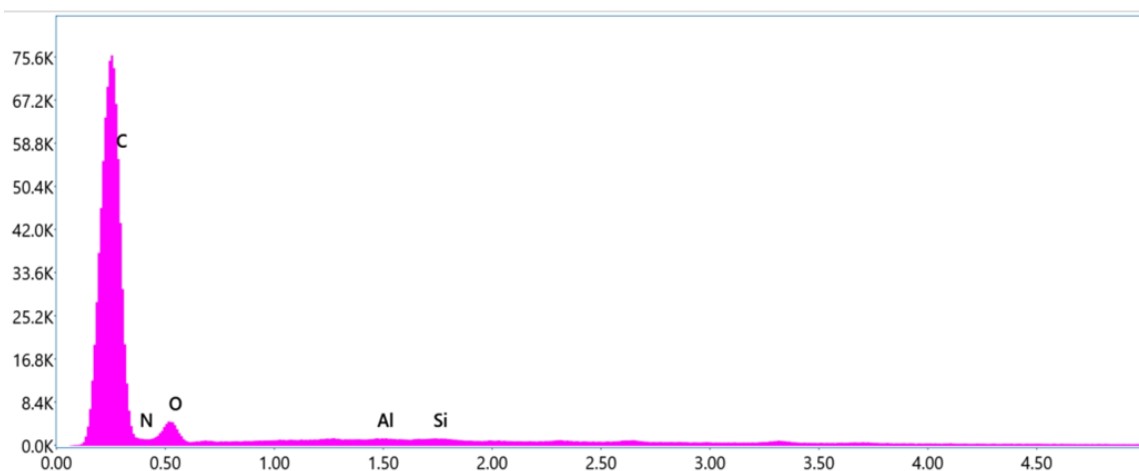


Figure 7. X-EDS spectrum

The X-EDS analysis in mapping mode was performed to visualize the distribution of chemical elements (Figure 8 shown this for the predominant elements of spectrum). Oxygen follows the carbon map because of the organic nature of substrate fibres and of the carbon-based and organic composition of the conductive treatment.

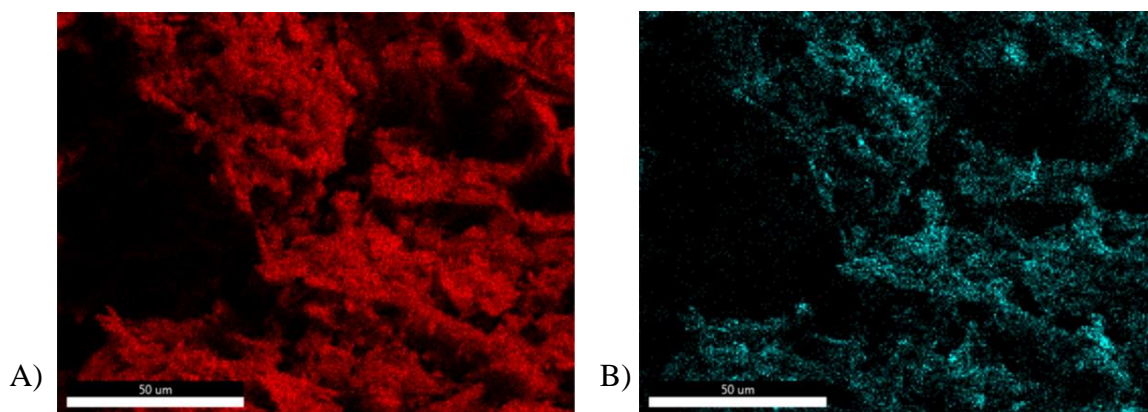


Figure 8. X-EDS maps for carbon (A) and oxygen (B)

CONCLUSIONS

The applied finishing shows an average impregnation depth, inside the textile substrate, of $\sim 285 \mu\text{m}$ and a pore size of $9.5 \mu\text{m}$ average, both with acceptable variations. Therefore, a good filling of the porous structure with an electrolyte solution could be allowed in a further step toward the supercapacitor or battery fabrication, leading to an ion mobility and interface area enough for a performance comparable with non-flexible devices. The found interval of fibre dimensions is placed between $8 \mu\text{m}$ and $22 \mu\text{m}$, thus revealing that the porous structure of conductive layer could only be formed inside the inter-yarns and, at most, inter-fibres spaces. This fact is concluded from the relatively comparable of the fibre dimension interval with the pore size in STD limits.

The electrode presents a clean chemical composition, the elements which could provide from the migration of the environmental mineral contaminants to the carbonic paste are missing in the acquired spectrum.

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BIOSHONES4ALL – INNOVATIVE GREEN MATERIALS, PROCESSES AND PRODUCTS

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The bioeconomy covers sectors and systems that rely on biological resources such as animals, plants and derived biomass and includes industrial and economic sectors that use biological resources and processes to produce bio-based products. A bio-based footwear economy using renewable biological resources sustainably, to produce biomaterials will help the industry reduce dependence on non-renewable resources. It will also address global commitments associated with the United Nations 2030 Agenda for Sustainable Development and the Paris Agreement by ensuring sustainable consumption and production patterns, combating climate change and its impacts. In footwear, the materials used, and waste produced are in general the highest contributors to the total greenhouse gas emissions. Research and innovation are core to pursuing 2030 targets. The sector needs to develop and deploy the ‘next generation’ of sustainable materials and processes, including biological and plant-based materials and man-made bio-based materials. Steps in this direction are being taken within BioShoes4All project that is developing namely leathers tanned with pine tree bark, wet-white tanned leather “bisphenols free”; coated textiles with over 65% biological carbon; soling materials incorporating up to 80% bio content; recyclable physically expanded lightweight soles, and new concepts of footwear, all tuned using life cycle assessment (LCA) in order to reduce the fossil, carbon or environment footprint.

Keywords: Footwear, bioeconomy, environmental footprint.

INTRODUCTION

The world is facing challenges that invite the footwear sector to accelerate its efforts to be sustainable by adopting inclusive, green and digital sustainable solutions and business models. Fashion businesses need to be resilient and help minimize depletion of the planet’s resources, greenhouse gas emissions, and climate change and prevent complex problems such as those posed by fossils overspend and hazardous substances. Globally regarding environmental issues footwear will benefit from tackling the products design, the impact of materials, production and distribution processes, recycling, and water, fossil resources and land usage. At consumption level, it calls for durable long-lasting goods, repair, reuse and circularity.

There are many potential areas of intervention and significant research and innovation needs to be done. Firm steps are being accomplished within BioShoes4All. It includes five areas of intervention: Biomaterials, Ecological Footwear, Circular Economy, Advanced Production Technologies, Training and Promotion (Figure 1), and it is developing new leathers, bio-based materials, recycled materials, traceability tools, production systems, footwear concepts and business models.

The project comprises 50 companies covering the whole footwear value chain including leather, polymers, soles, software, production equipment, leather goods, footwear and retail representation and leadership, plus 20 R&D bodies with complementary capabilities, coordinated by the CTCP, led by the Portuguese Footwear Cluster (Figure 2), and cofinanced by Next Generation EU and the Portuguese Recovery and Resilience Plan.



Figure 1. BioShoes4All main areas of research and intervention



Figure 2. BioShoes4All consortium and cofinancing

OBJECTIVES, METHODS AND RESULTS

Innovative Tannins and Leathers

Pine Tree Bark Tanned Leathers

Hides are biological *per se* but need to be chemically stabilized to be used in footwear. The stabilization process involves several steps being the tanning phase of the utmost importance. Tanning is done mainly using metals specially chromium, fossil derivatives, and to lesser extent using vegetable tannins, in many cases imported to Europe.

Chromium (III) tanning has been under scrutiny due to the possibility of oxidizing to chromium (VI) and hindering leather waste and post-consumer products recycling. In response to this concern, a great deal of research is carried out in recent years. BioShoes4All defined as target to develop up to 100% biological tanned leathers using local and agri-food, agro-industrial or forest biomass. Several possibilities were identified and are under study by leather and chemicals producers and R&D teams from ISEP, CTIC, UCP and CTCP.

These teams are working complementarily to identify useful biomass (e.g., composition, quantity), characterize, pretreat and extract tannins from different biomass (e.g., pine, olive,

coffee), develop bioproducts that can effectively tan the hides, develop the tanning processes, develop leathers with adequate functional and organoleptic properties, and carry out the new materials life cycle analysis.

Interesting results are being obtained using *Pinus pinaster* pine tree bark (Figure 3). These vegetable tanned leathers present good surface appearance and tear resistance and retraction temperature values of over 100 N and 70° C, respectively. Industrial application tests indicate may be used to produce footwear and leather goods.



Figure 3. Leather tanned with *Pinus pinaster* bark modified extract

Wet-White Bisphenol 'Free' Leather

Bisphenols have become an important topic of discussion as regards leather and footwear. Bisphenols are a family of very similar synthetic organic chemicals with two hydroxyphenyl functional groups that allow polymerization reactions to form larger molecules. Each type is identified by a CAS number, including Bisphenol-A (CAS 80-05-7), Bisphenol-B (CAS 77-40-7), Bisphenol-F (CAS 620-92-8) and Bisphenol-S (CAS 80-09-1). According to the project partners knowledge, none of these bisphenols are used directly as raw materials in leather manufacturing processes. However, Bisphenol-F and Bisphenol-S may potentially be found in some condensed chemical products as unwanted by products of secondary reactions of their production processes. In the leather industry, these condensed chemicals, or syntans, are mainly used in pre-tanning and retanning processes to enhance important leather characteristics such as softness, tear resistance or whitening effect. For these reasons, replacing them while maintaining the leather's performance represents a challenge.

This challenge inspired the project R&D team, led by Indutan, a sheep and goat leather manufacturer, to develop durable and functional leathers with bisphenol levels below 500 ppm (parts per million). Exhaustive work to carefully test, review and develop new leather processing recipes, resulted in new leather products in the high added values range with low bisphenol F and bisphenol S values, which fulfil the required specifications. Two examples of the new leathers' properties are shown in Table 1. From the results obtained, can be appreciated the new leathers are very similar to those previously produced by the company, and are ready to be produced on an industrial scale.

Table 1. New wet-white leathers bisphenol ‘free’ indicative properties

Parameter	Base	New 1	New 2
Bisphenol F(mg/kg)	BQL	58	40
Bisphenol S(mg/kg)	>10000	69	16
Shrinkage temperature (°C)	85	72	72
Leather “flower” strength - elongation (mm)	8.3	13.2	14.6
Leather “flower” load resistance (N)	165	328	446
Tear strength (N)	61	91	54
Elongation at break (%)	53	67	68
Tensile strength (N/mm ²)	19.3	37	18.6

BQL – Bellow quantification limit

Bio-Based Coated Textiles

In recent years the consumers demand for footwear made without leather has resulted in the search for biobased coated textiles which favor raw materials of biological origin. It is now possible to synthesize polyurethane coatings partly from raw materials of plant origin such as vegetable oils modified into polyols, which are then polymerized with reagents of fossil origin.

Portugal is one of the largest producers of olive oil and the actual olive stone represents an abundant biomass estimated to be 60,000 tons per year and currently without any other use other than burning to create energy. This has contributed to it being chosen by Monteiro Fabrics as a bio-filler to be incorporated into bio coating formulations. The ORIGIN material created within the BioShoes4All project is the result of the development of a BioTPU and water-based formulation containing olive stones applied to a cotton fibre textile base. Some relevant physical and mechanical characteristics are shown in Table 2 and fulfil the specifications for shoe uppers. It also has a proven 72% bio-based carbon content in accordance with ASTM D6866-22 Method B.

Table 2. ORIGIN olive composite physic-mechanical properties

Parameter		Specifications fashion shoes	Results
Tensile Strength (N/mm)	T	> 10	11.3
	TR	> 5	6.6
Elongation at break (%)	T	> 50	62.7
	TR	> 100	115.6
Tear strength (N)	T	> 25	29.4
	TR	> 20	26.2
Adhesion to the coating (N/5cm)	T	> 1	1.19

T – Warp, TR – Weft

The company, together with local partners, is also valorizing chestnuts byproducts. These biomaterials are crushed and ground into a fine powder and turned into an innovative material. The PEEL collection incorporates 60% plant-based materials including chestnut waste, organic cotton and natural oils, and 40% PVC. The material allows customers to create products without having to choose between cost, performance, aesthetics or sustainability. It is a technologically viable product, ready to be produced on a large scale and without compromising its performance.

Technical requirements for use in cemented footwear are all met including high Bally flex resistance (150000 cycles), Martindale abrasion (400000 cycles), resistance to friction (Crockmeter), among others. Peel’s 65% bio-based carbon content has been verified in accordance with ASTM D6866-22 Method B. Additionally, the material Life Cycle

Assessment (LCA) made by CTCP based in the EU footwear draft Product Environmental Footprint (PEF) methodology indicates a reduction of 19% in fossils resources used and a reduction of 9% of its carbon footprint (Figure 4; LCA from raw material extraction to PEEL production).

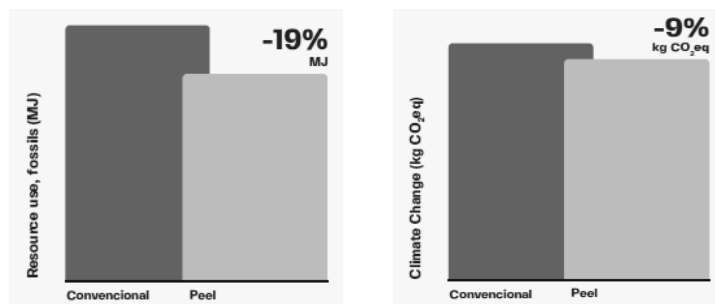


Figure 4. Biobased coated textile fossils resources and carbon footprint reduction

Bio and Ecosoles

BioPVC

The sustainability of materials has achieved unprecedented importance, particularly in the search for biobased and/or recyclable approaches. This is motivating BioShoes4All project partners to develop materials with high biobased content, that can be processed by injection molding to produce flexible and recyclable soles and complete footwear. One example is the LCR Coblex bioPVC incorporating bio plasticizers and additives claiming up to/more than 80% biobased content and the same level of performance of conventional materials, in terms of hardness, density, and flexion and abrasion resistances (Table 3). Additionally, the material LCA made by CTCP indicates a reduction of 36% in fossils resources used (LCA from raw material extraction to BioPVC pellets).

Table 3. BioPVC physical properties

Parameter	Specification	Results
Hardness (ISO 868, Shore (A))	60 - 65	63 - 64
Density (ISO 2781-met A, g/cm ³)	1.18 - 1.22	1.19-1.2
Abrasion resistance (ISO 20871, mm ³)	<250	106 - 115
Ross flex resistance (BS5131-Part 2.1, mm)	<0.4	0.0

E-blast “Super Critical” Physical Foaming TPU Material

An innovative approach is proposed by ALOFT that implemented after a detailed research and development with national and international partners, to the best of our knowledge, the first system used in the footwear and allied trade sectors in Europe to produce footwear components by E-blast “Super Critical” physical foaming process (N₂). The preliminary LCA analysis of E-Blast TPU done by CTCP indicates a Carbon footprint reduction of 75% comparing to standard compact TPU (Figure 5). Comparing to usual PU foam the new material has the advantage of being thermoplastic thus more easily recyclable and having no residues from chemical foaming agents in the final products. Physical tests are being carried out to verify the material properties after grind/injection cycles.

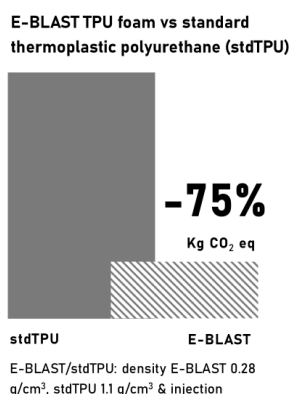


Figure 5. E-blast “Super Critical” foamed TPU carbon footprint reduction

BioShoes

BioShoes4All partners are developing concepts of footwear based in ecodesign approaches and new materials aiming to reduce products footprints based in the EU draft footwear Product Environmental Footprint (PEF) methodology. The PEF method assesses 16 impact categories (Table 4), covering climate change, acid rain, human toxicity, and particulate matter as well as impacts due to the use of water, land, and resources.

Table 4. Footwear environmental footprint impact categories assessed

EF Impact Category	Impact category Indicator	Unit
Climate change, total + fossil + biogenic + land use and land use change	Radiative forcing as global warming potential (GWP100)	kg CO ₂ -eq
Ozone depletion	Ozone Depletion Potential (ODP)	kg CFC-11-eq
Human toxicity, cancer	Comparative Toxic Units for humans	CTUh
Human toxicity, non-cancer	Comparative Toxic Units for humans	CTUh
Particulate matter	Impact on human health	disease incidence
Ionising radiation, human health	Human exposure efficiency relative to U235	kBq U235-eq
Photochemical ozone formation, human health	Tropospheric ozone concentration increase	kg NMVOC-eq
Acidification	Accumulated Exceedance (AE)	mol H ⁺ -eq
Eutrophication, terrestrial	Accumulated Exceedance (AE)	mol N -eq
Eutrophication, freshwater	Nutrients reaching freshwater end compartment (P)	kg P-eq
Eutrophication, marine	Nutrients reaching marine end compartment (N)	kg N-eq
Ecotoxicity, freshwater	Comparative Toxic Unit for ecosystems	CTUe
Land use	Soil quality index and others	Dimensionless
Water use	User deprivation potential	m ³ world eq
Resource use, minerals and metals	Abiotic resource depletion	kg Sb eq
Resource use, fossils	Abiotic resource depletion – fossil fuels, ADP	MJ

As shown in one example in Table 5, frequently, most relevant impact categories include “Climate change”; “Fossil resources use”; and “Minerals/metals resources use”.

Table 5. Footwear most relevant impact categories, stages and process (example)

Impact category	% Contribution	Life cycle stage	% Contribution	Component	% Contribution
Climate change	24,0%	Raw materials in final product	55,5%	Outsole	22,3%
				Insole	8,0%
				Interlayer	7,8%
				Insock	6,9%
				Upper	3,5%
		Raw materials that go to waste	3,0%	Interlayer	1,4%
				Waste	15,7%
		End of Life	7,3%	Transport	3,8%
Resource use, fossils	16,8%	Raw materials in final product	68,0%	Solid waste	3,2%
				Outsole	35,1%
				Insole	9,9%
				Insock	7,5%
		Waste	15,4%	Interlayer	5,6%
				Waste	12,0%
Resource use, minerals and metals	16,1%	Raw materials that go to waste	2,8%	Interlayer	1,3%
				Waste	84,0%
		Waste	90,9%	Waste	84,0%

The data obtained (Table 5) also details the environmental impact associated to the product “Life cycle stage” and “Materials, components and/or processes”, giving indications to make changes to reduce the products PEF. Among these, “Climate Change” is often chosen to present and discuss the environmental impact of shoe models. Figure 6 presents the results of the Climate Change impact category, Global Warming Potential indicator (GWP100), in kg CO₂ eq, calculated for an example pair of footwear before and after redesign. Within this study was possible to reduce the selected model carbon footprint (kg CO₂ eq) up to 36% considering the more sustainable version.

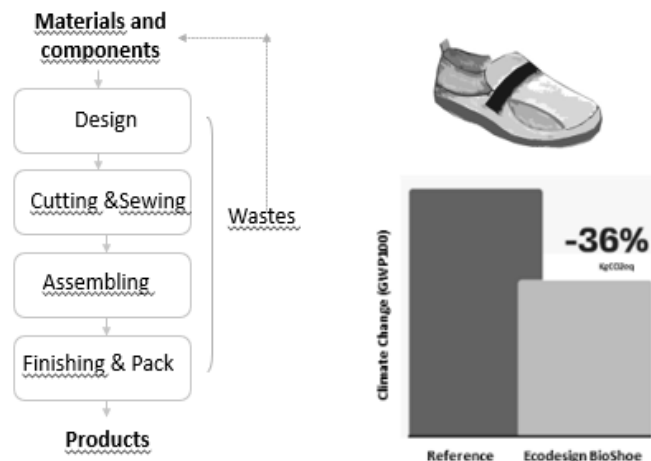


Figure 6. Redesigned footwear and carbon footprint reduction

CONCLUSIONS AND FUTURE WORK

The world is facing challenges that invite the footwear sector to accelerate its efforts to be sustainable by adopting inclusive, green and digital sustainable solutions and business models. Fashion businesses need to be resilient and help minimize depletion of the planet's resources, greenhouse gas emissions, and climate change and prevent complex problems such as those posed by fossils overspend and hazardous substances.

BioShoes4All is researching and deploying sustainable solutions for the footwear value chain. Globally regarding environmental issues the sector benefit from using bioeconomy renewable resources and adopting circular business models, tackling the products design, the impact of materials, production and distribution processes, recycling, and water, fossil resources and land usage. At consumption level, it calls for durable long-lasting goods, repair, reuse and circularity.

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For more information see the websites recuperarportugal.gov.pt. #ContruirOFuturo & <https://bioshoesforall.pt/>



EXPLORING THE EDUCATIONAL POTENTIAL OF VIDEO GAMES IN THE DIGITAL AGE

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In today's world, technology is a part of people's lives more than ever before. This has also led to the development of video games, which are used not only for relaxation and entertainment but also as a potential educational tool. As traditional methods used by teachers are becoming increasingly less engaging for students who are so accustomed to technology, digitalized education and the use of video games could improve the quality of the educational process. This paper investigates the impact of video games on education and analyzes how they can be effectively integrated into the teaching-learning-assessment process. The main objective is to explore the educational potential of video games, highlighting their benefits and challenges. The study employs a mixed-method approach, combining a review of relevant literature with case studies and interviews with teachers who have integrated video games into their educational programs. The results indicate that video games can enhance students' motivation, facilitate active learning, and develop cognitive and social skills. However, their effective use depends on the proper selection of games and their integration into activities that allow students to develop specific skills, not just have fun. The study contributes to the understanding of how video games can be used as an innovative pedagogical tool, offering new perspectives on their implementation in education. Video games represent a unique opportunity for education, but they require a careful and well-planned approach to maximize educational benefits and minimize potential risks.

Keywords: video games, digital education, interactive learning

INTRODUCTION

The 21st century is often considered the era of technology, which plays a crucial role in our lives today. Technology simplifies and streamlines many aspects of our work. Its influence is evident in almost every field, including education (Raja & Nagasubramani, 2018).

A considerable amount of financial resources are allocated to technology by schools, families and policy makers in the hope of improving educational outcomes. In addition to the investments made by schools, central governments often play an important role in providing or subsidizing computer equipment and Internet access (Bulman & Fairlie, 2016).

THE ROLE OF TECHNOLOGY IN MODERN EDUCATION

ICT, or "Information and Communications Technology," refers to technologies that facilitate access to information through telecommunications. Although similar to information technology (IT), ICT focuses mostly on communication technologies, including the Internet, wireless networks, mobile phones, and other means of communication. Currently, we have more opportunities to integrate ICT into teacher training programs, thus contributing to improving the quality of teaching. Effective use of these technologies in teacher training can significantly increase the quality of the educational process. A well-structured training program is essential to meet the needs of contemporary teachers who want to learn how to use ICT effectively in their teaching work (Ratheeswari, 2018).

Today, knowledge and information are essential for productivity, competitiveness and development. For this reason, countries focus on improving the quality of education, essential

for the development of human capital. It is important that education keeps pace with the rapid developments of the world, and information technology plays a central role in this process, offering new opportunities, especially through collective communication. However, developing countries fear that they are being left behind in the “Information Revolution” by allocating funds to technology without sufficiently preparing the ground for its effective use.

Modern education requires simple and effective technologies to meet its needs, and to properly integrate IT, policies are needed that include: expanding IT skills through educational programs, using IT to make institutions more efficient, supporting IT research, creating an appropriate climate for the use of IT in education and the promotion of collaboration between various institutions. It is also important to expand the culture of using IT in education, constantly evaluating their needs and effectiveness (Hamidi *et al.*, 2011).

Most educators will make the effort to integrate technology into their teaching only when they are confident that it will lead to substantial improvements in student learning outcomes. Therefore, for technology to drive educational change, it is essential to understand what learning outcomes can be improved by technology and the conditions under which these improvements can be achieved in practice. Providing clear guidance on how to effectively implement technology to enhance student learning is critical to its use as a catalyst for change in education (Means, 2010).

Video Games as an Educational Tool

Video games have become deeply integrated into the daily lives of millions of young people around the world, having a considerable impact on today’s culture and society. However, many authorities and most educators initially rejected them, focusing on the perceived negative effects. However, over the past two decades, extensive research has led to a more detailed, nuanced, and in-depth understanding of the impact of video games (De Aguilera & Mendiz, 2003).

Serious video games are valuable tools for learning specific strategies and acquiring knowledge, while contributing to the development of skills associated with the culture of the information society. This form of learning has the potential to have long-lasting effects (Gros, 2007).

Here are some examples of games developed for educational purposes as a result of this effort. Immune Attack (<https://www.sciencegamecenter.org/games/immune-attack>), a serious educational game created by FAS, Brown University and the University of Southern California, is a first-person strategy game designed to teach complex biology and immunology concepts to students by an alternative method.

Another example is Food Force (<https://www.myabandonware.com/game/food-force-m1f>), an educational game developed by the United Nations World Food Program in 2005. In this game, users are involved in food distribution missions in a country affected by famine, with the aim of helping her recover and become self-sufficient. Players assume the role of a scientist in a team of United Nations experts, including nutritionists, logistics officers, pilots, call officers and food procurement directors (Annetta, 2008).

Although video games and simulations (edutainment) are increasingly used in education, their mechanisms are still poorly understood. Most research has focused on comparing games to traditional teaching, but this approach can be problematic because each method represents different pedagogical strategies. These methods reflect distinct values of the instructional designer and are appropriate for different types of learning experiences (Squire, 2003).

Using Video Games in the Classroom

In 2002, a study at a public high school in Georgia evaluated AquaMOOSE 3D, a graphical environment designed to facilitate the exploration of three-dimensional mathematical concepts. The goal was to capitalize on the entertainment aspect of video games to create a more immersive learning environment compared to traditional methods. The research was conducted through a quasi-experimental study, comparing the AquaMOOSE intervention with classical curriculum-based instruction.

The researchers evaluated the impact of AquaMOOSE on students' understanding of the material in an experimental classroom. Four of the eight students interviewed found the software useless for learning, with some reporting that it was confusing and did not improve their understanding of mathematics. Although a few recognized some advantages, such as a clearer view of mathematical concepts, most of the feedback was negative.

End-of-year polls reflected similar views, with some students expressing dissatisfaction with AquaMOOSE, finding it confusing and ineffective. The discussion points out that prior to the study, students in the control group had expressed a desire to use the software and had high expectations due to their familiarity with video games. This created a sense of inequity as the control group did not have access to AquaMOOSE until the study was completed (Elliott *et al.*, 2002).

Video games are seen as promising for teaching and learning in the 21st century, but their acceptance by students is not guaranteed. A study of 858 high school students used an analytical model to examine and predict their acceptance of video games.

The results showed that the students do not form a homogeneous group in terms of the use of video games, there are notable differences between the groups. Some students do not play video games at all, and gender differences are evident, with males having a more favorable attitude toward the use of video games in education compared to females. The Technology Acceptance Model (TAM) has proven useful in the context of learning through video games, emphasizing the importance of perceptions of usefulness and ease of use. These results are consistent with other studies of technology acceptance in education, but contradict previous research on video games for entertainment, where perceived usefulness had less impact.

The study identified learning opportunities as a crucial factor in determining student preference for video games in the classroom, along with ease of use and usefulness. It is important to distinguish between utility and learning opportunities because they have a process-product relationship in education. Gender differences are not directly related to video game preference, being mediated by experience and ease of use, with experience having a significant role in shaping students' perceptions (Bourgonjon *et al.*, 2010).

Another study exploring the relationship between adolescents' video game use and their school behaviors and performance in different settings was conducted on a sample of 508 adolescents, boys and girls, randomly selected from secondary schools in Tehran, Iran. Data were collected through a questionnaire completed by parents at the end of the school year regarding school performance, student behavior, and video game use. Data analysis was performed using descriptive statistics, the contingency coefficient, and chi-square tests.

The results indicated that most teenagers are interested in video games, with 76.8% of them playing occasionally. Girls with older mothers tend to use video games more frequently than those with younger mothers. Boys whose mothers are homemakers and who have portable devices in their rooms spend more time playing video games and show higher levels of aggression. In addition, girls who frequently play video games exhibit unusual behaviors and a different mental state. Both boys and girls reported a significant percentage of yelling and binge eating related to time spent playing video games.

The table below records data on the behavior of students playing video games.

Table 1. Variables related to the effects of video games on behavior and school performance

Variable	Option	Total
Student's ability to talk to his/her parents about his/her problems	Very much or much	323(65.5%)
	Moderate	136(27.6%)
	Little or very little	34(6.9%)
Student's behavioral and mental status	Hyperactive	29(5.9%)
	Too happy	149(30.5%)
	Normal	310(63.6%)
The sum of effect of the use on student's behavioral & mental status	Very much or much	53(11.2%)
	Moderate	127(26.7%)
	Little or very little	240(50.5%)
	I do not know	55(11.6%)
The quantity of change in student's behavior due to game	Too violent	11(2.4%)
	Moderately violent	99(21.7%)
	Not become violent	347(75.9%)
The quantity of change in student's self-control due to games	Completely loosed	6(1.3%)
	Moderately loosed	69(15.1%)
	Without change	381(83.6%)
The amount of student's dreaming due to games	Very much or much	23(4.7%)
	Moderate	67(13.8%)
	Little or very little	161(33.1%)
	He/she does not dream.	235(48.4%)
Overeating and shouting during using games	Overeating	27(5.6%)
	Shouting	66(13.6%)
	Both of them	27(5.6%)
	None of them	365(75.2%)
The quantity of change in student's scholastic performance due to games	Better than past.	27(5.9%)
	Worse than past.	53(11.5%)
	Not changed	380(82.6%)

Source: adapted from Dirandeh *et al.*, 2015

Thus, video games have a considerable impact on adolescents' behavior, but not on their school performance. In addition, social factors influencing health significantly affect how adolescents interact with video games (Dirandeh *et al.*, 2015).

METHODOLOGY

The study employs a mixed-methods approach to investigate the educational potential of video games by analyzing existing studies on the use of video games in education to identify their benefits and challenges, examining cases where video games have been integrated into educational programs to assess their impact on student motivation and learning, and conducting interviews with eight teachers from Romania, representing both private and public schools, to gather their opinions on the use of video games in education. The interviews explored the frequency of use, perceived advantages and disadvantages, and the types of video games considered suitable for lessons. The collected data are analyzed to provide insights into how video games can be effectively integrated into the teaching and learning process.

ROMANIAN TEACHERS' OPINION REGARDING THE USE OF VIDEO GAMES IN EDUCATION

A number of 8 teachers were interviewed regarding the integration of video games in the courses they taught. A number of 5 teachers teach in a private school, and 3 of them teach in a state school, both from Bucharest, Romania. The interviews took place by means of telephone discussions; the answers of the teaching staff being noted by the researcher during the discussions. The interviews took place in August 2024, according to an interview guide made by the author of the present study. The interview guide was composed of six main questions. Below are the collected questions and answers.

1. *Do you think that video games can contribute positively to the educational process? If so, in what way?*

Teachers' responses to this question varied, reflecting both their enthusiasm and skepticism about the benefits of using video games in the educational process. Those who argued in a positive way for the use of video games highlighted the fact that they can help improve student motivation and facilitate learning through interactive experiences. For example, one of the teachers at the private school mentioned that he used video games to explain some difficult physics concepts. He also said he has seen an increase in students' interest in his subject following the use of video games.

2. *How often have you used video games in class?*

All of the 8 teachers claimed to have used video games in the classroom at least once. However, only 3 of them said they used them consistently. This may be due to the specifics of the subject taught by the teacher, his skills, the interest of the students or other factors related to the materials and teaching aids.

3. *What do you think are the main advantages and disadvantages of using video games in education?*

One of the main advantages mentioned by all the teachers was related to the increase in students' motivation and interest in the taught subject. Also, many teachers have mentioned that learning has become much more practical due to the fact that in video games you can simulate various real or hypothetical situations that put students in various poses from which they can learn more easily. In addition, depending on the discipline taught, teachers also mentioned the development of certain skills through video games such as: critical thinking, problem solving, creativity, decision making, but also communication and teamwork skills.

Some teachers expressed concern that video games could be distracting or addictive to students, stressing the importance of using them sparingly and within well-defined educational contexts. A teacher from the state school pointed out the logistical difficulties and lack of resources needed to implement these methods in mainstream education, adding that continuous training of teachers would be essential for the success of this initiative.

4. *Are there certain types of video games that you think are more suitable for use in lessons? If so, what are they and why?*

The teachers interviewed provided some examples of suitable video games for use in the classroom. It should be noted that, depending on the discipline taught, each teacher mentioned a certain type of game. The table below shows the games listed by the teachers and their type.

Table 2. Examples of video games used by teachers

Serious games	Simulation Games	Adventure and Interactive Storytelling Games:	Augmented Reality (AR) and Virtual Reality (VR) Games
Minecraft Education Edition	The Sims	Life is Strange	Google Expeditions
Working with Water	RollerCoaster Tycoon	Tell Me Why	Tilt Brush
Little Learning Machines	Flight Simulator		VR Lab

Source: author's own research

5. *How do students react to the integration of video games in the learning process? Have you noticed a change in their academic engagement or performance?*

All the teachers reported observing a significant increase in student engagement when video games were integrated into the learning process. They also noted that video games contributed to a deeper understanding of difficult concepts, as students could apply theoretical knowledge in a practical and interactive way. On the other hand, some teachers mentioned that although student engagement increased, they did not always see a significant improvement in academic performance. They also highlighted that integrating video games into the curriculum must be done carefully, emphasizing content and utility, so that the games do not become a distraction but an effective learning method.

6. *To what extent do you think additional teacher training is needed to effectively use video games in education?*

All the teachers agreed and even strongly supported the fact that teachers in Romania need training courses to help them use video games in the classroom as effectively as possible to increase the quality of the educational act.

Therefore, the opinions of the teachers interviewed suggest that, although there is an increased interest in the use of video games in education, their widespread implementation would require not only material resources, but also a change of mentality among teachers and decision-makers in the educational system. It is also necessary to train teachers so that they can use technology in the classroom in the most effective way.

CONCLUSIONS

In conclusion, video games possess significant educational potential, offering both advantages and challenges when integrated into the learning process. The literature review indicates that video games can boost student engagement and motivation, although concerns about their effectiveness and the risk of distraction remain. Case studies show that, with careful implementation, video games can positively influence student learning outcomes, especially in terms of motivation and active participation. Interviews with Romanian teachers reveal a cautiously optimistic view, with educators acknowledging the potential of video games to enhance traditional teaching methods. However, they stress the need to choose appropriate games and maintain a balanced approach to ensure educational goals are achieved. Overall, the study suggests that video games can be a valuable educational tool, but their integration requires thoughtful planning and consideration of both their benefits and potential challenges.

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THE EFFECT OF CERIUM DIOXIDE NANOPARTICLES ON THE *Bradyrhizobium japonicum* POPULATION

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One of the effective approaches involves the use of biological compositions containing nitrogen-fixing bacteria, particularly from the genus *Bradyrhizobium*, and cerium dioxide nanoparticles (CDNs), which prove particularly promising for biological research due to their minimal toxicity. The object of the research was the soy rhizobia strain *Bradyrhizobium japonicum*, which was deposited in the D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine under registration number IMV B–7194. The work used CDNs, particle size 4–6 nm, $\xi \approx +10$ mV. Methods of regression analysis of variance and experimental designs were used to assess dependence between bacterial titer of *B. japonicum* IMV B–7194 and several factors: CDNs concentration, substrate (mannitol) concentration and duration of culturing. Statistical analysis was carried out using TIBCO Statistica 14. On the basis of the obtained model, it was established that the duration of exposure (cultivation) of CDNs was the most important factor ($F = 8.08$, $p \leq 0.001$), with its increase, the number of cells increases linearly. At the same time, an increase in CDNs concentration ($F = 3.0722$, $p \leq 0.006$) should lead to a decrease in cell titer compared to the positive effect of mannitol ($F = 6.19$, $p \leq 0.001$), with other factors constant. It was determined that the effect of the studied nanoparticles in different concentrations had a non-linear dose-dependent nature.

Keywords: cerium dioxide nanoparticles, nitrogen-fixing bacteria, *Bradyrhizobium*.

INTRODUCTION

Today, among the pressing agriculture problems technogenic influence and overloading of soils, which lead to the death of their nitrogen-fixing biota and the appearance of extraneous, including pathogenic microorganisms, dangerous for plants and people, are attracting more and more attention. One of the consequences is also the imbalance of nitrogen and its transformations in agroecosystems. This process can have a negative impact on the development and yield of cultivated plants, in particular, the important leguminous fodder crop of soybeans (Suman *et al.*, 2022).

Considering the need to grow soybeans and the problem of nitrogen imbalance in the soil, there is an urgent need to create modern ecological technologies. An effective method is the use of biological formulations (Hadas, 2014). Currently, the world market has a wide range of inoculants based on nitrogen-fixing bacteria for the processing of leguminous crops, including soybeans. Pre-sowing seeds inoculation with biological preparations based on nitrogen-fixers contributes too many factors, in particular: release of antibiotic substances by prokaryotes, which prevents infection by phytopathogens; increasing the photosynthesis activity; increasing the yield of the crop compared to the crop without treatment; increasing protein accumulation in legume seeds. In addition, the use of bacterial inoculants is an alternative to mineral nitrogen fertilizers, the production of which is quite energy consuming and expensive (Agbowuro *et al.*, 2021).

Nodule bacteria are most often used for the production of inoculants. These microorganisms have an individual selective ability to infect different types of leguminous

plants, i.e. specificity to a certain species. Thus, only bacteria of the genus *Bradyrhizobium* are able to form symbiotic relationships with soybeans and nodules on the roots of the plant (Bogino *et al.*, 2015). Therefore, the basis of inoculants is often the species *Bradyrhizobium japonicum*, which is actively used in the production of bacterial inoculants for the pre-sowing treatment of soybean seeds. In addition, this species is used by science as a model organism for which genetic engineering methods are actively being implemented to create new highly efficient nitrogen-fixing strains capable of increasing the yield of leguminous crops (Sundh *et al.*, 2021). However, the slow growth rate of *B. japonicum* presents several challenges in agricultural biotechnology, particularly in the fields of microbial inoculants (Krutylo, 2016).

In recent years, the use of nanoparticles in agriculture is gaining more and more popularity (Pansambal *et al.*, 2023). Nanoparticles are defined as particles smaller than 100 nm in size. In particular, nanoparticles, taking part in electron transfer processes, enhance the action of enzymes that convert nitrates into ammonium nitrogen, intensify cell respiration, photosynthesis, synthesis of enzymes and amino acids, carbohydrate and nitrogen metabolism (Nosrati *et al.*, 2023).

It should be noted that nanocrystalline materials with a wide spectrum of action include nanobiomaterials based on cerium dioxide. Despite considerable interest, the biological activity of cerium dioxide has not been sufficiently studied. Until recently, almost no attention was paid to this compound, since cerium dioxide is insoluble in water and biological fluids. Due to oxygen non-stoichiometry and low toxicity, cerium dioxide nanoparticles (CDNs) are extremely promising objects for biological research (Younis *et al.*, 2016; Zhang *et al.*, 2019).

The aim of our study was to research a possible mathematical dependence between microbial growth of *Bradyrhizobium japonicum*, concentration CDNs, bioprocess time and mannitol concentration.

MATERIALS AND METHODS

Bradyrhizobium japonicum strain IMB B-7194 from the collection of the D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine. An aqueous colloidal solution of CDNs (particle size 4-6 nm, $\xi \approx +10$ mV) was obtained by hydrothermal treatment of the solution for 50 hours in the temperature range of 100÷200°C.

Cultivation of *B. japonicum* was carried out in Erlenmeyer flasks with a volume of 750 ml (with a medium volume of 100 ml) with periodic cultivation at 220 rpm at a temperature of 28-30°C, for 72-96 hours in a mannitol-yeast liquid nutrient medium (g/l): NaCl – 0.1; K₂HPO₄ · 3H₂O – 0.5; MgSO₄ · 7H₂O – 0.2; FeCl₃ – 0.01; mannitol – 10.0; calcium gluconate – 1.5; yeast extract – 2.0; pH 7.0. At the initial stages of cultivation, the initial optical density of the suspension of microorganisms was OD₆₇₀ = 0.1.

All studies were carried out in at least three repetitions. The obtained data were statistically processed by generally accepted methods of variation statistics.

The dependence of the growth of *B. japonicum* bacteria on the participation of CDNs was evaluated using regression analysis methods in the theory of experimental planning according to the full factorial plan 3^k. This approach makes it possible to estimate the linear and quadratic effects of factors (X_1, X_2, X_3) on the indicator Y within the framework of one model and to express it in the form of a regression equation:

$$y = a_0 + \sum_{i=1}^n a_i x_i + \sum_{i=1}^n a_{ii} x_i^2 + \sum_{i=1}^n \sum_{j>i}^n a_{ij} x_i x_j \quad (1)$$

where: a_0 – constant, a_i – linear coefficient, a_{ii} – quadratic coefficient, and a_{ij} – second-order interaction coefficient.

Statistical processing of the data of the planned experiment (calculation of regression coefficients, analysis of variance (ANOVA) and construction of response surfaces was carried out using the trial version of the Statistica program (TIBCO Software Inc., <https://www.tibco.com/>) using the DOE library. Coefficients were considered statistically significant at $p \leq 0.05$.

RESULTS

During the cultivation of *B. japonicum* in a liquid nutrient medium with different amounts of CDNs, the dynamics of cell titer growth of the studied bacteria were established. Taking into account the data of previous studies, we used nanoparticles in the most physiologically expressed concentrations of 1 μM and 1 mM for biotesting the effect of CDNs. In the course of the work, *in silico* optimization of the parameters of the mathematical model of the response of *B. japonicum* to the presence of CDNs was carried out. The study was conducted according to the plan of a full factorial design (FFD) (Table 1).

Table 1. Mathematical model factors for assessing the CDNs impact

Factors	Factor levels		
	-1	0	+1
Concentration of cerium dioxide nanoparticles (X_1), M	0	10^{-6}	10^{-3}
Duration of action of cerium dioxide nanoparticles (X_2), days	3	10	17
Concentration of mannitol in nutrient medium (X_3), g/l	5	10	15

The plan of the experiment to study changes in the intensity of the number of *B. japonicum* bacteria at different concentrations of CDNs, as well as options for combining factors and the obtained results are shown in Table 2.

Table 2. Experiment planning matrix according to the FFD

№	Optimization factors			Optimization parameter
	X_1^*	X_2^*	X_3^*	Cell titer, CFU/ml
1	0	3	5	1,60E+07
2	0	3	10	2,40E+08
3	0	3	15	2,14E+08
4	0	10	5	4,40E+08
5	0	10	10	9,00E+08
6	0	10	15	2,20E+09
7	0	17	5	1,40E+09
8	0	17	10	1,80E+09
9	0	17	15	3,50E+09
10	0,000001	3	5	1,83E+07
11	0,000001	3	10	1,04E+08
12	0,000001	3	15	5,60E+08
13	0,000001	10	5	3,80E+08
14	0,000001	10	10	1,25E+09
15	0,000001	10	15	1,40E+09
16	0,000001	17	5	7,40E+08
17	0,000001	17	10	2,54E+09
18	0,000001	17	15	4,90E+09
19	0,001	3	5	1,50E+07

№	Optimization factors			Optimization parameter
	X_1^*	X_2^*	X_3^*	Cell titer, CFU/ml
20	0,001	3	10	8,90E+07
21	0,001	3	15	1,30E+08
22	0,001	10	5	2,90E+08
23	0,001	10	10	6,20E+08
24	0,001	10	15	1,26E+09
25	0,001	17	5	4,30E+08
26	0,001	17	10	7,53E+08
27	0,001	17	15	1,90E+09

* Note:

X_1 – concentration of cerium dioxide nanoparticles, M

X_2 – duration of action of cerium dioxide nanoparticles, days

X_3 – concentration of mannitol in the nutrient medium, g/l

The level of significance of the effects (linear, quadratic and interaction effects) was determined by the analysis of variance (ANOVA), which showed that the concentration of cerium dioxide nanoparticles (X_1) and the duration of action of nanoparticles (X_2) had a significant ($p \leq 0.02$) effect on the cell titer, but lower than the concentration of mannitol ($p \leq 0.01$). For a visual assessment of the effects of variance analysis, a Pareto diagram is presented in (Fig. 1), on which the effects are arranged in descending absolute value. This diagram shows that the linear effects of the duration of cultivation and mannitol concentration have the maximum reliable effect, and the effect of CDNs has the minimum.

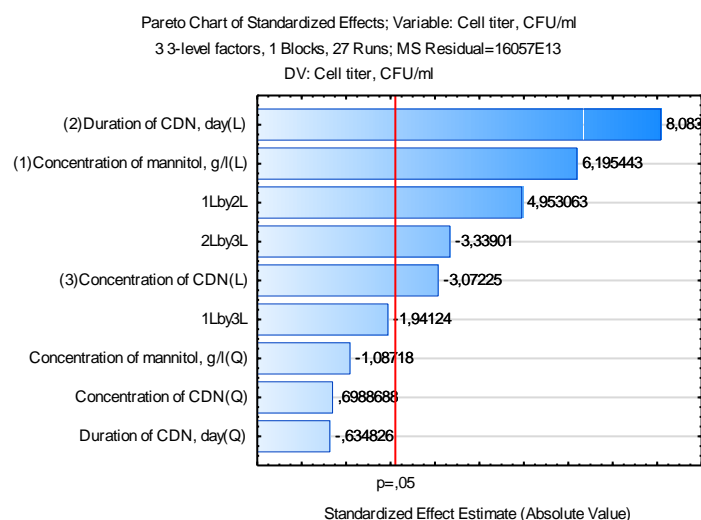


Figure 1. The influence of the researched factors on the *B. japonicum* titer

During the initial data analysis, a regression equation was obtained, which has the form of a quadratic polynomial of the second order, taking into account only statistically significant effects:

$$Y = 9,43 \times 10^8 - 5,8 \times 10^8 X_1 + 1,62 \times 10^9 X_2 + 1,24 \times 10^9 X_3 - 6,63 \times 10^8 X_1 \times X_2 - 1,14 \times 10^9 X_2 \times X_3 \quad (2)$$

From the obtained model, it turns out that the duration of CDNs exposure (cultivation) turned out to be the most important factor ($F = 8.08$, $p \leq 0.001$), with its increase, the number of cells should increase linearly. At the same time, an increase in CDNs concentration ($F = 3.0722$, $p \leq 0.006$) should lead to a decrease in cell titer compared to the positive effect of mannitol ($F = 6.19$, $p \leq 0.001$), with other factors constant.

According to the response surface of the titer of *B. japonicum* bacteria (Fig. 2), the effect of low CDNs concentrations only increases with increasing duration of cultivation with nanoparticles. The obtained data allow us to assert the positive effect of micromolar concentrations of CDNs on the growth of bacteria, but less pronounced than the effect of mannitol.

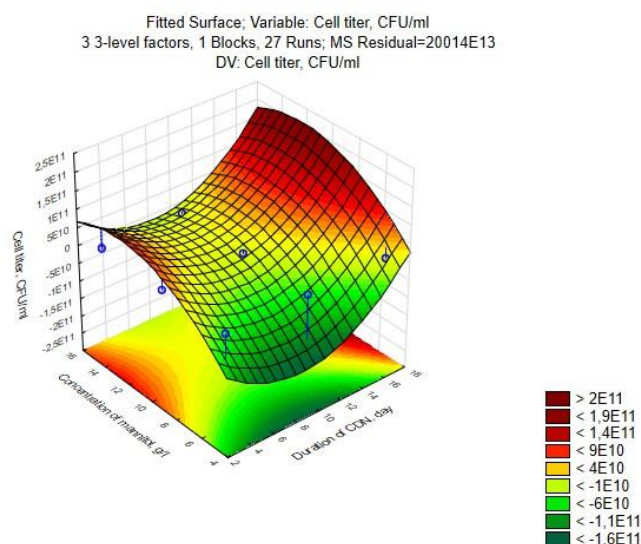


Figure 2. The response surface of *B. japonicum* titer, as functions of the studied factors

The conducted work made it possible to determine the optimal values for the studied factors within the framework of this study. (Fig. 3).

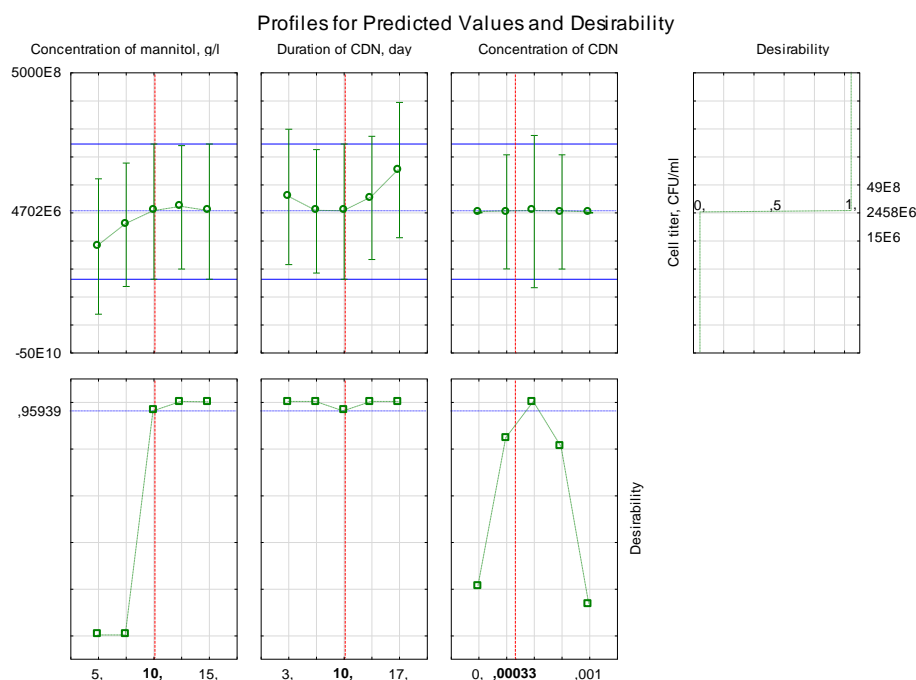


Figure 3. Profiles of predicted values to obtain optimal values of the studied factors

As a result of our work, the stimulating effect of low concentrations of CDNs on the growth of the studied bacteria was shown, which is enhanced by the presence of a substrate in the medium. The calculated maximum cell titer at these levels of exposure was determined at

a nanoparticle concentration of 330 μmol and a mannitol concentration of 10 g/L in the medium during a ten-day exposure to the CDNs.

CONCLUSIONS

Throughout the course of this study, results were obtained that enabled the formulation of several conclusions:

Based on bacterial growth indicators, it was determined that cerium dioxide nanoparticles at concentrations ranging from 1 micromole to 1 millimole had the most significant effect on cell titer after their introduction into the culture medium, as compared to the control group ($p < 0.01$).

- The absence of a direct concentration dependence between CDNs and the ability to grow was noted.

- As part of our experimental work, we obtained optimal value calculations for the investigated factors, which revealed: the duration of CDNs action is 10 days, with a concentration of nanoparticles of 330 μmol and a concentration of mannitol in the medium of 10 g/L.

Thus, the use of the model gram-negative microorganism *B. japonicum* allows for biotesting of cerium dioxide nanoparticles, and makes it possible to assess their effect in the concentration range of 1 micromole to 1 millimole. The research is promising and requires comprehensive study for the creation of new nanotechnologies and the possible application of these nanomaterials in complexes with microorganisms during the inoculation of nodular nitrogen-fixing bacteria in the pre-sowing processing of soybeans.

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WOUND DRESSINGS FILMS BASED ON THE CITRIC ACID MODIFIED STARCH

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The research focuses on obtaining polymer films based on citric acid-modified starch (MSt) in combination with polyvinyl alcohol (PVA) as a base for wound dressings with prolonged drug release. Starch modification was carried out with 0.5 and 1 mol/l citric acid solutions at a temperature of 40°C for 1.5, 2.0 and 2.5 hours. 10% solutions of MSt and PVA were mixed in ratios of 25:75, 50:50 and 75:25 to obtain films by the solution casting method. The influence of citric acid concentration and processing time on the physical-mechanical properties of the films was studied using an automated tensile testing machine ZwickRoell Z2.5TH1. The peculiarities of the interaction between PVA and MSt at different modification durations were determined by Fourier-transform infrared spectroscopy. It was established that the duration of modification and the concentration of citric acid affect the physical-mechanical properties of the films. It was found that increasing the concentration of citric acid contributes to obtaining stronger films. The duration of starch modification with citric acid does not significantly affect the sorption properties of the films, which were evaluated by the amount of water vapor sorption. The sorption properties of the films largely depend on the ratio of MSt and PVA. Thus, films with an MSt/PVA ratio of 75:25 demonstrate an optimal set of properties. The obtained films can be a base for wound dressings with prolonged drug release.

Keywords: modified starch, wound dressings, citric acid.

INTRODUCTION

Wound healing is a critical aspect of healthcare, where effective wound dressings play a crucial role in facilitating healing and preventing complications. Traditional dressings often lack the capability for sustained drug release, necessitating frequent changes and potentially hindering the healing process. To address this limitation, researchers have developed advanced wound dressings capable of controlled and prolonged drug release (Dhivya *et al.*, 2015).

Starch is a natural and abundant polysaccharide, has garnered attention as a potential material for wound dressings due to its biocompatibility, biodegradability, and low cost (Falcão *et al.*, 2022). However, native starch exhibits certain limitations, including poor mechanical properties and rapid degradation. To overcome these challenges, starch can be modified to enhance its functionality and tailor its properties for specific applications (Torres *et al.*, 2013).

Citric acid is a natural organic acid, has emerged as a promising starch modifier. Citric acid reacts with the hydroxyl groups of starch, forming ester linkages that lead to the crosslinking of starch molecules (Yu *et al.*, 2005). This modification process can significantly improve the mechanical properties, water resistance, and stability of starch (Gonzalez Seligra *et al.*, 2016).

The introduction of an ester group into the cellulose backbone through the esterification reaction of hydroxyl groups imparts hydrophobicity to cellulose, making it more compatible with non-polar polymers and solvents. The use of carboxylic acids, such as citric acid (CA),

for cellulose modification offers a less environmentally aggressive approach. CA serves as a cost-effective and stable esterification agent, capable of altering the hygroscopic nature of cellulose or nanocellulose through direct chemical modification, thereby enhancing hydrophobicity. Moreover, as emphasized by several authors, it is an esterification agent that can overcome the toxicity and cost associated with other esterification agents. Several authors have also reported the use of anhydrides as esterification agents, such as succinic, acetic, butyric, and hexanoic anhydride.

CA, being a tricarboxylic acid, reacts with cellulose through the attachment of a carboxyl group via esterification with a cellulose hydroxyl group. Further reaction via esterification with another cellulose hydroxyl group can lead to cross-linking between cellulose chains, resulting in cellulose with increased hydrophobicity.

Polyvinyl alcohol (PVA) is a synthetic polymer known for its excellent film-forming ability, biocompatibility, and non-toxicity. PVA is widely used in various biomedical applications, including wound dressings (Dash *et al.*, 2011).

Research Objectives

In this study, polymer films based on citric acid-modified starch (MSt) in combination with polyvinyl alcohol (PVA) were developed. The aim of this research is to create a composite material that leverages the individual advantages of both components, resulting in films with desirable properties for wound dressings with prolonged drug release. The influence of various modification parameters, such as citric acid concentration and modification time, on the physical, mechanical, and sorption properties of the films have been researched. The interaction between MSt and PVA using FTIR spectroscopy also have been investigated to gain insights into the molecular structure and properties of the composite films.

It is planned to improve wound treatment technologies by optimizing the composition and properties of polymer films based on starch, offering innovative solutions for long-term drug delivery and improving the results of wound healing.

MATERIALS AND METHODS

Starch Modification

Potato starch (CAS 9005-25-8) modification was conducted using two different concentrations of citric acid (CAS 77-92-9) solution (0.5 mol/l and 1 mol/l). For each concentration, the modification was performed at a constant temperature of 40°C but with varying reaction times of 1.5, 2.0, and 2.5 hours. This approach aligns with previous studies demonstrating the effectiveness of citric acid in altering starch properties (Yu *et al.*, 2005). Different reaction times were chosen to investigate the effect of the modification duration on the final characteristics of the film.

Film Preparation

10% (w/v) solutions MSt and polyvinyl alcohol (PVA 17-99) were prepared to obtain films of varying composition. This concentration was selected based on previous research indicating suitable film-forming properties for both starch and PVA (Mali *et al.*, 2005). Then these solutions were mixed in three different ratios (w/w): 25:75, 50:50, and 75:25 (MSt:PVA). To form films from these mixtures the solution casting method was employed as widely used technique for obtaining polymer films due to its simplicity and versatility (Gutiérrez *et al.*, 2015).

Characterization and Analysis

The tensile strength and elongation at break of the films were evaluated using an automated tensile testing machine ZwickRoell Z2.5TH1. These properties are crucial in understanding the film's ability to withstand stress and deformation, which are relevant for wound dressing applications (Bergo *et al.*, 2007; Ghanbarzadeh *et al.*, 2010; Ibrahim *et al.*, 2019). Molecular interactions between modified starch (MSt) and polyvinyl alcohol (PVA) within the films, Fourier-transform infrared (FTIR) spectroscopy was used.

RESULTS AND DISCUSSIONS

Evaluation of Mechanical Properties

For each film sample, five replicate specimens were prepared according to standard testing methods (ASTM D882) (Anseth *et al.*, 1996). By analyzing the tensile strength and elongation at break data, valuable insights into the mechanical behavior of the film and its suitability for wound dressing applications can be gained (Fig.1). Higher tensile strength values signify a stronger and more durable film, while higher elongation at break values indicate a more flexible and deformable film.

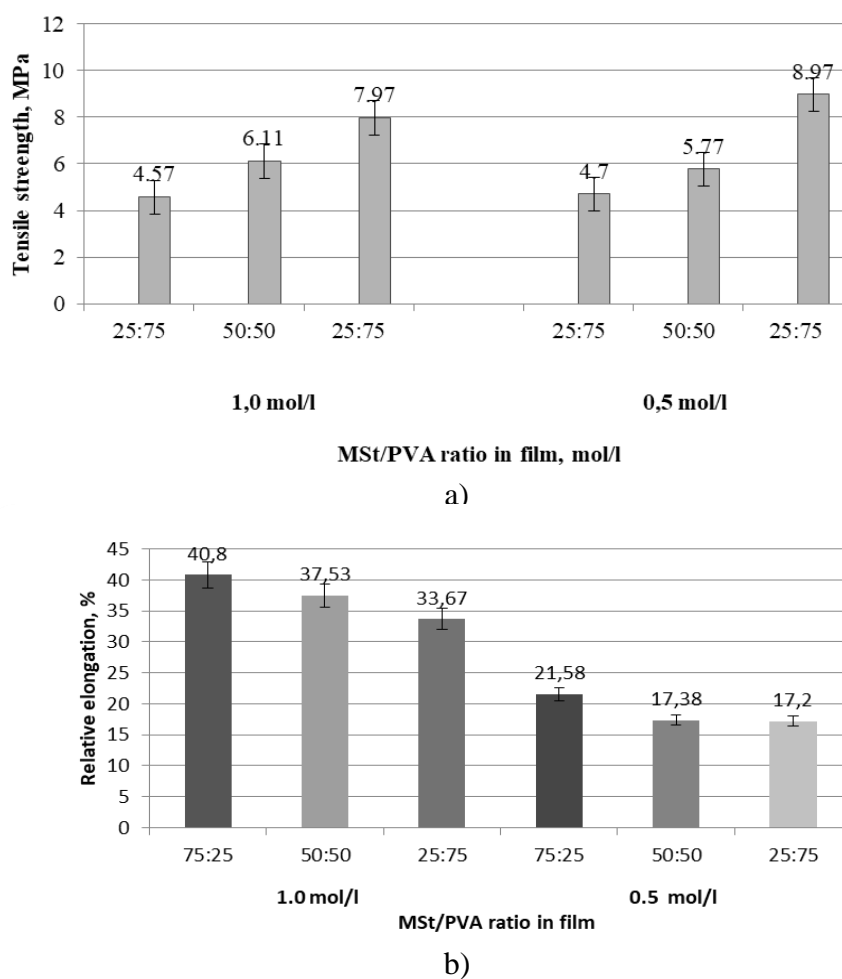


Figure 1. Tensile strength (a) and elongation (b) of film on different ratios of MSt/PVA

Processing time and citric acid concentration also influence the physical-mechanical properties of the films. It was determined that samples with a citric acid concentration of 0.5 mol/l have a tensile strength ranging from 4.7 to 8.97 MPa, and a relative elongation at break ranging from 17.2 to 21.58%. For samples treated with citric acid at a concentration of 1 mol/l, the tensile strength ranges from 4.57 to 7.97 MPa, and the relative elongation at break ranges from 33.67 to 40.8%.

Based on the research results, it can be established that with an increase in starch processing time, functional groups are added that influence the PVA crosslinking process. With an increase in concentration, the resulting films are more elastic.

Fourier-Transform Infrared (FTIR) Spectroscopy Analysis

To further investigate the molecular interactions between modified starch (MSt) and polyvinyl alcohol (PVA) within the films, Fourier-transform infrared (FTIR) spectroscopy was used.

The spectra were analyzed to identify characteristic absorption bands associated with MSt, PVA, and potential new bonds formed due to the modification process (Fig.2).

Specifically, the appearance of new absorption bands in the ester carbonyl region (around 1730-1750 cm^{-1}) would indicate the formation of ester bonds between the hydroxyl groups of PVA and the carboxyl groups of citric acid in the modified starch. Judging by the curves, these peaks appeared at first, and gradually disappeared, which indicates their participation in the interaction. On the upper curve (75:25 MSt/PVA, 1 mol/l), the peak is larger, on the lower one (25:75 MSt/PVA, 0.5 mol/l) it is almost absent due to scale of modification and percentage of MSt in films, that indicates successful modification.

This esterification reaction is expected to contribute to the cross-linking of starch molecules and the enhanced mechanical properties observed in the films.

Furthermore, changes in the intensity and position of absorption bands associated with hydroxyl groups (around 3200-3600 cm^{-1}) could provide information about the extent of hydrogen bonding interactions between MSt and PVA. These interactions play a crucial role in determining the film's overall structure, stability, and sorption properties.

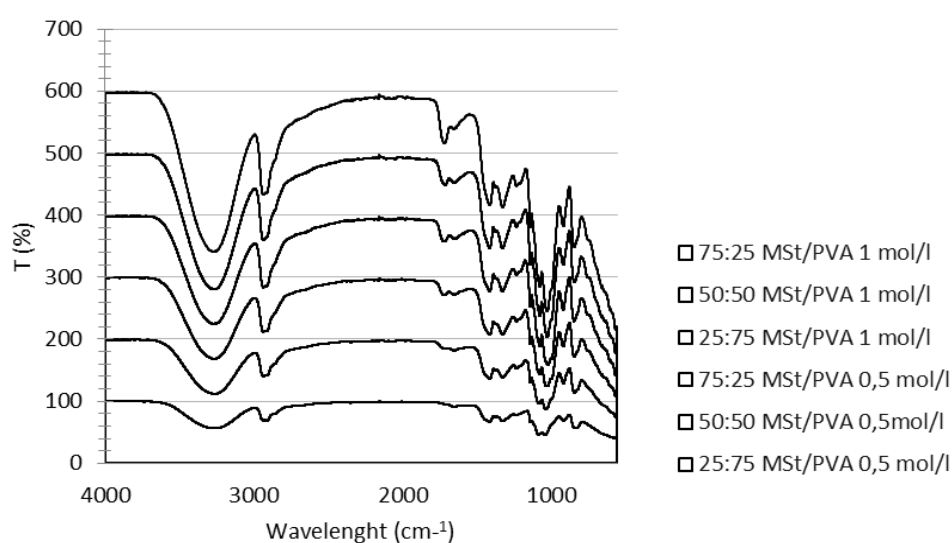


Figure 2. FTIR spectra of films with different MSt/PVA ratios and modification conditions

All six graphs exhibit a similar profile with a broad minimum in the range of 1000-1700 cm^{-1} , which corresponds to the vibrations of C-O and C-C bonds in the polymer chains, thus indicating successful modification. A deeper minimum signifies greater absorption and, consequently, a higher concentration of these bonds. In the spectrum of the 75:25 MSt/PVA 1 mol/l sample, a small peak (maximum %T) is also observed in the 3300-3500 cm^{-1} region, which corresponds to the vibrations of O-H bonds. This may indicate the presence of residual water molecules in the modified material.

By comparing the FTIR spectra of films with different MSt/PVA ratios and modification conditions, it is possible to discern the influence of these factors on the molecular interactions and, consequently, the properties of the resulting films.

CONCLUSION

This research investigated the development of polymer films based on citric acid-modified starch (MSt) and polyvinyl alcohol (PVA) for potential application in wound dressings with prolonged drug release. The study systematically evaluated the effects of citric acid concentration and modification time on the physical-mechanical properties and sorption characteristics of the films.

The results demonstrated that the modification process significantly influenced the film properties, with higher citric acid concentrations and longer modification times leading to improved mechanical strength and elasticity. FTIR analysis confirmed the formation of ester bonds between MSt and PVA, indicating successful cross-linking and enhanced structural integrity.

Processing time and citric acid concentration also influence the physicochemical properties of the films. It was determined that samples with a citric acid concentration of 0.5 mol/L citric acid had tensile strength of 4.7-8.97 MPa and elongation at break of 17.2-21.58%. Samples with 1 mol/L citric acid had tensile strength of 4.57-7.97 MPa and elongation at break of 33.67-40.8%.

Based on the research results, it can be established that with an increase in starch processing time, functional groups are added that influence the PVA crosslinking process. With an increase in concentration, the resulting films are more elastic and stronger. Films based on modified starch are more homogeneous and transparent.

Among the studied formulations, films with an MSt/PVA ratio of 75:25 exhibited the most favorable combination of properties, including optimal mechanical performance and sorption characteristics. These findings suggest that the developed films hold significant promise as a novel material for wound dressings, offering potential advantages in terms of drug delivery and healing outcomes.

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ANTIFUNGAL ACTIVITY OF ENDOPHYTIC BACTERIA ASSOCIATED WITH ANTARCTIC VASCULAR PLANTS

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Plants harbor a wide diversity of microorganisms, which play a crucial role in their growth, survival and establishment by conferring enhanced resistance to abiotic stress, allowing plants to grow in extreme conditions. Endophytic bacteria, residing within plant tissues, often exhibit antimicrobial properties. This study investigated the antifungal potential of endophytic bacteria isolated from Antarctic vascular plants. Isolates were screened for their inhibitory activity against a panel of plant pathogenic fungi. Among studied bacteria only 3 strains have shown broad spectrum of antifungal activity – *Arthrobacter psychrochitiniphilus* 15.6, *Pseudomonas yamanorum* 24.4, and *Hafnia* sp. 25.2. The results highlight the potential of Antarctic endophytic bacteria as a promising source of novel bioactive compounds for the development of sustainable biocontrol agents. However, there is a need to identify specific compounds responsible for inhibiting phytopathogens growth. Understanding the mechanisms of antifungal activity is crucial for the effective application of these bacteria in agriculture and other fields.

Keywords: endophytic bacteria, antifungal activity, Antarctic plants

INTRODUCTION

Microorganisms in the Antarctic region have garnered significant attention due to their potential to produce novel bioactive compounds. Extremophilic bacteria and fungi from this region have demonstrated remarkable adaptations to the harsh environmental conditions, which include cold temperatures, high salinity, and intense ultraviolet radiation (Corrêa & Abreu, 2020). In recent years, there has been a growing interest in exploring the diversity and bioprospecting potential of these microorganisms, particularly their antifungal properties (Brunati *et al.*, 2009; Godinho *et al.*, 2013).

Endophytic bacteria, which are microorganisms that reside within plant tissues without causing any apparent harm to the host, have emerged as a promising source of antifungal compounds (Deshmukh *et al.*, 2018). These endophytes have the ability to produce a diverse array of secondary metabolites, including volatile organic compounds, that can inhibit the growth of pathogenic fungi (Deshmukh *et al.*, 2018).

The diverse and unique microbial communities found in Antarctica, including those associated with endemic plant and algal species, represent an underexplored source of bioactive compounds (Deshmukh *et al.*, 2018). Endophytic bacteria, which reside within the tissues of host organisms without causing any apparent harm, have been identified as a

particularly promising group for the discovery of novel antifungal agents (Deshmukh *et al.*, 2018).

Studies have shown that cold-tolerant fungal genera, such as *Penicillium*, *Aspergillus*, *Beauveria*, and *Cladosporium*, isolated from benthic mats in Antarctic lakes have demonstrated potent antimicrobial activities, including the production of novel antibiotics (Brunati *et al.*, 2009). These findings suggest that the endophytic bacterial communities associated with Antarctic plant and algal species may also harbor the potential to produce a wide range of antifungal compounds with diverse structural and functional characteristics.

This research paper aims to investigate the antifungal activity of endophytic bacteria isolated from Antarctic vascular plants.

MATERIALS AND METHODS

Plant Samples Collection

Plant samples were collected during the 25th Ukrainian Antarctic Expedition (January-April 2020) along the Western part of the Antarctic Peninsula (WAP). To isolate endophytic bacteria, surface sterilization of the plants was performed according to Barra *et al.* (2016) with modifications.

Bacterial Species Used in the Study

For this study, 12 bacterial cultures isolated from *D. antarctica* and *C. quitensis* samples were investigated (Iungin *et al.*, 2023) and represented in Table 1.

Antifungal Activity Studies

Antifungal activity of bacterial isolates was tested with the agar disk-diffusion method (Elkahoui *et al.*, 2012). Six phytopathogenic fungi cultures were obtained from the National collection of D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine (Department of Physiology and Systematics of Micromycetes), *Nigrospora oryzae* 15966, *Fusarium solani* 50718, *Nectria inventa* 3041, *Botrytis cinerea* 16884, *Sclerotinia sclerotium* 16883, and *Rhizoctonia solani* 16036. A 5-day mycelium of pregrown fungi culture was placed in the middle of the Petri dish with a sterile needle. Overnight bacterial cultures were inoculated equidistantly from the fungus and incubated for five days at 25 °C. The results were presented as a percentage of fungal growth inhibition.

RESULTS AND DISCUSSION

Endophytic Bacteria

The habitat of the vascular plants and the plant communities they create is largely defined by the environmental extreme conditions of the Antarctic continent, which include low temperatures, high levels of UV radiation, and low water availability. These harsh conditions likely select for endophytic bacteria that are adapted to produce a wide range of bioactive secondary metabolites, including those with antifungal properties. Mean temperature in Antarctic is around -10 °C, so bacteria were cultivated at 4 °C and 15 °C to mimic the environmental conditions. However, previous studies have shown the ability of isolated endophytic strains (Table 1) to grow at wide temperature range, suggesting their unique origin and the presence of an intermediate host, such as mammals or birds.

Table 1. Endophytic bacteria associated with Antarctic vascular plants

Species name	Strain number	Host plant	Place of isolation
<i>Siminovitchia terrae</i>	9.1	<i>D.antarctica</i>	Lahille Island
<i>Pseudomonas salomonii</i>	10.1	<i>C. quitensis</i>	Lahille Island
<i>Psychrobacter arcticus</i>	10.4	<i>C. quitensis</i>	Lahille Island
<i>Arthrobacter psychrochitiniphilus</i>	15.6	<i>D.antarctica</i>	Ronge Island
<i>Arthrobacter psychrochitiniphilus</i>	16.7	<i>D.antarctica</i>	Ronge Island
<i>Agreia</i> sp.	23.2	<i>D.antarctica</i>	Santos Peak, Graham Passage
<i>Pseudomonas yamanorum</i>	24.4	<i>D.antarctica</i>	Santos Peak, Graham Passage
<i>Hafnia</i> sp.	25.2	<i>D.antarctica</i>	Galindez Island, Argentine Islands
<i>Pseudomonas</i> sp.	26.2	<i>D.antarctica</i>	Galindez Island, Argentine Islands
<i>Pseudoarthrobacter</i> sp.	26.7	<i>D.antarctica</i>	Galindez Island, Argentine Islands
<i>Brachybacterium</i> sp.	39.12	<i>C. quitensis</i>	Lagotellerie Island
<i>Kocuria salsicia</i>	40.1	<i>D.antarctica</i>	Lagotellerie Island

Half of the studied isolates (10.4, 25.2, 26.2, 26.4, 26.7, 39.12) demonstrated psychrophilic growth ability. However, the majority of isolates showed active biomass growth at 37°C and 42°C. These findings broaden our understanding of plant-microbe interactions in Antarctic vascular plants within the context of the ecosystem's overall functioning.

Antifungal Activity

Endophytic bacteria, which reside within plant tissues without causing any apparent harm to the host, have garnered significant attention in recent years due to their remarkable ability to produce a diverse array of secondary metabolites and enzymes that exhibit potent antifungal properties (Deshmukh *et al.*, 2018; Midhun and Mathew, 2021). These endophytic microorganisms have evolved alongside their plant hosts, establishing symbiotic relationships that enable them to thrive in the nutrient-rich environment within the plant tissues (Chadha *et al.*, 2014).

Among the isolated bacterial strains, only three – *Arthrobacter psychrochitiniphilus* 15.6, *Pseudomonas yamanorum* 24.4, and *Hafnia* sp. 25.2 – showed antifungal activity against phytopathogenic fungi (Table 2). The other strains had no effect on the growth of the selected fungal strains.

Table 2. Endophytic bacteria antifungal activity

Fungal species name	Inhibition of fungal growth, %		
	<i>Arthrobacter psychrochitiniphilus</i> 15.6	<i>Pseudomonas yamanorum</i> 24.4	<i>Hafnia</i> sp. 25.2
<i>Nigrospora oryzae</i> 15966	11.13 ± 1.73	–	18.30 ± 1.90
<i>Fusarium solani</i> 50718	33.00 ± 8.00	20.33 ± 1.15	–
<i>Nectria inventa</i> 3041	–	5.10 ± 0.21	21.85 ± 2.03
<i>Botrytis cinerea</i> 16884	18.28 ± 1.58	–	18.90 ± 1.90
<i>Sclerotinia sclerotium</i> 16883	21.61 ± 2.13	11.43 ± 0.57	–
<i>Rhizoctonia solani</i> 16036	5.96 ± 0.16	3.37 ± 0.2	20.08 ± 0.21

By exploring the diversity and metabolic capabilities of these endophytes, the study seeks to identify potential sources of novel antifungal agents that could be developed for various applications, such as in medicine, agriculture, and industry.

The antifungal activity of endophytic bacteria is attributed to a variety of mechanisms, including the production of antimicrobial compounds, the induction of host plant defense mechanisms, and the competition for resources (Zhang *et al.*, 2022). One of the primary mechanisms of antifungal activity in endophytic bacteria is the synthesis of secondary metabolites, such as phenolic compounds, alkaloids, and terpenoids, which can inhibit the

growth and development of pathogenic fungi (Deshmukh *et al.*, 2018; Midhun and Mathew, 2021). These bioactive molecules can disrupt fungal cell membranes, interfere with enzymatic processes, and hinder the synthesis of essential cellular components, thereby effectively controlling fungal infections (Chadha *et al.*, 2014; Zhang *et al.*, 2022).

Additionally, endophytic bacteria can stimulate the host plant's innate immune system, triggering the production of defense-related compounds and the activation of signaling pathways that enhance the plant's resistance to fungal pathogens (Fadiji and Babalola, 2020). By colonizing the plant's internal tissues, endophytic bacteria can also outcompete pathogenic fungi for essential nutrients and space, thereby limiting their ability to establish infections (Fadiji and Babalola, 2020).

Ongoing research continues to uncover the diverse mechanisms employed by endophytic bacteria in their antifungal activities, highlighting their potential as a promising source of novel antimicrobial agents and biopesticides for sustainable agriculture and human health applications (Deshmukh *et al.*, 2018; Midhun and Mathew, 2021).

CONCLUSIONS

In conclusion, endophytic bacteria possess a remarkable arsenal of mechanisms that contribute to their potent antifungal properties. These include the production of antimicrobial secondary metabolites, the induction of host plant defense responses, and the competitive exclusion of fungal pathogens. The findings of this research could contribute to the development of novel antifungal agents derived from endophytic bacteria isolated from Antarctic plant species.

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IMPROVEMENT OF PARCHMENT TECHNOLOGY

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Parchment is a unique biogenic material, which essentially differs from other types of leather in its technology, structure and properties. Despite its ancient origin, and today, due to its strength and durability, it is used in producing musical instruments, interior items, decorations, and restoration of rare items. At the same time, the problems of restoring ancient or developing modern technology for maintaining parchment from animal skins for the past decades have not been cared for by anyone due to the limited number of restoration specialists and technologists. Known parchment technologies involve the use of significant amounts of calcium hydroxide in *beamhouse* processes, which leads to the formation of sludge, as well as sodium sulfide, which pollutes industrial wastewater with sulfur-containing compounds harmful to aquatic organisms. The purpose of the work is to improve the parchment technology by reducing the cost or eliminating the use of these environmentally hazardous materials. To achieve this purpose, traditional physical-chemical and modern instrumental methods of analysis are used. Based on the results of laboratory studies and production tests, the sheep parchment technology has been improved, which provides enzymatic soaking and use of the mineral zeolite during liming. Compared with the known technology, the developed technology completely eliminates sodium sulfide from the *beamhouse* processes, reduces the consumption of calcium hydroxide by a third, ensures more rational use of material and raw material resources, and improves the composition of wastewater.

Keywords: parchment, technology, properties

INTRODUCTION

Cultural heritage represents a national treasure evolving from the culture and spirituality of people. Therefore, it should be constantly restored, protected and developed by all generations as a priceless heritage. A very important category of museum cultural heritage are leather and parchment items, which are a source of valuable historical information: book bindings, manuscripts, charters etc. (Miu *et al.*, 2020). However, like any organic structure, this material is subject to destructive processes through physical and chemical (light, humidity, temperature, pollutants, etc.), biological and microbiological factors, leading to changes in the structure of the polypeptide chain (Plavan *et al.*, 2010). The resistance of parchment to negative influences, including aging, is largely determined by the manufacturing technology (Kolesnyk *et al.*, 2018). Considering that parchment is still in demand today for restoration work, production of unique documents, musical instruments, souvenirs, jewelry, etc., there is a need to restore, and even more likely, improve this technology.

An analysis of the technological features of leather parchment (hereinafter simply “parchment”) production has revealed the importance of the hide processing method, the choice of raw materials and chemicals (di Curci, 2003; Dzendzelyuk, 2015; Adakina *et al.*, 2018; Fourneau *et al.*, 2020). Since traditional tanning and post-tanning processes are not required in the manufacture of parchment, special attention should be paid to the soaking and ash processes, which largely determine the properties of the finished leather: its strength, elasticity, density, thickness, and condition of the front surface.

Known parchment manufacturing technologies involve the use of a significant amount of calcium hydroxide in the preparatory processes, which leads to the formation of lime sludge and sodium sulphide, which, although it helps speed up the process and prepare the hide for further processing, significantly pollutes industrial effluents with sulphur-containing compounds harmful to aquatic life. Therefore, there is an urgent need to reduce the consumption of harmful materials and find more environmentally friendly ones.

The studies of many scientists are devoted to the improvement of beamhouse processes (Zhang *et al.*, 2021; Danylkovych and Lishchuk, 2022; Morera *et al.*, 2022; George *et al.*, 2014; Nazer *et al.*, 2006). A generalized analysis of the literature shows that the most promising areas for improving biotechnological processes in the production of natural leather are associated with enzymatic treatments, which improve the quality of products and production culture, and reduce the environmental impact; due to this, they are traditionally classified as “clean technologies” (Saran *et al.*, 2013; Zhang *et al.*, 2022; Nyakundi *et al.*, 2022; Jayakumar *et al.*, 2022). Among the latest developments, the works of Kozar *et al.* (2014), Sepehri *et al.* (2020), Mokrousova *et al.* (2015), Sakalova and Khodanitska (2023) are of interest, devoted to the study of natural minerals in the form of montmorillonite, kaolin, zeolite, which, due to their structural features and high sorption capacity, are successfully used as sorbents, fillers, and various auxiliary materials in the leather, food and other industries.

In previous studies, to expand the range of chemical materials and finished products and increase the level of environmental friendliness of parchment production, the authors have established:

- peculiarities of interaction in the collagen-enzyme preparation system during soaking, which consist in the interaction of the preparation with protein functional groups, which contributes to increased enzyme activity and watering of the hide during soaking (Kolesnyk and Andreyeva, 2020a; Kolesnyk, 2021);

- features of sorption-desorption processes in the calcium hydroxide-zeolite system, which regulate the concentration of calcium hydroxide in the solution, its gradual effect on the skin, resulting in a more uniform distribution and better fixation of reagents in the dermis structure, increased strength and yield of the skin by area (Kolesnyk *et al.*, 2022);

- rational parameters of the preparatory processes: consumption of the proteolytic enzyme preparation Pellvit C during soaking is 0.2-0.8 g/l, and calcium hydroxide and zeolite during ashing is 5.0 and 3.5 g/l, respectively (Kolesnyk and Andreyeva, 2020b).

Taking into account the above, this study aims to improve the sulphide-free technology of parchment production using a modern enzyme preparation and domestic natural mineral zeolite in the preparatory processes in compliance with the principles of environmental protection and resource efficiency while ensuring the quality of this unique type of leather.

MATERIALS AND METHODS

The work used both new and common leather and chemical materials in the tanning industry:

- *model collagen preparation* – high molecular weight fibrous protein preparation “GELIOS 11” (TU U 15.8-13848909-001-2008, TOMIG LLC), obtained from purified untanned shingles from cattle hides; contains about 93 % of purified collagen in dry matter;

- *sheep raw materials* using the wet-salted canning method;

- *finished leather* (parchment);

- *Pellvit C* – proteolytic enzyme preparation (manufacturer TFL Ledertechnik GmbH, Germany). The pH value of a 10% solution is 5,3, the activity by the casein number method is 59.9 units/g. When assessing the ability of the drug to break down certain target substrates

(gelatin, collagen, fibrinogen) using enzyme electrophoresis in polyacrylamide gel, the presence of fragments with a molecular weight MW of 97 units was established. IR spectroscopic studies indicate that the mentioned enzyme preparation has a polyfunctional nature (the presence of hydroxyl, amine, imine, peptide and some other functional groups in the structure), due to which it is able to interact with collagen, the main component of the dermis (Fig. 1, Table 1) (Kolesnyk *et al.*, 2020);

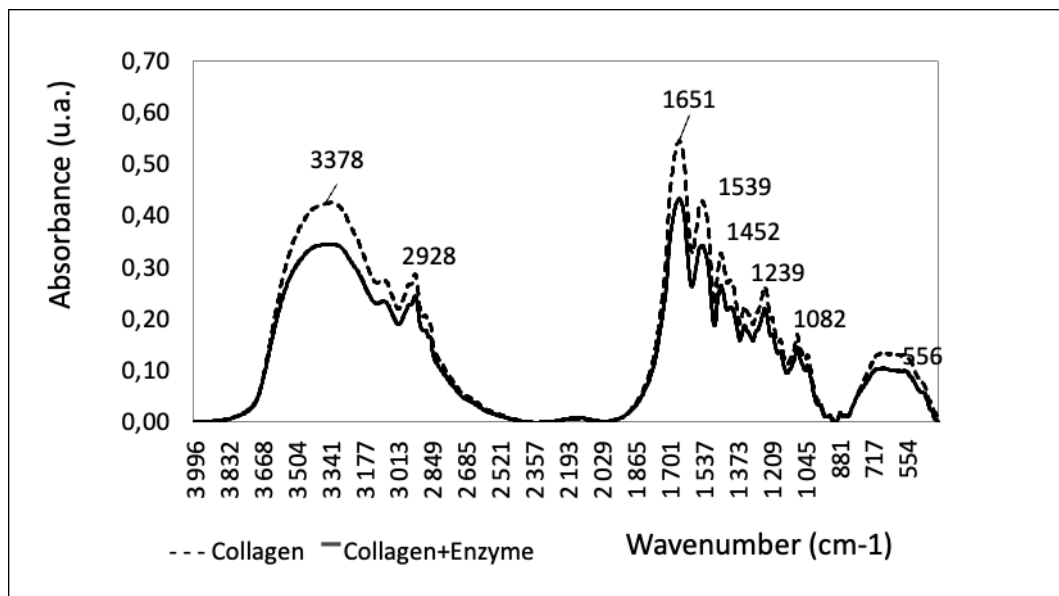


Figure 1. IR spectrograms of collagen preparation before and after treatment with an enzyme preparation

Table 1. Change in relative optical density in IR spectrograms of collagen preparation after treatment with enzyme preparation

Wavenumber (cm^{-1})	Interpretation of absorption bands	Experimental data*	
		collagen	collagen + enzyme
3378	Amide A (ν NH); imines (ν C=N), overlapping inter- and intramolecular hydrogen bonds of the side chains; ν OH	<u>3378</u>	<u>3335</u>
		1,74	2,06
		<u>2928</u>	<u>2928</u>
2928	Alkanes (CH_2); alkenes (ν CH)	1,00	1,00
		<u>1651</u>	<u>1651</u>
1651	Amide I, acids and their derivatives ($\text{C}=\text{O}$); alkenes (ν C=C)	3,45	3,50
		<u>1539</u>	<u>1542</u>
1539	Amide II; carboxylic acids (COO^-)	2,28	2,28
		<u>1452</u>	<u>1452</u>
1452	Alcohols (δ OH); substituted alkenes (δ CH); alkanes (ν CH_3)	1,57	1,25
		<u>1336</u>	<u>1336</u>
1336	Alkanes (δ CH_3)	1,00	1,77
		<u>1239</u>	<u>1238</u>
1239	Amide III; amini second, third (ν CH); ethers (ν SOS); alcohols (δ C=O, ν C=O)	1,23	1,17
		<u>1082</u>	<u>1082</u>
1082	Primary, secondary, tertiary alcohols (ν CO)	0,76	0,52
		<u>556</u>	<u>561</u>
556	Amide VI	0,57	0,60

*Note: numerator – frequency λ , cm^{-1} ; denominator – relative optical density.

- *zeolite* is a natural mineral of the Sokirnitskoye deposit (Ukraine), which belongs to the group of minerals of volcanic sedimentary origin with similar composition, framework aluminosilicates of alkali and alkaline earth metals. According to energy dispersive X-ray fluorescence analysis, the mineral under study contains 65.88% SiO_2 , no less than 8.5% Al_2O_3 , is characterized by an average content of Fe_2O_3 (5.66%), a significant content of alkaline earth (CaO, MgO) and alkaline compounds, 68 and 5.27%). Based on microscopic studies carried out using an MBS-9 microscope, it was found that the average particle size of the mineral is 150 microns. It has been experimentally proven that zeolite does not swell either in water or organic solvents. The pH value of an aqueous solution (or rather a suspension), depending on the concentration (2.5-10.0 g/l), is at the level of 5.7-6.0. The mineral content is 88.1%, moisture – 4.9%.

The hypothesis of the delayed effect of calcium hydroxide on leather raw materials with the addition of zeolite as a substance with high adsorption capacity has been investigated. The hypothesis suggests the following mechanism: the absorbing complex of the zeolite sorbs calcium hydroxide ions, and after some time the reverse process gradually occurs – the zeolite gives back Ca^{2+} and OH^- ions to the solution. This is confirmed by the results of studying the kinetics of calcium hydroxide sorption-desorption by zeolite depending on the concentration of lime and mineral. In this case, the nature of ion sorption and desorption depends on the concentration of both components of the system. Thus, in calcium hydroxide solutions with a concentration of 5.0 g/l, the lowest degree of lime sorption-desorption is observed at a zeolite concentration of 1.5 g/l; the highest degree of sorption - at a zeolite concentration of 3.5 g/l, and desorption – 5.0 g/l (Figure 2).

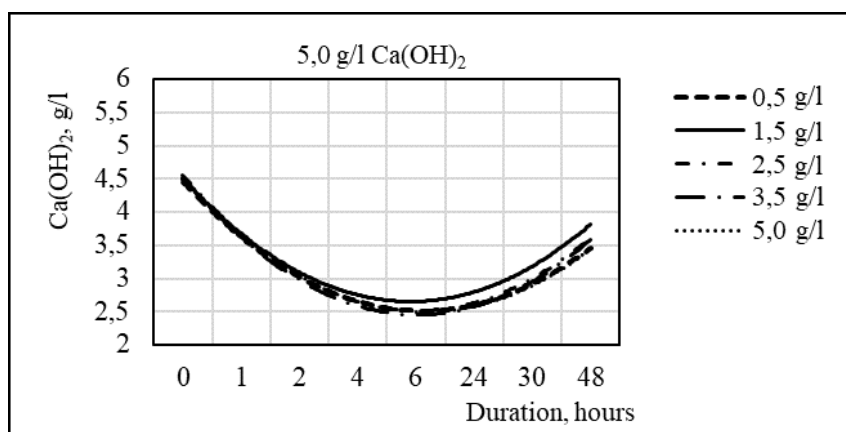


Figure 2. Kinetics of calcium hydroxide sorption-desorption depending on the consumption (concentration) of zeolite

- *other chemical materials common in leather production.*

To achieve these goals, the work uses traditional physicochemical and modern instrumental methods of analysis, including spectroscopic, energy-dispersive X-ray fluorescence (XRF), microscopic, disk electrophoresis in the «enzyme-electrophoresis» modification.

To conduct physical and mechanical tests and chemical analysis, samples of raw materials and finished leather were taken according to the requirements of regulatory documentation: preparation and sampling – ISO 2418:2017; determination of thickness – ISO 2589:2019; determination of tensile strength and relative elongation – ISO 3376:2008; determination of welding temperature (settling) – ISO 3380:2008; determination of the mass fraction of moisture – ISO 4684:2005; determination of the mass part of minerals (ash) – ISO 4047:2006; determination of nitrogen content (mass part of pelt substance – ISO 5397:2006;

determination of the mass part of calcium hydroxide – DSTU 13538-68; determination of the mass part of aluminum oxide – ISO 11885:2007.

RESULTS

Taking into account the results of our research (Kolesnyk *et al.*, 2020; Kolesnyk and Andreyeva, 2020a; Kolesnyk and Andreyeva, 2020b; Kolesnyk, 2021; Kolesnyk *et al.*, 2022), the technology for making parchment from sheep raw materials has been improved (Fig. 3).

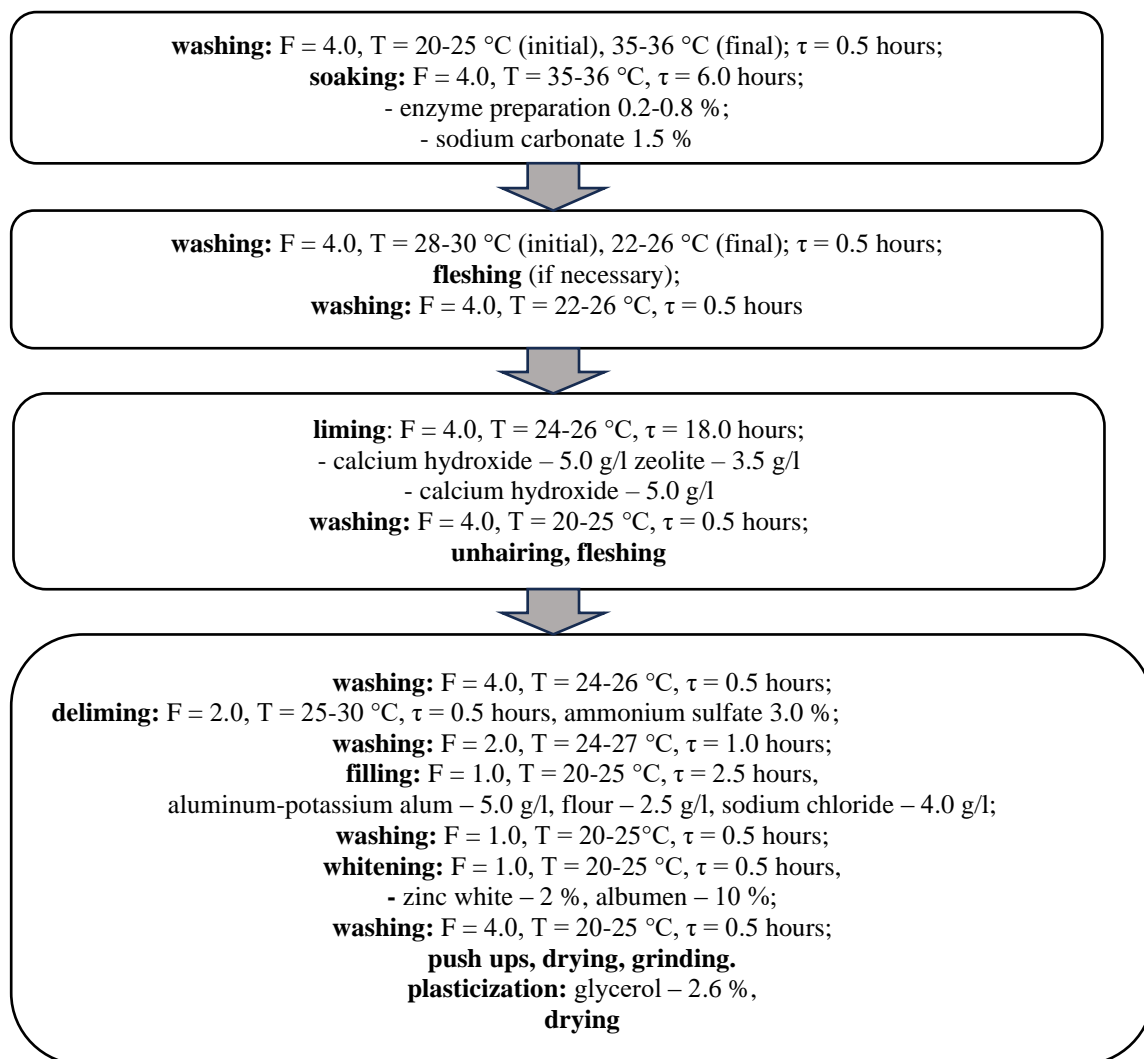


Figure 3. Scheme of improved technology for the production of sheepskin parchment

In the conditions of the Kyiv leather enterprise Chinbar PrJSC, an improved technology for the production of parchment for various purposes (for the manufacture of musical instruments and souvenirs, restoration work) was tested using the wet-salted preservation method of sheepskin as a raw material.

Enzymatic soaking was carried out after washing the raw materials at a float (F) of 4.0 with a gradual increase in temperature to 36 °C for 0.5 hours. First, the enzyme preparation Pellvit C was dosed, and 10 minutes after its dissolution, sodium carbonate; material consumption was determined taking into account the activity of enzyme preparation and the

mass of raw materials. After soaking for 6.0 hours, washing was carried out at F 4.0, temperature 28-30 °C (initial), 22-26 °C (final) for 0.5 hours and fleshing in the raw materials.

The liming-unhairing process was carried out according to the following parameters: F 4.0, temperature 24-26 °C, duration 18.0 hours; consumption of calcium hydroxide 5.0 g/l, natural mineral zeolite – 3.5 g/l; in this case, these materials were previously combined in an aqueous solution for 24.0 hours. In order to increase the degree of the diamond branch and give the parchment the necessary elastic-plastic properties, another 5.0 g/l of calcium hydroxide was dosed 6.0 hours before the end of the liming process. Next, at F 4.0, temperature 20-25 °C, the branch was washed for 0.5 hours. After mechanical removal of the hair and slowing, the branch was washed again (F 4.0, temperature 20-25 °C, duration 0.5 h).

The beamhouse processes in the control batch were carried out using a known technology, therefore the consumption (liquor concentration) of sodium sulfide during soaking and sulfide liming was 0.7 and 9.0 g/l, respectively, and the consumption (liquor concentration) of calcium hydroxide during liming was 15.0 g/l.

Further processes and operations for all batches were carried out according to the well-known technology for the production of parchment, which involves drying of deliming pelt on frames and final finishing by sanding, plasticizing and bleaching.

The deliming process was carried out at F 2.0, temperature 25-30 °C, for 0.5 hours using ammonium sulfate – 3.0%. After the latter, washing was performed (F 2.0, temperature 24-27 °C, duration 1.0 hour).

During the next filling, potassium alum was used – 5 g/l, wheat flour – 2.5 g/l and sodium chloride – 4 g/l (F 1.0, temperature 20-25 °C, duration 2.5 hours). At the end of the filling process, washing was carried out (F 1.0, temperature 20-25 °C, for 0.5 hours).

The bleaching process was carried out according to the following parameters: F 1.0, temperature 20-25 °C, duration 0.5 hours; consumption of zinc white – 2.0%, albumin (dry egg white) – 10.0% with the following washing (F 4.0, temperature 20-25 °C, duration 0.5 hours). After washing, squeezing, drying (in a tense state), grinding and plasticization (glycerin – 2.6%) were performed.

Table 2. Comparative assessment of parchment production technologies

Index	Technology	
	famous	new
<i>Leather:</i>		
Tensile strength, 10 MPa	5.80	6.46
Relative elongation at stress 10 MPa, %	20.7	22.5
Thickness, mm	0.69	0.67
Shrinkage temperature, °C	69.0	69.5
Mass part, %:		
- moisture	13.9	14.1
- pelt substance *	84.6	85.4
- minerals *	8.10	7.49
Output by area, %	92.5	93.0
<i>Spent solution after preparatory processes:</i>		
Sodium sulfide content, g/l	2.90	–
Calcium hydroxide content, g/l	4.18	2.66

Note: * in terms of absolutely dry substance

No complications were identified during the processing of the experimental batch. According to organoleptic assessment, indicators of chemical analysis and physical and mechanical tests, pelt and the finished parchment were not inferior to the control ones: thus, pelt had a clean surface grain without hair residues, signs of pipeness, drawn grain and other

defects; the finished leathers were stronger, had a more uniform clean surface grain and a higher yield in area. For the environmental assessment of the developed technology, an analysis of the spent liming solutions was carried out, which revealed (Table 2) an improvement in their composition in the case of the improved technology.

Thus, the test results confirmed the effectiveness of the improved parchment manufacturing technology, which consists of eliminating sodium sulfide from the technological cycle, reducing consumption and more rational use of raw materials (calcium hydroxide consumption decreases by 30%, and area yield increases by 0.5%), improving the physical and mechanical properties of the skin (increasing tensile strength by 10.2%) and the composition of wastewater (lack of sulfides, reducing the calcium hydroxide content in the waste ash solution by 1.6 times).

The technological efficiency of the new development can be explained, firstly, by the peculiarities of interaction in the collagen-enzyme preparation system during soaking, which consist of the interaction of the enzyme with nitrogen-containing substances, hydroxyl and carboxyl groups of the protein, which contributes to an increase in its activity and hydration of the skin; secondly, by sorption-desorption processes in the calcium hydroxide-zeolite system, due to which the concentration of calcium hydroxide in the solution is regulated, its gradual effect on the skin is carried out, as a result of which a more uniform distribution and better fixation of reagents in the structure of the dermis are ensured, strength indicators, and leather yield by area are increased.

The ecological imperative of the developed technology is based on a decrease in the amount or even complete elimination of environmentally hazardous materials from circulation. The expected economic effect from the introduction of the new technology will be UAH 1.97 per 1 m² due to a more rational use of scarce and expensive today raw leather and chemical materials.

CONCLUSION

The technology for the production of sheep parchment for various purposes (for the manufacture of musical instruments and souvenirs, for restoration work) has been improved. The developed technology was tested in the conditions of an existing tannery. The results of the work have a certain environmental and socio-economic effect since they provide:

- reducing the negative impact on the environment due to the exclusion of sodium sulfide from the technological cycle and reducing the consumption of calcium hydroxide;
- more rational use of raw materials and material resources.

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EFFECT OF LONG-TERM STORAGE ON THE PROPERTIES OF AN ENZYME-CONTAINING PREPARATION FROM FISH WASTE

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The demand for fish products in Europe is gradually increasing, but at the same time fish stocks are declining due to stress factors for aquatic populations, as a result of illegal and unregulated fishing. At the same time, fish processing generates a significant amount of waste, the discharge of which is unprofitable both for the industry as a whole and for individual entrepreneurs, and, in addition, it provokes environmental pollution. Since fish waste contains microelements and various nutrients, it is a valuable raw material resource that is used in the production of fat, flour, animal feed, cosmetics and pharmaceuticals, etc. In this case, the main emphasis is on the mineral, protein- and fat-containing components of waste, but the presence of enzymes in them is practically not taken into account. Previously, it was reported on the production, study of the properties and the possibility of using an enzyme-containing preparation (ECP) obtained from fish waste for leather processing. The purpose of this study is to determine the effect of long-term (for 4 years, in a refrigerated chamber at a temperature of 7-8 °C) storage on the technological capabilities of this preparation. The experiment was conducted in laboratory conditions on the heifer skin, which was bated with ECP at a rate of 0.2, 0.4, 0.6 and 0.8% of the sample weight. The effect of ECP on the chrome leather indicators was studied using traditional and modern analytical methods. Based on the results of chemical analysis and physical and mechanical tests of leather, the suitability of ECP obtained from fish waste for processing leather at the bating stage was established even after such a long storage.

Keywords: enzyme-containing preparation from fish waste, storage, properties.

INTRODUCTION

Existing fish collection and processing methods produce a significant amount of fish waste worldwide each year. It is estimated that, on average, two-thirds of all fish catches are by-products that are discarded. Fish by-products and processing waste pose serious disposal problems for the fishing industry. Therefore, the waste generated from fish processing must be utilised and treated properly to sustain the industry and preserve the environment. In this regard, numerous instruments have been adopted in the European Union to minimise the environmental impact of fisheries within the framework of Integrated Coastal Zone Management (Sappasith *et al.*, 2024).

Processed fish waste has found many applications, among which the most important are animal feed, biodiesel/biogas, dietary products (chitosan), natural pigments (after extraction), food packaging (chitosan), cosmetics (collagen), chromium immobilisation, soil fertilisation and food moisture maintenance (hydrolysates) (Arvanitoyannis & Kassaveti, 2008). The main attention is paid to mineral, protein- and fat-containing components of wastes, the presence of enzymes in them is practically not taken into account, although fish processing wastes, especially digestive organs, have a huge biotechnological potential as sources of such enzymes as proteases, lipases, chitinase, alkaline phosphatase, transglutaminase, hyaluronidase, acetylglycos-aminidase and others. These enzymes can find a variety of applications in the seafood industry, including isolation and modification of proteins and marine oils, production of bioactive peptides, acceleration of conventional fermentation,

cleaning and separation of shellfish, scaling of fish, removal of membranes from fish roe, extraction of flavourings, shelf life extension, texture modifications, removal of extraneous odours, and quality control directly or as components of biosensors. Enzymes from fish and shellfish from cold habitats are particularly useful because they can function at comparatively lower temperatures, thereby saving energy and protecting food products (Venugopal, 2016; Shahidi & Janak Kamil, 2001; Imran, 2022).

Enzymatic methods have become an important and indispensable part of the processes used by modern industry to produce a large range of goods, including leather goods (Kopytina *et al.*, 2022; Kopytina *et al.*, 2023). The production, properties and potential use of an enzyme-containing preparation (ECP) derived from fish waste for leather treatment have been previously reported. The preparation was obtained according to the following scheme: grinding - degreasing - extraction - coarse fractionation of extract proteins with separation from other proteins and impurities by filtration or centrifugation - fractionation - purification. The preparation obtained was a powder of brown coloured fine fibrous substance, which dissolved reasonably well in warm water and had an activity of 450 units/g according to the precipitation method. No complications were detected when using the preparation for bating sheepskin, the obtained leather semi-finished product (pelt) was soft, plastic, with a clean surface grain by organoleptic evaluation (Atamanova *et al.*, 2020). Using chromatographic and electrophoretic analysis methods, the presence of several fractions of active proteolytic enzymes in the preparation was established, and using the IR spectroscopy method, its multifunctional nature (the presence of certain functional groups and bonds), the ability to interact with collagen and the reagents used for its processing (Andreyeva *et al.*, 2001). Since the effectiveness of enzymes and enzyme preparations largely depends on many factors, including the duration of storage, the purpose of this study was to determine the effect of long-term (four years, in a refrigerated chamber at a temperature of 7-8 °C) storage of enzyme-containing preparation obtained from fish waste on its properties and technological capabilities.

MATERIALS AND METHODS

Materials

The work uses the above-mentioned enzyme-containing preparation from fish waste, as well as leather and chemical materials common in leather production:

- *pelt* obtained from cattle hide (skin) after soaking and liming processes according to the method of producing chrome-tanned leather for footwear uppers; mass part of moisture – 28.4%, shrinking temperature – 57 °C, thickness – 1.84 mm;
- *chrome tanned leather* for shoe uppers without finishing (Crust), made from this pelt;
- *enzyme-containing preparation* from fish waste (ECP), which was stored under polyethylene film in a refrigerator at a temperature of 7-8 °C for four years;
- *other chemical materials used in leather production*: ammonium sulphate (DSTU 7370:2013), sodium chloride (DSTU 8056:2015), sodium bicarbonate (DSTU 3893:2016) and sodium carbonate (DSTU 7274:2012), sulphuric (DSTU 2184:2018) and acetic (DSTU 13189: 2019) acids, dry chromium tannin (DSTU 2726-94), Sulphirol C – anionic fatliquor based on sulphated fish oil (Smit & Zoon, the Netherlands), condensed tannins of quebracho extract (China).

Methods

To achieve this goal, traditional physical and chemical methods of analysis and a modern spectroscopic (spectrophotometric) method were used in the study. For physical and mechanical tests and chemical analysis, samples of raw materials and Crust were selected and analysed in accordance with the requirements of regulatory documents: preparation and sampling – ISO 2588:2022; determination of the mass part of moisture – ISO 4684:2005; determination of the mass part of minerals (ash) – ISO 4047:2006; determination of nitrogen content (mass fraction of carbonaceous matter) – ISO 5397:2006; determination of chromium oxide content – ISO 5398-1:2007; determination of the content of substances extractable by organic solvents – ISO 4048:2006; determination of tensile strength and relative elongation – ISO 3376:2008; determination of porosity – ISO 2589:2019; measurement of thickness – ISO 2589:2019; determination of shrinkage temperature – ISO 3380:2008.

To evaluate the effect of the enzyme preparation on the structure of the biogenic material (leather semi-finished product) during enzymatic treatment, a modified gelatin melting method was used to control the liming and bating processes, which are aimed at removing soluble protein substances from biogenic raw materials. The essence of the method is that a 0.5 g weight of the crushed semi-finished product (in terms of absolute dry matter) is placed in a 100 mL flask with a lapped lid and 50 mL of distilled water is poured in. The flask is closed and kept in a thermostat at 70 °C for 2 h with periodic stirring, and then cooled. The contents of the flask are filtered through a cotton filter into a 100 mL volumetric flask, thoroughly rinsed with distilled water, which is poured onto a filter to wash the melted gelatin. Then add 1 mL of 10% sodium hydroxide solution and 5% copper sulphate solution to the volumetric flask. The volume is made up to the mark with distilled water. The flask was transferred to a dark place, where it was kept for 20 min, after which the optical density of the solution was measured using a spectrophotometer at a wave length $\lambda = 520$ nm in a 10-mm-wide cuvette (ULAB 102 UV spectrophotometer, China).

The gelatin concentration, g/L, is determined by extrapolating from the calibration curve based on the results of determining the optical density of gelatin solutions of the set concentration: 0.10, 0.15, 0.20, 0.25, 0.30, 0.42, 0.52 and 0.75 g/L.

Process Recipe for Leather Semi-Finished Product

The research was carried out in laboratory conditions in glass containers on a special installation, the design of which allows maintaining the required temperature and constant rotation. Production parameters of chrome tanned leather for shoe uppers:

1. Washing: water – 200 %, temperature 28-38 °C, duration 40 min;
2. Deliming: water – 200 %, ammonium sulphate 2.2%, temperature 38 °C, duration 60 min;
3. Bating: in deliming bath; product ECP 0.2% (group 1), 0.4% (group 2), 0.6% (group 3), 0.8% (group 4); temperature 38 °C, duration 60 min;
4. Washing: water – 200%, temperature 30-25 °C, duration 30 min;
5. Pickling: water – 80%, temperature 20-23 °C, sodium chloride 6.0%, sulphuric acid 0.8% 10 minutes after the salt dosage, duration 90 min;
6. Tanning: in a spent pickling solution, dry chrome tanning agent (basicity 36-42%) 2.2%, sodium bicarbonate 0.4% 3 h after the start of tanning, temperature 20-23 °C, duration 9 h;
7. Laying: duration 12 h;
8. Washing: water – 100-150%, temperature 35 °C, duration 20 min;
9. Neutralization: water – 250%, temperature 35 °C, sodium bicarbonate 1.5 %, duration 60 min;

min;

10. Washing: water – 200%, temperature 35 °C, duration 40 min;
11. Fatliquoring: water – 200%, temperature 55 °C, anionic fatliquor 6.0% (100 % fat), acetic acid 0.3% by weight of fatliquor (at the end of fatliquoring), duration 60 min;
12. Retanning: in the spent fatliquoring bath, temperature 35 °C, quebracho (in terms of tannins) 2.0%, duration 40 min;
13. Washing: water – 200%, temperature 30 °C, duration 10 min.
14. Laying, sammy-out, drying, tempering, staking.

Material consumption was calculated from the weight of semi-finished product samples.

RESULTS AND DISCUSSION

After long-term storage in polyethylene film at a low temperature, the preparation did not lose its appearance: there was no change in consistency and color, nor the appearance of an unpleasant smell. pH of the 10% solution was 4.5.

When assessing the activity of the preparation by the precipitation method, a slight decrease in its activity was found relative to the initial value at a temperature of 40 °C (420 units/g vs. 450 units/g), which indicates a certain stability of the structure and properties of the studied object. The effect of temperature on the activity of the preparation was evaluated in the range of 20-60 °C, which is quite acceptable for liquid physico-chemical processes of leather production. It was found that the activity of the preparation increased with an increase in temperature from 20 to 40 °C. With a further increase in temperature, there was a tendency to decrease this indicator (Fig. 1).

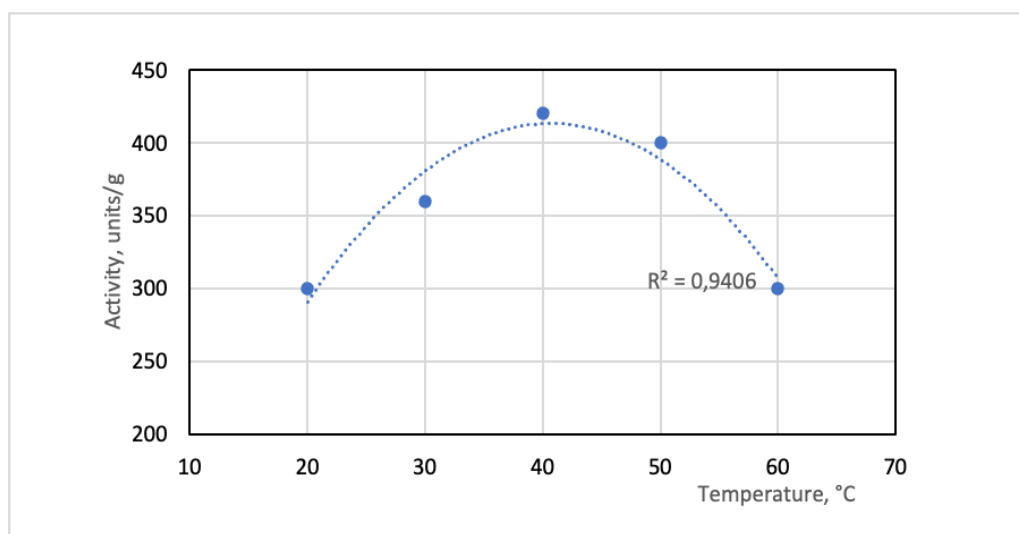


Figure 1. Effect of temperature on the ECP activity after storage for 4 years

The next series of experiments was devoted to determining the technological capabilities of the investigated enzyme-containing preparation after long-term storage. It should be noted that when determining the melting of gelatin, even before working with the photospectrometer, the color of the solutions under study signaled different amounts of nitrogen in different groups (Fig. 2). This indicated the influence of the conditions of enzymatic treatment on the degree of removal of interfibrous proteins from the structure of the dermis. Indeed, with an increase in the consumption of the enzyme preparation from 0.2 to 0.8 %, gelatin melting (i.e., loosening of the structure) doubled, regardless of the duration of

bating. At the same time, the more active effect of the ECP is detected after 0.5 h of bating, and after bating for 1.0 h, the gelatin melting rate decreases by 31.8-37.7 % (Fig. 3).



Figure 2. Flasks with analytical solutions prepared for use on the spectrophotometer (first four flasks on the left – after 0.5 h of bating, last four flasks on the right – after 1.0 h of bating)

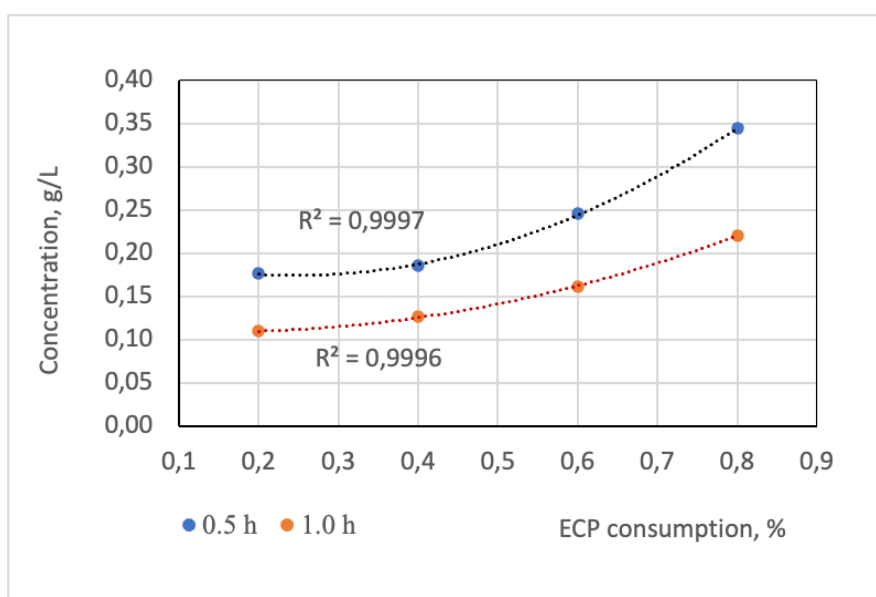


Figure 3. Effect of bating conditions on gelatin melting

The results of chemical analysis and physico-mechanical tests of leather materials in the form of pelt and Crust are shown in Table 1, which shows that the use of the ECP for bating, even after prolonged storage, allows to obtain high-quality leather material that fully meets the requirements of regulatory document in terms of consumer properties (strength, elongation, hydrothermal resistance, chemical analysis).

The absence of a difference between the strength of the leather as a whole and the strength of its front layer indicates a more even distribution of components in the dermis and is an indirect confirmation of the improvement of the cutting properties of the leather, i.e. more rational use of scarce raw materials.

Table 1. Indicators of pelt and finished leather (Crust)

Indicator	Value				Regulatory document **
	group 1	group 2	group 3	group 4	
PELT:					
Mass part of the pelt substance, %*	78.6	77.4	76.9	76.0	-
CRUST:					
Mass part, %:					
- moisture	15.2	15.2	15.6	15.7	10-16
- chrome oxide*	5.3	5.3	5.0	5.5	≥3.5
- substances extracted with an organic solvents*	7.2	7.0	7.5	7.8	3.7-10
Tensile strength, MPa	15.6	15.5	15.3	15.1	≥15.0
Tension at the appearance of cracks in the grain, MPa	15.6	15.5	15.3	15.1	13.0
Elongation under tension 10 MPa, %.	27.0	29.0	30.8	31.0	20-40
Porosity, %.	58.0	66.0	67.0	68.5	-
Thickness, mm	1.78	1.80	1.81	1.75	-
Shrinking temperature, °C	117	118	117	118	-

Note: *In terms of absolutely dry substance;

**DSTU 2726-94. Leather for shoe uppers. Technical specifications (for cow, bovine, pigskin).

CONCLUSION

Enzymatic methods have become an important and indispensable part of the processes used by modern industry for the production of a wide range of goods. The aquatic environment contains a huge pool of diverse genetic material and, therefore, represents a significant potential for various sources of enzymes. In recent years, research has been carried out on the study of enzymes of fish and aquatic invertebrates, interesting applications related to marine enzymes have appeared. Enzymes obtained from fish processing waste may have distinctive features that make them more suitable for industrial applications, as fish live in a wide range of temperature conditions and have characteristics that distinguish them from warm-blooded animals. Thus, to maximise the efficient use of aquatic resources, these enzymes can be extracted from fish processing waste and used as value-added products or processing aids in a variety of economic applications.

The effectiveness of enzymes and enzyme preparations largely depends on many factors, including the duration of storage. We have experimentally confirmed the stability in time of the structure and properties of the enzyme-containing preparation obtained from fish waste, its compatibility with dermal collagen, i.e. the possibility of using it for skin treatment. Further research in this area is planned. The expected results include expanding the range of materials for the production of natural leather, more rational use of natural resources, and reducing the harmful impact on the environment as a result of the production activities of fish processing and tanneries.

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SURFACTANT INFLUENCE ON THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES AS POTENTIAL ANTIMICROBIAL TREATMENT FOR TEXTILES

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The abundance of the scientific literature reporting the performances of the metal and metal-oxide nanoparticles promoted further studies regarding the new synthesis strategies that combine the advantages of low costs and environment-friendly methods. Herein, the synthesis of zinc oxide nanoparticles (ZnO NPs) is presented, using different ratios of zinc precursor and a tension-active compound, sodium dodecyl sulfate (SDS). The fabricated dispersions were used as antimicrobial treatment for a textile fabric consisting of a knitted 95% cotton / 5% elastane. The deposition was performed using the padding method. The treated fabrics were evaluated in terms of antimicrobial activity, against *Escherichia coli* and *Staphylococcus aureus*, and physico-mechanical properties (mass per unit area, horizontal and vertical density, air permeability and hydrophilicity). The antimicrobial efficiency was correlated with the critical micelle concentration (CMC) of the surfactant used. The satisfactory effect was obtained when the concentration of SDS was above this concentration. The size of the inhibition zone varied in accordance with the zinc precursor content, in the case of gram-negative bacteria and showed disorderly behavior for the gram-positive bacteria. The mass per unit area and the horizontal and vertical density did not suffer significant modifications after treatment deposition. However, the air permeability decreased due to the presence of the long hydrocarbon chains from the surfactant molecular structure, while its ionic part led to an improved hydrophilicity.

Keywords: textiles, nanoparticles, antimicrobial.

INTRODUCTION

The increasing progress in nanoscience research provided a solid fundament for exploiting the properties of nanomaterials in different fields and for different applications (Baig *et al.*, 2021). Metal and metal-oxide nanoparticles are the subject of many studies evaluating their potential in areas such as medicine (for drug delivery, diagnostics, tissue engineering, antimicrobial properties), environment bioremediation (as pollutants removers, sensors, catalysis), agriculture (as pesticides and herbicides, food safety, water purification), material science (as protective coatings) electronics (for energy storage) etc. (Altammar, 2023). In textiles science, nanomaterials have been applied for textile finishing (manufacturing antimicrobial and self-cleaning textiles), on the one hand (Vats *et al.*, 2023; Dejene & Geletaw, 2023), and for environment bioremediation (textile wastewater treatment), on the other hand (Al-Araji *et al.*, 2023; Munir *et al.*, 2023). Among metal nanoparticles tested for their antimicrobial properties, silver nanoparticles (AgNPs) are the most popular, due to their versatility and high efficiency at low cost. Moreover, by synthesizing AgNPs using *green* methods, the environment-friendly property is added to the list of their advantages (Lite *et al.*, 2022; Lite *et al.*, 2023). Gold nanoparticles have been also studied for their antimicrobial properties and environment-friendly fabrication. However, despite their high efficiency, the high cost of the gold precursor (gold salts), represents a disadvantage (Si *et al.*, 2020). The antimicrobial mechanism consists of disrupting the membrane of the

microorganism by producing reactive oxygen species and by binding the -SH groups of the constituent proteins (Abdul-Reda Hussein *et al.*, 2024). In addition to their metal homologues, the metal-oxide nanoparticles present also the photocatalytic effect. Titanium nanoparticles (TiO₂ NPs) (Anbumani *et al.*, 2022), magnesium oxide nanoparticles (MgO NPs) (Khan *et al.*, 2022), calcium oxide nanoparticles (CaO NPs) (Jadhav *et al.*, 2022), zinc oxide nanoparticles (ZnO NPs) (Jayachandran *et al.*, 2021; Kim *et al.*, 2020), copper oxide nanoparticles (CuO/Cu₂O NPs) (Shehabeldine *et al.*, 2023) were reported to exhibit antimicrobial properties and are promising candidates for producing antimicrobial treatments for textile finishing. For this work, the synthesis of ZnO NPs was performed, using different ratios between the surfactant and the zinc precursor. The resulting dispersions were deposited on textile fabrics and the antimicrobial effect was evaluated. Moreover, it was determined how the physico-mechanical properties of the treated textile fabric were altered by the obtained dispersions.

EXPERIMENTAL

Reagents, Solutions and Materials

The precursor of the zinc oxide consisted of zinc acetate dihydrate. The surfactant selected for this study was sodium dodecyl sulfate (SDS), an anionic surfactant, with a long hydrocarbon chain. Sodium hydroxide 0.02M was used for pH adjustment. All reagents were purchased from Merck and were of analytical purity. The textile fabric used consisted of a knitted 95% cotton / 5% elastane (navy blue color) – made of threads Nm 50/1 having horizontal density, Ht = 13 threads no./cm and vertically density, Vt = 19 threads no./cm. The binder used in the finishing process was based on acrylic resin (PERMUTEX® RA-9260) and was purchased from Stahl Europe B.V.

Sample Preparation

Synthesis of ZnO NPs Treatment

Solutions of the zinc precursor and surfactant were prepared, to a final volume of 600 mL. Different molar ratios of these elements were used, according to Table 1. The pH of each solution was adjusted to ~11, using NaOH 0.02M, until they turn into milky-white dispersion.

Table 1. Molar ratios zinc precursor/SDS used for ZnO NPs synthesis

Sample code	Zn(OAc) ₂	SDS
0.5:1	0.5	1
1:1	1	1
2:1	2	1
3:3	3	3
5:5	5	5
10:10	10	10
1:2	1	2
2:2	2	2
1:10	1	10
5:10	5	10

Treatment Deposition on Textile

The textile materials (20 cm × 30 cm) were treated with the obtained dispersions, after 24 h from their preparation, by the padding method. For this process, a laboratory-scale paddler instrument BVHP 2 (Roaches, West Yorkshire, UK), equipped with two rollers, was

used. Each sample was passed two times through the padder, then, they were subjected to a drying operation, for 4 min, at 100°C, followed by a curing step, for 2 min, at 150°C, using a drying/curing/heat-setting unit, model TFO/S 500 mm (Roaches, West Yorkshire, UK). The binder emulsion was prepared up to a concentration of 20 g/L. For each textile sample, 200 mL of the binder emulsion was used, directly in the padder bath.

Sample Characterization

Antimicrobial Assessment

To perform a qualitative antimicrobial assessment, against *Staphylococcus aureus* ATCC 6538 (gram-positive) and *Escherichia coli* ATCC10536 (gram-negative), the agar-well diffusion method was used, according to SR EN ISO 20645/2005. A volume of each strain was spread on the entire Petri dish surface. The textile samples (10 mm in diameter) were placed in the center of the plates, on the surface of the nutrient medium. The plates were incubated at 37°C for 24h. Inhibition zones were calculated according to the following formula:

$$IZ = (D - d)/2 \quad (1)$$

where IZ represents the diameter of the inhibition zone (mm), D – the total diameter of the specimen plus the inhibition zone (mm), and d – the diameter of the specimen (mm).

Physico-Mechanical Properties

The treated fabrics were characterized in terms of mass per unit area (SR EN 12127-2003), density (SR EN 1049-2:2000), permeability to air (SR EN ISO 9237:1999), and hydrophilicity, measured using the drop test method, according to the Romanian Standard SR 12751/1989 standard.

RESULTS AND DISCUSSION

Antimicrobial Assessment

The results of the tested samples are presented in Table 2, for both bacteria strains. According to the standard SR EN ISO 20645:2005, the criteria for inhibition zones are the following: if the size of the inhibition zone is zero and the sample shows visible bacteria contamination, the effect is evaluated as unsatisfactory. When the contamination is minimal, the effect is at the efficiency limit. The effect is evaluated as satisfactory when there is no bacteria growth observed on the sample (even if the size of the inhibition zone is zero). When the size of the inhibition zone is higher than zero, the antimicrobial effect can be further quantified.

The samples evaluated with a satisfactory effect correspond to the ratio precursor:surfactant of 1:10, 5:10, and 10:10. The common aspect of these samples is represented by the high content of surfactant. This phenomenon is related to the behavior of the surfactant at its critical micelle concentration (CMC) (IUPAC). In the case of SDS surfactant, the value of the CMC is 8×10^{-3} mol/L (Dominguez *et al.*, 1997). The formation of micelles prevents growth and aggregation of the ZnO NPs. Table 3 illustrates the Petri dishes containing the samples evaluated with the satisfactory effect.

Table 2. Antimicrobial assessment

Sample code	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Inhibition zone (mm)	Effect evaluation	Inhibition zone (mm)	Effect evaluation
0.5:1	0	Unsatisfactory	0	Unsatisfactory
1:1	0	Unsatisfactory	0	Unsatisfactory
2:1	0	Unsatisfactory	0	Unsatisfactory
3:3	0	Unsatisfactory	0	Unsatisfactory
5:5	0	Unsatisfactory	0	Unsatisfactory
10:10	3.5	Satisfactory	6	Satisfactory
1:2	0	Unsatisfactory	0	Unsatisfactory
2:2	0	Unsatisfactory	0	Unsatisfactory
1:10	2.5	Satisfactory	3.5	Satisfactory
5:10	3	Satisfactory	2.5	Satisfactory

When comparing the sizes of the inhibition zone (Figure 1), the general trend observed is of direct proportionality between zinc precursor and antimicrobial effect. This effect is in accordance with previous studies (Sirelkhatim *et al.*, 2015; Li *et al.*, 2020). In the case of *Escherichia coli* (gram-negative bacteria), the values of the inhibition zone size corresponding to the ratio zinc precursor:surfactant 1:10, 5:10, and 10:10 are 2.5, 3, and 3.5 mm, while for *Staphylococcus aureus* (gram-positive bacteria), the values were 3.5, 2.5, and 6 mm. Therefore, the treatment presented higher efficiency for gram-positive bacteria. Moreover, while for *E. coli* the effect was of a slight increase in efficiency with the zinc content increase, for *S. aureus*, the efficiency reached a minimum efficiency for the 5:10 ratio. This phenomenon could be explained based on the cumulative effect of the particles' size and content. While the increase if the content promotes a higher antimicrobial efficiency, the increase in the particle size or the formation of aggregates leads to a decrease in efficiency (Palanikumar *et al.*, 2014; Álvarez-Chimal *et al.*, 2022).

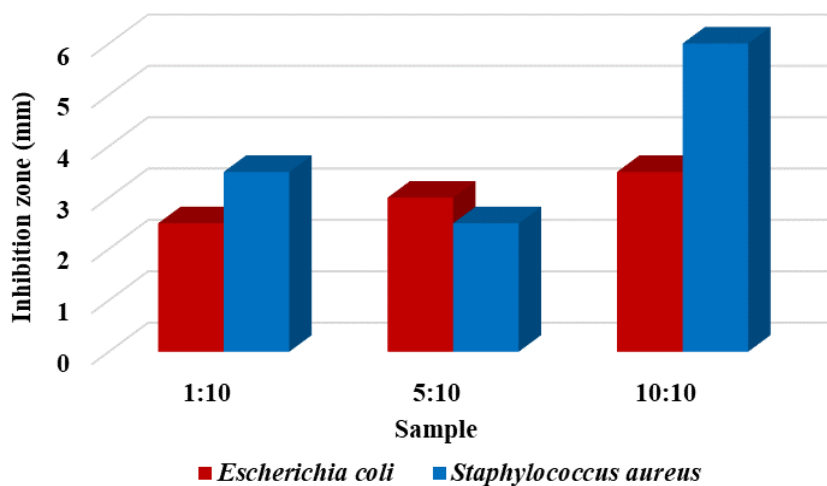
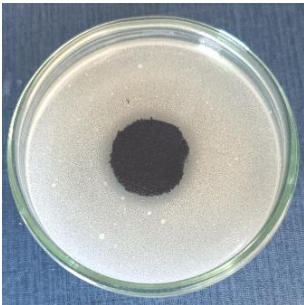
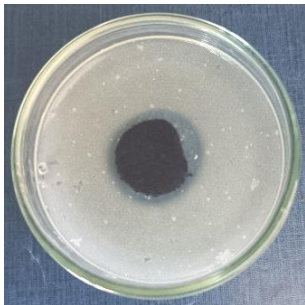
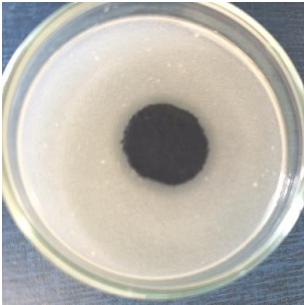
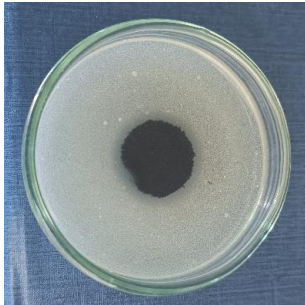
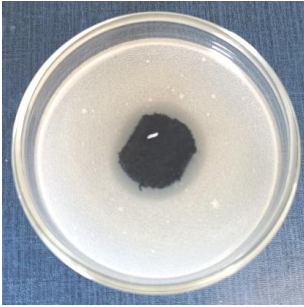
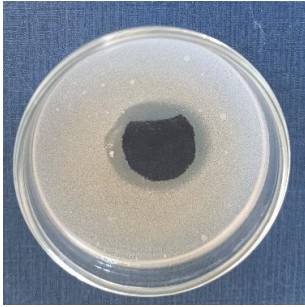


Figure 1. Sizes of the zone of inhibition formed on Petri dishes inoculated with microbial strains and incubated with textile samples treated with ZnO NPs

Table 3. Images of Petri dishes inoculated with the tested microbial strains and incubated with textile samples treated with ZnO NPs

Bacteria strain Sample	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
1:10		
5:10		
10:10		

Physico-Mechanical Properties

The physico-mechanical characteristics (Table 4) showed little modification of mass per unit area, density, and air permeability, while the hydrophilicity suffered a decrease to a minimum of 52%.

Except for sample 5:10, the mass per unit area was not modified after depositing the treatment, suggesting that the antimicrobial efficiency is obtained even at a minimum loading of the material. The horizontal and vertical density decreased by 5.4% and 0.5% respectively, and it was maintained for all samples. This might be due to the textile fibers relaxation after applying the treatment and might also explain the lack of increase in mass per unit area (Laureckienė & Milašius, 2017; Li *et al.*, 2021). The air permeability decreased by 30-33%, while hydrophilicity increased by 28-52%. The presence of the tension-active compound sodium dodecyl sulfate contributed to the decrease of air permeability due to its long hydrocarbon chain, while the ionic part of the molecule bonded to the functional groups of the textile material led to an improved hydrophilicity. However, even with these improvements,

the material remained hydrophobic, which led to the conclusion that the initial character of the material is not drastically changed after applying the treatment.

Table 4. Physico-mechanical characteristics of the treated textiles compared to reference

Sample	Fabric mass per unit area (g/m ²)	Density		Air permeability, 100 Pa (l/m ² /s)	Hydrophilicity (s)
		Horizontal (threads no. /10cm)	Vertical (threads no. /10cm)		
Reference	175	148	191	305.3	105
1:10	175	140	190	203.6	50
5:10	169	140	190	213	75
10:10	175	140	190	204.3	70

CONCLUSION

Textile fabric samples of a knitted 95% cotton / 5% elastane (navy blue color) were treated with synthesized ZnO NPs of different ratios zinc precursor:SDS surfactant. The color of the material was intentionally selected in order to evaluate the effect of the white-milky treatment deposition. The first observation after applying the treatment was that this did not alter the color of the material. By assessing antimicrobial efficiency, a strong relation between the content of the surfactant and the antimicrobial effect of the resulting ZnO NPs dispersion was demonstrated. This behavior was attributed to the critical micelle concentration of surfactant used. In the case of gram-negative bacteria *E. coli*, a general trend of increase in efficiency was observed with the increase of zinc precursor (from 2.5 to 3.5 mm IZ), while for the gram-positive bacteria *S. aureus*, the effect was cumulative between the antagonist antimicrobial effect at the increase of the ZnO particles size and its total content (the minimum IZ was 2.5 mm for the ratio 5:10 and the maximum was 6 mm, for the ratio 10:10). The physico-mechanical characteristics were not significantly modified in terms of mass per unit area and density (horizontal and vertical). However, the air permeability decreased by 30-33% as a result of treatment deposition, while the hydrophilicity was improved.

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COLLAGEN/ALBUMIN-BASED MATRICES DESIGNED FOR VAGINAL ADMINISTRATION OF A NON-STEROIDAL ANTI-INFLAMMATORY DRUG

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The aim of this study was to develop some spongy matrices based on collagen and albumin, loaded with a non-steroidal anti-inflammatory drug (NSAID). The formulations were designed as vaginal drug delivery systems with potential applications in some gynaecological conditions associated with inflammation. Spongy matrices were obtained by lyophilization of the corresponding hydrogels, prepared by mixing a type I collagen gel with various proportions of albumin, followed by incorporating aceclofenac as the drug model and glutaraldehyde as a cross-linking agent. The spongy matrices were characterized through various morphological, biological and biopharmaceutical analyses. SEM analysis displayed the specific microstructure of the biopolymers and the presence of aceclofenac in the polymeric network. Swelling capacity and enzymatic degradation revealed good absorption properties and resistance to collagenase for collagenic spongy matrices, while the addition of albumin led to a limited swelling capacity and a rapid degradation profile due to albumin water solubility. The kinetic analysis showed a non-Fickian drug release mechanism for all matrices. By correlating all the obtained results, it can be stated that spongy matrices based on collagen, albumin and aceclofenac can represent a starting point in the development of new therapeutic systems for vaginal administration, considering the introduction of a third polymer in order to improve the resistance and stability of the systems, and therefore their therapeutic performance.

Keywords: collagen/albumin spongy matrices, aceclofenac, vaginal administration

INTRODUCTION

Many gynaecological conditions, which include bacterial vaginosis, endometriosis or gynaecological cancers, are often accompanied by inflammatory processes (Mitchell and Marrazzo, 2014; AlAshqar *et al.*, 2021; Garg *et al.*, 2023). Moreover, persistent inflammation encountered in such conditions has been associated with infertility and pregnancy complications (Ravel *et al.*, 2021).

Regarding the treatment of these pathologies, both systemic and local treatment options are currently available. However, in recent years, localized treatment has received special attention due to a series of advantages such as the minimization of adverse effects and the targeted action of these therapies (Thapa *et al.*, 2022). Regarding vaginal drug delivery systems, natural polymers play an important role in the development of such systems, based on distinctive features such as biocompatibility, biodegradability and non-toxicity (Pandey *et al.*, 2020; Jain *et al.*, 2024).

Collagen is the main component of the extracellular matrix and is responsible for ensuring tissue strength, being found in connective tissue that forms structures such as the skin, tendons, bones or ligaments (Kong *et al.*, 2023). As a biomaterial, it possesses several important characteristics such as biocompatibility, lack of toxicity and biodegradability (Tudoroiu *et al.*, 2023), alongside with its versatility which allows its processing in different pharmaceutical forms, such as spongy matrices, films or semi-solid formulations. Moreover, it has a great number of applications in regenerative medicine, tissue engineering, wound healing and controlled drug delivery systems (Marin *et al.*, 2018).

Albumin is one of the most important proteins in the body, with important implications in drug transport and blood colloidal osmotic pressure regulation (Belinskaia *et al.*, 2021). Moreover, it is a great candidate for developing drug delivery platforms, based on its biocompatibility and non-immunogenicity. An important advantage of albumin compared to other biopolymers is related to the presence of charged amino acids on its surface, allowing the proper loading of hydrophobic drugs. Furthermore, albumin is widely used in drug delivery of anticancer treatments, based on its interaction with some abundantly expressed receptors associated with several cancers (Asrorov *et al.*, 2024).

Therefore, the aim of this study was the development and evaluation of some collagen/albumin-based spongy matrices, loaded with a NSAID, with potential applications in the adjuvant treatment of some gynaecological conditions associated with inflammation.

MATERIALS AND METHODS

Materials

The collagen-based hydrogels were obtained by using the collagen gel preparation technique of Collagen Department, Division Leather and Footwear Research Institute, National Research and Development Institute for Textile and Leather, Bucharest (INCDTP). Thus, type I fibrillar collagen gel of approximately 2.30% (w/w) concentration and acidic pH was extracted from calf hide using the established methodology. In order to obtain a collagen gel with a concentration of 1.0% and physiological pH (7.4), 1M sodium hydroxide solution was gradually added to the initial collagen gel, under continuous mechanical stirring.

Reagents used in the present study were purchased as follows: aceclofenac (ACF) from MP Biomedicals, albumin, glutaraldehyde (GA) and sodium hydroxide from Merck, glacial acetic acid and sodium acetate from Chemical. Distilled water was used both in the preparation stage and in the performed analyses, and all other chemicals used were of analytical grade.

Methods

Preparation of Hydrogels Based on Collagen, Albumin and Aceclofenac

In order to prepare the combined collagen/albumin-based hydrogels, albumin powder was added to the previously obtained 1% collagen gel (pH 7.4), and the mixture was subjected to appropriate homogenization until its complete incorporation. Then, both aceclofenac (0.2 or 0.4%) and the crosslinking agent represented by glutaraldehyde were incorporated. The composition of the prepared hydrogels (Fig. 1) is shown in Table 1.

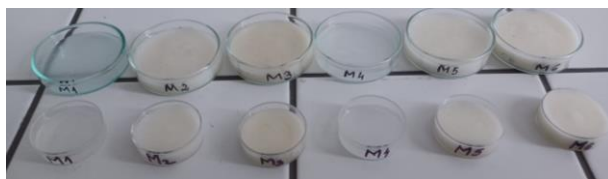


Figure 1. Hydrogels based on collagen, albumin and aceclofenac, crosslinked with GA

Table 1. Composition of the prepared hydrogels and related spongy matrices

Code	Collagen, %*	Albumin, %*	ACF, %*	GA, %*
CA1	1	0	0.2	0.005
CA2	1	2.5	0.2	0.005
CA3	1	5	0.2	0.005
CA4	1	0	0.4	0.005
CA5	1	2.5	0.4	0.005
CA6	1	5	0.4	0.005

* The amount of collagen albumin, ACF and GA are reported to 100 g hydrogel

Obtaining Process of the Spongy Matrices

The spongy matrices were obtained by lyophilization of previously prepared hydrogels, using a Delta LSC 2-24 apparatus (Martin Christ, Germany). The lyophilization program was the one established within the Collagen Department of the Leather and Footwear Research Institute, INCDTP, Bucharest. The lyophilization program lasted 48 hours using a freezing temperature of -40°C during the first 10 hours of the process.

Scanning Electron Microscopy (SEM)

Morphological analysis of the spongy matrices based on collagen, albumin and aceclofenac was performed using a Hitachi Tabletop Microscope TM 4000 Plus (Hitachi, Japan). All samples were analyzed without being coated with a conductive layer and using a voltage of 15 kV. Detection was performed using the back-scattering (BSE) mode.

Swelling Capacity

The evaluation of the swelling capacity of spongy matrices based on collagen, albumin and aceclofenac was performed using a previously described methodology (Tihan *et al.*, 2019) and acetate buffer, AB (pH 5.5) as the absorption medium, in order to simulate the conditions encountered at the cervico-vaginal level. The experiment was conducted in duplicate and the swelling capacity was calculated using Equation (1):

$$\text{Swelling capacity (g/g)} = (W_t - W_i)/W \quad (1)$$

where W_t corresponds to the weight of the swollen matrix at specific time points, and W_i is the initial weight of the spongy matrix, in dry form.

Enzymatic Degradation

In order to evaluate the enzymatic degradation of the spongy matrices, a previously reported methodology was used (Tihan *et al.*, 2019). The degree of enzymatic degradation was determined by evaluating the weight loss recorded by the spongy matrices, in a predetermined time interval. The experiment was performed in duplicate and weight loss (%) was calculated using Equation (2):

$$\text{Weight loss (\%)} = (W_0 - W_t)/W_0 \times 100 \quad (2)$$

where W_0 represents the weight of the spongiuous matrix after soaking in phosphate buffer (initial weight) and W_t – the weight of the matrix soaked in collagenase solution, measured at different time intervals.

In vitro Release Kinetics of Aceclofenac

The assessment of the *in vitro* release kinetics of aceclofenac from the spongiuous matrices was carried out by using an experimental “sandwich” type device, which was attached to a dissolution apparatus with paddles, as previously described (Barbaresso *et al.*, 2014), using AB pH = 5.5 at 37°C as release medium. Samples collected at different predetermined time intervals were spectrophotometrically analyzed at a wavelength of 275 nm (representing the absorption maximum of ACF). Furthermore, in order to establish the release mechanism of aceclofenac from the spongiuous matrices, the experimental data were fitted using the Power Law model (Equation 3) and the Higuchi model (Equation 4):

$$m_t/m_\infty = k \cdot t^n \quad (3)$$

$$m_t/m_\infty = k \cdot t^{0.5} \quad (4)$$

where m_t/m_∞ corresponds to the fraction of drug released at different time points (t), k – the kinetic constant, and n – the release exponent.

RESULTS AND DISCUSSION

SEM analysis revealed the characteristic surface morphology of the designed spongiuous matrices. Thus, depending on the specific composition of each sample, significant differences can be observed in the structure of the analyzed matrices. The SEM images obtained for the CA1-CA6 spongiuous matrices are displayed in Figure 2.

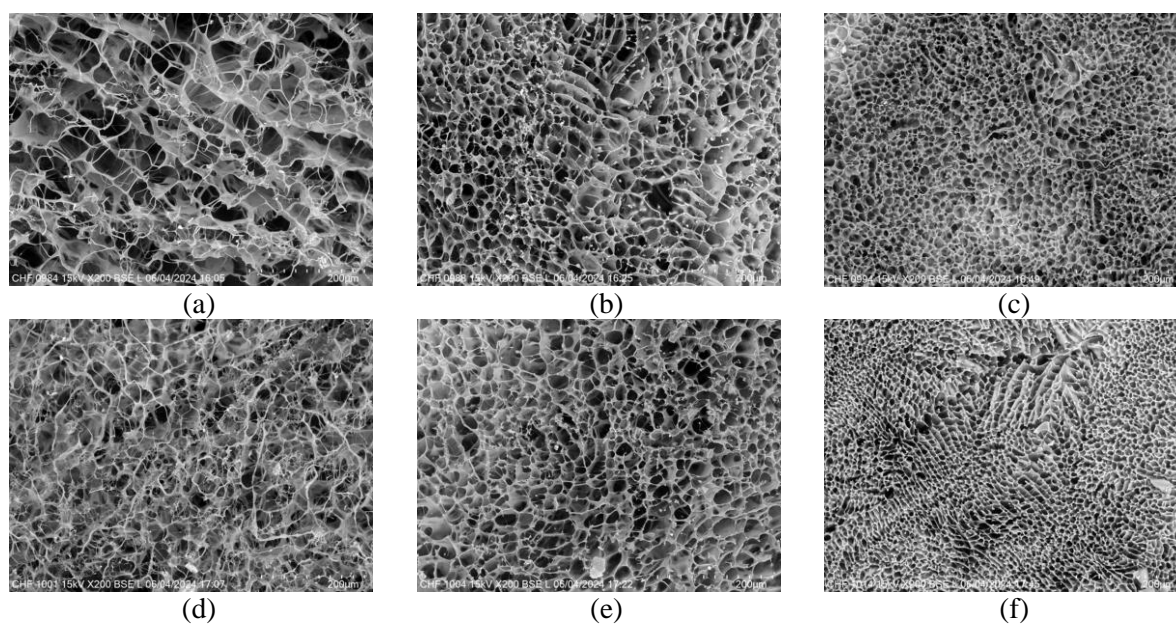


Figure 2. SEM images of spongiuous matrices based on collagen, albumin and aceclofenac

Analyzing the obtained SEM images, certain morphological characteristics specific to each polymer can be noticed. The matrices based on collagen and aceclofenac (CA1 and

CA4) showed the characteristic microstructure of collagen matrices, namely, numerous pores having different shapes and sizes and a non-uniform distribution, interconnected by a network of collagen fibers. The addition of albumin generated a series of changes in the microstructure of the matrices, having an increasingly compact appearance, with the increase in the proportion of albumin in the samples. Compared to the collagen matrices, the matrices based on collagen and albumin (CA2, CA3, CA5 and CA6) showed a different pore architecture, presenting an intermediate structure between that of collagen and albumin, the latter being characterized by a denser, lamellar-type polymeric network, with interconnected pores, being more numerous and having smaller sizes with the increase in the proportion of albumin. Regarding the active substance, the images show the presence of aceclofenac, both embedded in the microporous structure and in free form, on the surface of the matrices.

The swelling capacity of the spongy matrices is a very important quality attribute in the case of vaginal administration, since they must quickly hydrate in contact with the limited volume of vaginal fluid available at the site of administration, gradually transforming into the original hydrogel and releasing the drug (Furst *et al.*, 2015). The swelling capacity of all spongy matrices, at different time intervals, is shown comparatively in Figure 3.

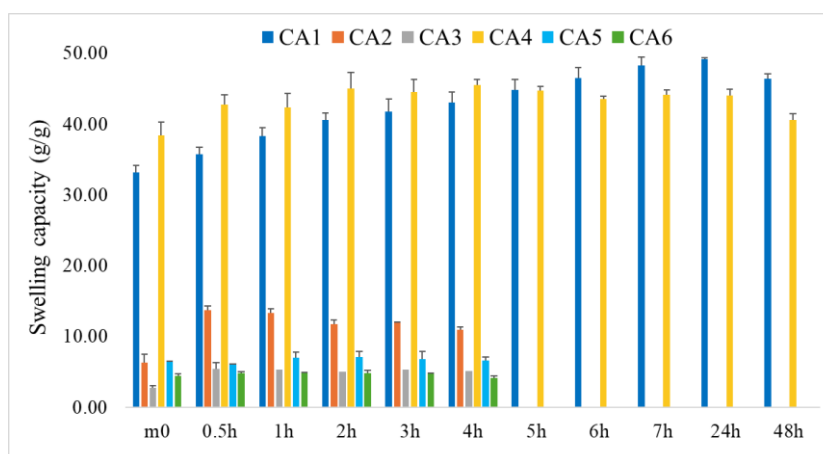


Figure 3. The swelling capacity of the spongy matrices CA1-CA6

The maximum swelling capacity recorded for each individual spongy matrix varied between 4.82 g/g (CA6 matrix) and 49.08 g/g (CA1 matrix). Among the 6 designed spongy matrices, CA1 and CA4, showed the best absorption capacity, showing the characteristic behaviour of collagen matrices. On the other hand, the addition of albumin led to a marked decrease in the swelling capacity, as well as a faster loss of structural integrity, gradually transforming into the original hydrogels, once the threshold of 4 hours is exceeded.

Another important attribute of collagenic spongy matrices is their resistance to enzymatic degradation. Analyzing the obtained results, it can be stated that the CA1 and CA4 matrices, having only collagen and ACF in their composition, had the highest resistance to enzymatic degradation, preserving their structural integrity over a period of 24 hours, compared to matrices that had albumin in their composition, which lost their structural integrity in about 2 hours. However, correlating the results with those obtained in the evaluation of the swelling capacity, the rapid loss of structural integrity can also be associated with the soaking of the samples in the collagenase solution, followed by their gradual disintegration. The weight loss recorded by CA1-CA6 spongy matrices over a period of 24 hours is shown comparatively in Figure 4.

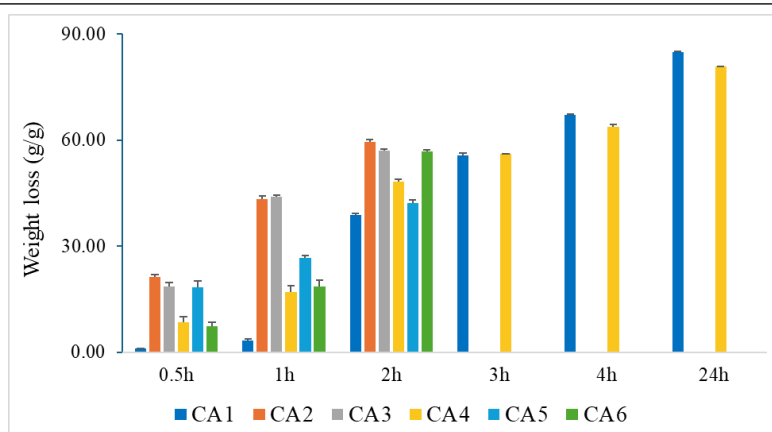


Figure 4. Weight loss (%) recorded by the spongy matrices

The weight loss recorded by CA1 and CA4 samples was comparable between the two matrices, reaching a maximum of 84.94% and 80.78%, respectively, after 24 hours. In this case, doubling the drug concentration in sample CA4 resulted in a decrease in weight loss of approximately 5%. The same trend was observed in the case of collagen/albumin-based matrices, respectively, a slower degradation correlated with the increase of drug concentration in the sample. As for the CA3 and CA6 matrices, no significant influence of drug concentration on the recorded weight loss values was noticed.

For a complete characterization of the biopolymeric drug delivery systems, an analysis of the *in vitro* release kinetics of aceclofenac (model drug) from the designed spongy matrices was performed. Thus, the influence of the spongy matrices' composition on the release kinetics of aceclofenac was studied by comparing the obtained cumulative *in vitro* drug release profiles (Figure 5).

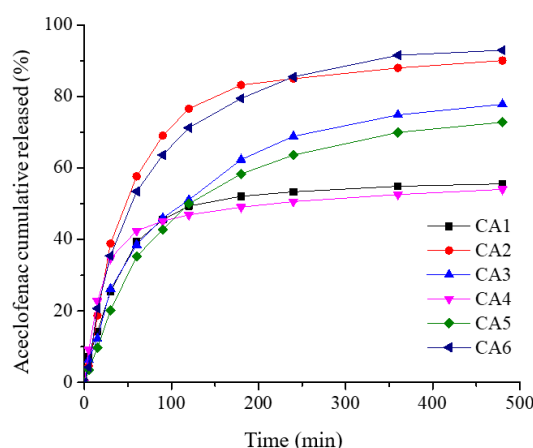


Figure 5. Cumulative release profiles of aceclofenac from CA1-CA6 spongy matrices

Thus, a difference can be observed in the shape of the release profiles of aceclofenac from collagen matrices (CA1 and CA4) compared to those corresponding to collagen/albumin-based matrices (CA2, CA3, CA5, CA6), correlating well with swelling capacity and enzymatic degradations results. However, all release profiles showed a biphasic release mechanism of aceclofenac, highlighting an initial burst-release effect, corresponding to the rapid release of aceclofenac found in free form on the surface of the matrices, in

accordance with the images obtained within the SEM analysis. This phenomenon was obvious within the first 60 minutes of the experiment, and is particularly useful in the rapid control of pain and inflammation at the site of administration. The percentage of drug released in the first 60 minutes varied between 35.30 and 57.65%, the most pronounced burst-release effect being observed for CA2 matrix. After the initial burst-release phase, the release of the drug from the spongy matrices took place gradually during the next 7 hours of the experiment, this second stage of drug release being useful in the long-term control of inflammatory processes associated with various gynaecological conditions with cervico-vaginal localization.

Regarding the maximum percentage of aceclofenac released from the spongy matrices, it varied between 54.03 (CA4) and 92.93% (CA6). The lowest values were recorded for collagen matrices (CA1 – 55.54%; CA4 – 54.03%), these results correlating well with swelling capacity and enzymatic degradation results, where an extended preservation of the structural integrity of these matrices could be observed, which can be associated with a slower degradation of the structural support and a gradual release of the drug. For collagen/albumin-based matrices, the maximum percentage of aceclofenac released was higher, with values between 72.81 (CA5) and 92.93% (CA6), correlated with increased degradation of these matrices and therefore faster release of the incorporated drug.

Subsequently, to establish the release mechanism of aceclofenac from the spongy matrices (CA1-CA6), the experimental data were fitted with the Power Law and Higuchi kinetic models. Considering the correlation coefficients values, it can be observed that the release of aceclofenac from the matrices verified the Power Law model, the coefficients having higher values in this case (0.9534 - 0.9840). Moreover, by analyzing the kinetic parameters, it can be seen that the release exponent (n) recorded values between 0.24 and 0.43 (< 0.5), which denotes a complex (non-Fickian) release mechanism of aceclofenac from the matrices. The values of the correlation coefficients obtained for Higuchi and Power law models, the kinetic parameters (k, n), as well as the maximum percentage amount of aceclofenac released from the matrices, are shown in Table 2.

Table 2. Correlation coefficients for Higuchi and Power law models, kinetic parameters and maximum percentage of aceclofenac released from the spongy matrices

Sample	Correlation coefficient		Kinetic constant, k (1/min ⁿ)	Release exponent, n	Drug released, (%)
	Higuchi Model	Power Law Model			
CA1	0.9097	0.9538	0.101	0.30	55.54
CA2	0.9232	0.9534	0.130	0.33	90.06
CA3	0.9763	0.9840	0.068	0.41	77.83
CA4	0.8793	0.9595	0.141	0.24	54.03
CA5	0.9733	0.9786	0.056	0.43	72.81
CA6	0.9526	0.9716	0.110	0.36	92.93

CONCLUSIONS

By correlating all the obtained results, it can be stated that spongy matrices based on collagen, albumin and aceclofenac could represent promising drug delivery systems intended for intravaginal administration in some gynaecological conditions associated with inflammatory processes. However, in order to improve the performance and stability of these systems, future studies will aim to introduce a third polymer, which will lead to an increase in the mechanical strength and stability of the designed matrices, and at the same time, conferring a mucoadhesive character, an important quality attribute which allows extending

the intravaginal retention time of the formulations, thus increasing the therapeutic efficiency of these biopolymeric systems.

Acknowledgement

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MICROORGANISMS DEGRADING POLYURETHANE FOR FOOTWEAR WASTE VALORISATION

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Polyurethane (PU) is a widely used polymer in footwear manufacturing, contributing to components such as outsoles, insoles, adhesives, or finishing agents, among others. Currently, 90% of discarded footwear ends up in a landfill. According to the European Directives concerning textile and footwear waste disposal, solutions are required for recovering and valorizing their different materials. Among them, polyurethane is gaining a growing interest. In this sense, strategies involving the action of microorganisms and their enzymes on the structure of polyurethane are being explored to gain insight into its revalorization. One of the key aspects is the discovery of new microorganisms capable of degrading polyurethane, thereby broadening the possibilities of identifying specific enzyme activities or combining them to target different types of polyurethanes. In this study, the isolation of microorganisms (fungi and bacteria) capable of degrading the ester polyurethane model Impranil® DLN was carried out from mature compost samples. Four bacteria and four fungi were identified, and their degradative capacity was subsequently tested using ester-type and ether-type polyurethane foams. The degradative capacity was assessed through Fourier transform infrared spectroscopy and thermogravimetric analysis, in addition to observation via scanning electron microscopy (SEM). Among the identified species, *Gymnasciella dankaliensis* demonstrated the capacity to degrade the ester-type foam. This is the first work that relates this fungus to PU biodegradation.

Keywords: biodegradation, polyurethane, microorganisms.

INTRODUCTION

Polymers are produced massively worldwide, reaching nearly 400 million tons in 2021 (Plastics Europe, 2022), and it is expected to triple by 2060 (Investigate Europe, 2023). Among these, polyurethane (PU) accounts for 5.5% of the total plastic production (Plastics Europe 2022). It is a versatile plastic with applications in numerous areas. In footwear, polyurethane is widely used in components such as soles, insoles, and for adhesives and coatings. PU is synthesized from polyols and isocyanates. Based on the polyols used, PU can be divided into two major types: polyester polyurethane and polyether polyurethane.

Due to its environmental resistance, PU poses a significant pollution challenge, and its biodegradation has become a topic of great interest to help address the problem in an eco-friendly way. In this context, discovery and characterization of highly efficient PU degrading microbes and enzymes is essential for developing green plastic recycling processes. In this regard, microorganisms with degradative capabilities have been identified from various sources, generally from soils (Wu *et al.*, 2023). This study aims to isolate and identify PU-degrading microorganisms from mature compost in order to further study its degradative mechanism and consider them as a biological tool for the degradation of PU.

In this work, mature compost was used as a source for isolating bacteria and fungi capable of degrading Impranil® DLN (Impranil), which is a protected anionic aliphatic PU-polyurethane colloidal dispersion, with 40% solid dispersed in 60% water. This dispersion is commonly used in handbags, shoe uppers, and shoe lining materials (Windermuth, 1956, Traeubel, 1988) and has been reported in previous studies as a PU model for an initial isolation (Biffinger *et al.*, 2015).

First, microorganisms that cause the visual dissolution of Impranil (also referred to as “clearing”) (Biffinger *et al.*, 2015) were isolated on agar plates (Jin *et al.*, 2021). After microorganism identification, additional degradation assays with an ester and ether-type PU foams were performed to confirm if these microorganisms effectively degraded PUs different from Impranil. While all eight identified microorganisms efficiently degraded Impranil, only the fungus identified as *Gymnasciella dankaliensis* degraded the ester-type PU foam, according to SEM, FTIR and TGA analysis. None of them exhibited degradative capacity on the ether-type PU foam assayed in this work, which is consistent with the fact that ether PUs are more biologically stable and the ether group has higher hydrolysis resistance than the ester group (Jin *et al.*, 2021).

MATERIALS AND METHODS

Polymers studied: The specific polyurethane used in the study was Impranil® DLN (Covestro AG, Germany). The polyester-based foam consisted of a blend of a slightly branched polyester based on adipic acid with a combination of methylene glycol and diethylene glycol, and a polyester prepolymer with its isocyanate part from MDI. The polyether-based foam consisted of a blend of a propoxylated polyether triol with ethylene oxide initiated with glycerin and a polyether prepolymer with its isocyanate part from MDI. Both formulations were prepared without mineral fillers or additives and using an amine catalyst.

Source of microorganisms: Mature compost was provided by the Polytechnic School of Orihuela (EPSO) of the Miguel Hernández University of Elche University of Alicante (Spain).

Microorganism Isolation and Molecular Identification

The isolation medium for all Impranil and PU foam assays degradation was M9 minimal medium with agar (per liter ultrapure [RO] water, in grams): Na₂HPO₄ (33.9), KH₂PO₄ (3.0), NaCl (0.5), NH₄Cl (1.0), and 2.0 mM MgSO₄, 0.10 mM CaCl₂, adjusted to pH 7.2, and 1% Impranil (v/v) or Impranil discs, which were obtained by allowing a 100 microliter drop of the polymer to dry, as the sole carbon source. Compost was diluted 1:5 in a saline solution and agitated for 2h (120 rpm). Diluted compost suspension was applied onto the surface of the agar plates/Impranil discs (100 µl/plate or /disc) and incubated at 30, 35 or 58°C. Clearing spots in the agar surface were subsequently developed and microorganisms grew on and around the discs. Microorganisms' samples were taken individually and cultured in new M9-Impranil plates, allowing their isolation. Once isolated, genomic DNA was extracted, and amplified by PCR, using universal primers 8F (Edwards *et al.*, 1989) and 1492R (Stackebrandt *et al.*, 1993) for bacteria, and primers P-ITS1 and P-ITS4 for fungi (White *et al.*, 1990). Amplicons were then purified and sequenced with SANGER technology, and a BLAST search against the rRNA/ITS database of type material was conducted to determine the closest phylogenetic relatives.

Polyurethane Foam Degradation

Degradation of polyurethane foams was performed by placing samples on top of the agar plates containing M9, thus foam being the sole carbon source. Samples were incubated at 35°C or 30°C, depending on the isolated microorganism. The temperature of 58°C was discarded for these experiments, as we were not able to isolate any microorganisms under these conditions. After the designated incubation period (120 days), samples were sterilized for 15 minutes with a sodium hypochlorite solution, followed by two rinses with deionized water and finally allowed to dry before analysis.

Samples Images

Photographs of the polymer samples (control and biodegraded) were obtained with a Canon EOS M10.

FTIR Analysis of Polyurethane Degradation

Fourier transform infrared (FTIR) spectroscopy was employed to analyze samples (Impranil discs and PU foams) using a Varian 600-IR spectrometer (Varian Australia PYT LTD, Australia) with a diamond prism by attenuated total reflection (ATR). Sixteen scans were averaged at a resolution of 4 cm⁻¹, covering a wavenumber range of 500-4000 cm⁻¹.

SEM

Sample colonization and surface modifications were analyzed using a scanning electron microscope (SEM) Phenom proX, at a 500X magnification. Samples were disinfected prior to the analysis, with 5% sodium hypochlorite followed by two rinses in distilled water.

Thermogravimetric Analysis (TGA)

The thermal stability of the PUs was evaluated using a thermobalance equipment, TGA 2 STARe System thermobalance (Mettler-Toledo AG, Switzerland) with STARe software. Samples (7-10 mg) were heated from 30 to 600°C at a heating rate of 10°C/min under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Impranil Colonization and Degradation

Impranil discs and agar plates inoculated with the compost solution were cultured at 30, 35 and 58°C, the latter temperature being maintained in temperature-controlled composting processes (although composting in field conditions can reach up to 70°C) (Vico *et al.*, 2024). Microbes generating clarification halos or growing on the discs were isolated and cultured separately. Microbial isolation was successful at 35 and 30°C, while no microorganisms were isolated at 58°C. Among the isolated microorganisms, only the fungus *A. fasciculatus* was isolated at 30°C, while the rest were isolated at 35°C. While the colonization of Impranil discs was observed visually (Figure 1), and under SEM analysis (Figure 2), degradation was confirmed through FTIR analysis of the samples. As is shown in Figure 3, the greater the colonization, the greater the changes in the FTIR spectra. A remarkable decrease of the C=O stretching vibration (1728 cm⁻¹) of the urea/urethane groups is observed as colonization proceeds. The increase of the intensity of N-H stretch (3321 cm⁻¹), N-H bending (1623 cm⁻¹), and C-N stretching (1532 cm⁻¹) vibrations indicates the formation of amines during polyurethane biodegradation.



Figure 1. Colonized Impranil discs after incubation with mature compost

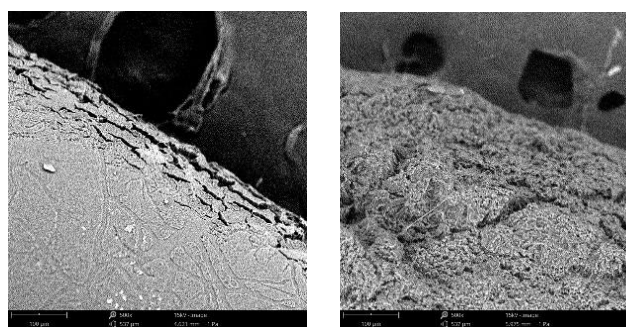


Figure 2. SEM images of the Impranil discs at 500x magnification. Left, control sample. Right, disc incubated for 40 days with the compost extract

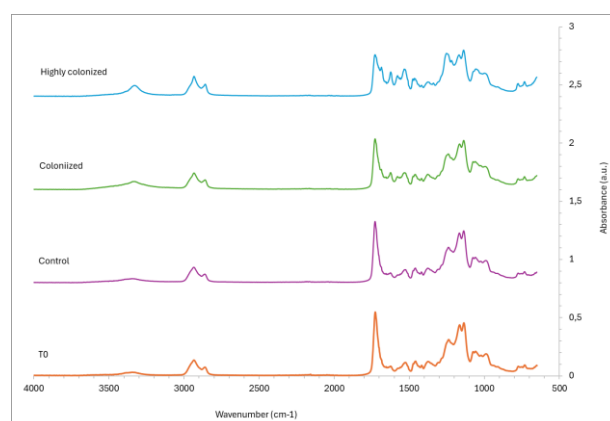


Figure 3. IR images showing degradation of the Impranil discs after 40 days incubation at 35°C

Microorganisms' Isolation and Identification

The isolates were identified as shown in Table 1. % identity is also displayed, based on 16S rRNA gene sequence for bacteria and ITS for fungi. All the microorganisms were isolated at 35°C, except for *A. fasciculatus*, which was isolated at 30°C. Among the isolated microorganisms, only *R. rhodochrous*, generally found in soil, had been previously associated to biodegradation in rubber (Andler *et al.*, 2022), low-density polyethylene (Rose, 2020) and plasticizers (Nalli *et al.*, 2002). As for the rest of microorganisms isolated from Impranil, none of them had been previously related to PU biodegradation.

Table 1. Identified microorganisms

Type	Name	% identity
Bacteria	<i>Rhodococcus rhodochorus</i>	99.99
	<i>Bordetella petrii</i>	99.5
	<i>Limosilactobacillus pontis</i>	99
	<i>Krasinilnikoviella muralis</i>	98.6
Fungi	<i>Gymnascella dankaliensis</i>	99.8
	<i>Arachniotus flavoluteus</i>	98.8
	<i>Aspergillus rugulosus</i>	100
	<i>Aspergillus fasciculatus</i>	99.8

Polymer Foam Degradation

Although Impranil has been frequently used as a model for the initial isolation of PU-degrading microorganisms, there are studies that suggest that this capacity should be confirmed

by testing additional PUs, as a positive activity observed for Impranil might not necessarily correlate with a degradative capacity in other PUs (Biffinger *et al.*, 2015). While Impranil is a thermoplastic polyurethane, PU foams are thermosetting and therefore more difficult to recycle by conventional mechanical methods. Therefore, the initially isolated microorganisms were cultured in the presence of an ester-type (27 Asker C hardness) and an ether-type PU foam (4 Asker C hardness), both formulations typically used in footwear.

Ester-PU foam samples cultured for 120 days along with some of the isolated microorganisms such as *A. rugulosus* and *A. fasciculatus* showed initial signs of growth, especially samples inoculated with *G. dankaliensis*. However, others (*I. nagnjinensis*, *A. flavoluteus*, *L. pontis*, *R. rhodochrous*, *B. petrii*) were not able to use the samples as the sole carbon source, despite being isolated as Impranil degrading microorganisms in our previous screening. Ether-PU foam used in this work was not suitable for any of the microorganisms. Considering the variety of PUs than can be synthesized depending on their precursors and additives, this does not necessarily imply that these microorganisms lack the capacity to degrade any other PU formulations, which is an issue that is currently being addressed in additional experiments. The image shows the appearance of the ester-PU foam used in this work incubated exposed to (right) and without (left) *G. dankaliensis* inoculum (Figure 4).

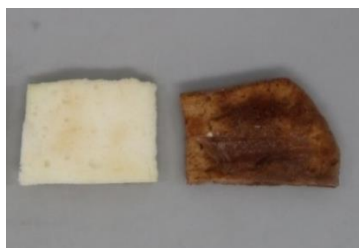


Figure 4. Colonisation of *G. dankaliensis* of the ester-PU sample after 120 days (right), compared to the sample incubated without any microorganism (left)

As SEM images show (Figure 5), the ester-PU foam that was exposed to *G. dankaliensis* revealed breakage between the foam's pores, along with a high number of cracks. PU foam incubated with the rest of microorganisms and ether-PU foam showed no relevant modifications in the same period (images not shown). This correlates to previous works stating that ether PU is more biologically stable, and the ether group presents higher hydrolysis resistance than the ester group (Jin *et al.*, 2021).

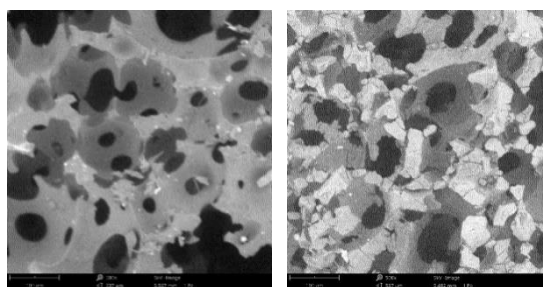


Figure 5. SEM images of the ester PU incubated with *G. dankaliensis*. Control (left). Biodegraded (right)

FTIR spectra comparisons between the control sample and the *G. dankaliensis* biodegraded sample are shown in Figure 5. Both spectra were baseline corrected and normalised based on the peak at 2950 cm^{-1} , which corresponds to C–H stretching vibrations. According to Bhavsar *et al.* (2024), the proportional change in these components is minimal due to the comparative abundance of C–H bonds in the polymer molecules and, therefore, can be used for normalisation

and comparison of the spectra of the blank and the biodegraded sample. There is an increase in the intensity of the peaks at 3311 cm^{-1} , corresponding to N-H stretching, and 1595 and 1531 cm^{-1} , corresponding to N-H bending/C-N stretching, for the biodegraded sample. These increases can be associated with amine formation after polyurethane hydrolysis. The peak at 1726 cm^{-1} is associated with the C=O of the free carbonyl of the urethane decreases in the biodegraded sample. This also indicates the decomposition of the polyurethane by hydrolysis.

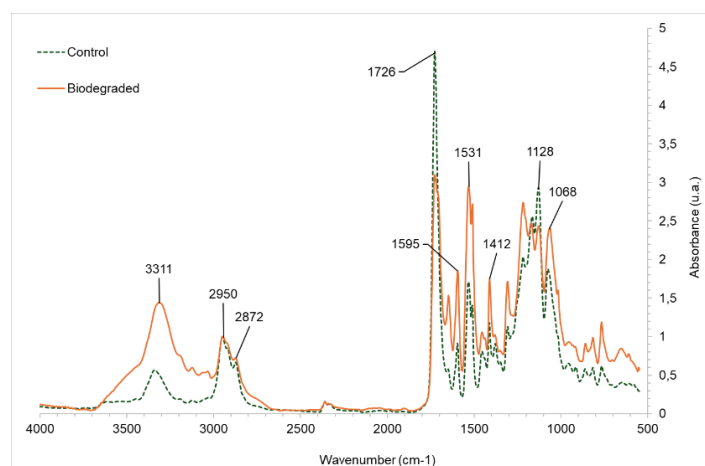


Figure 6. FTIR spectra comparison of the blank and the biodegraded samples

Thermal stability was assessed by thermogravimetric analysis (TGA). Polyurethanes exhibit a segmented structure comprising soft (S) and hard segments (H). The soft segments, typically derived from polyols, impart flexibility, while the hard segments, formed from diisocyanates, provide rigidity and thermal stability. Thermal stability of polyurethane depends on the type and structure of the H-S to S-S ratio (i.e. NCO/OH ratio). As the NCO/OH ratio increases, the thermal stability and crystallinity increases due to increase in the hydrogen bond interaction in the H-S (Szycher, 1999). As shown in Figure 6, the thermal stability of the biodegraded sample is lower than the control sample. If the derivatives of the TGA curves are analyzed (Figure 7), in both cases, a peak above $356\text{--}370^\circ\text{C}$ is observed, which may be attributed to the decomposition of the hard segments. A peak above $395\text{--}405^\circ\text{C}$ is also noted, which may be due to the decomposition of the soft segments. The area under the curve of the derivative of weight loss as a function of temperature, in the range of 220 to 380°C , can be correlated with the amount of hard segments present in the sample, whereas the area under the curve between 380 and 480°C can be associated with the quantity of soft segments. Since soft segments are more prone to biodegradation than hard segments (Pfohl, 2022), the lower content of soft segments in the biodegraded sample compared to the control sample indicates a higher biodegradation in the former.

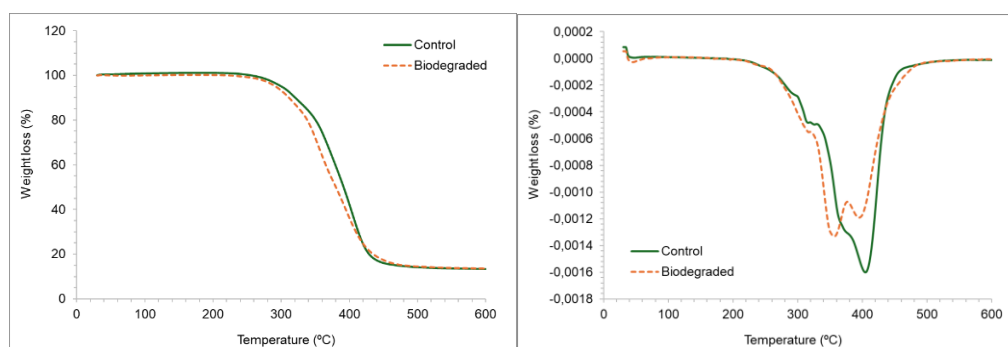


Figure 7. Left. TGA curve comparison of the control and biodegraded samples. Right. DTGA curve comparison of the control and biodegraded samples

In the present study, *G. dankaliensis* (NCBI:txid166389), isolated from mature compost, demonstrated the capacity to degrade an ester-PU foam. This fungus had not been previously reported as polyester PU-degrading fungi in the literature. Degradation of PU by *G. dankaliensis* can be attributed to rupture of the ester bond, as evidenced by the IR spectra. Thermogravimetric analysis showed a predominant degradation of the soft segments of the polymer, which is consistent with previous findings for other fungi (Jin *et al.*, 2021). This finding contributes to the catalog of species described as PU-degrading microorganisms (Liu *et al.*, 2021). However, further research is needed to study the mechanism of ester-PU foam degradation by *G. dankaliensis*, identifying the different by-products obtained and the specific enzymes involved in this activity. This will support future strategies for PU waste valorization, applicable not only for the polyurethanes used in the footwear industry, but also extensible to all types of polyurethanes.

CONCLUSIONS

The study presented here successfully isolated and identified a variety of microorganisms, including both bacteria and fungi, from mature compost samples, capable of degrading the ester-type polyurethane model Impranil® DLN. Among the microorganisms tested, *Gymnasciella dankaliensis* demonstrated capacity to degrade an ester-type polyurethane foam, marking the first instance of this species being linked to PU biodegradation. The results highlight the varying degradative capacities of microorganisms depending on the chemical structure of the polyurethane, with ester-type PU being more susceptible to microbial attack than ether-type PU due to the inherent chemical differences, particularly the greater hydrolysis resistance of ether groups, but also for the distribution and arrangement of these bonds in the different polyurethanes (Rajan *et al.*, 2024; Su *et al.*, 2023).

This research adds valuable knowledge to the growing field of biological polyurethane degradation, suggesting that specific microorganisms, such as *G. dankaliensis*, could serve as potential tools for the valorization of footwear waste containing polyurethane. The ability to target and degrade specific types of polyurethanes presents a sustainable alternative to traditional chemical and mechanical recycling methods. However, additional studies are required to fully understand the mechanisms of PU degradation, including identifying the enzymes involved and exploring the potential for upscaling this process for industrial applications. Further research will be essential to enhance the efficiency of microbial PU degradation, not only for footwear waste but for broader applications in polyurethane recycling and valorization.

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IMPACT OF EDUCATIONAL POPULARIZATION OF GENETICS ON THE DEVELOPMENT OF SOCIETY

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Apparently, there are practically no areas of science and activity in the social sphere that are not, to one degree or another, related to genetics. Current trends and advancements of post-genomic technologies initiate the geneticization of society – the awareness of different segments of the population about the basics of genetics and genetic technologies. These advancements hold great promise for unlocking new insights into genomics and improving our understanding of personalized lifestyle. An educational survey allows gathering feedback and opinions from all the parties involved in the education process, from students to teachers. Recollecting information is vital to make decisions that improve the quality of the educational experience. In 2024, at the Department of Biotechnology, Leather and Fur, for the first time, Kyiv National University of Technologies and Design students participated in the questionnaire study dedicated to International DNA Day, celebrated on April 25. The objective of the work is to analyze the level of awareness of students, their relatives and teachers in the field of genetics. The questionnaire was carried out using the Google-form. 173 people were invited to take the questionnaire. The questionnaire consisted of nine questions, such as «What modern achievements of genetics do you know?», «Do you support human genetic editing?», etc. Looking forward, the improvements in the science and genetic knowledge in the information field of various layers of society can develop a sustainable process of geneticization of the country's population.

Keywords: questionnaire, geneticization, biotechnology.

INTRODUCTION

Genetics, as a discipline, is an essential part of the modern world. However, analyzing the interaction between genetics and society can be complex. Genetics is not just a biological phenomenon but has for decades been a cultural phenomenon as well. From films to advertising and even public health announcements, many find themselves aware of the increasing pervasiveness of the field of genetics in contemporary society. Studying genetic metaphors gives one insight into the increased role genetics plays in the socio-cultural sphere today. The genomic imaginary is often constructed by science popularizes or communicators who introduce scientific concepts to popular consciousness. Despite the intentions of the popularizes, this genomic imaginary can impact the way civil society and legislators understand the role of genetics (Mannette, 2021).

The concept of geneticization entered circulation through the work of Lippman, a radical epidemiologist and dedicated activist for women's health. Over the course of three papers (1991, 1992, 1994), she developed a detailed account of geneticization as 'an ongoing process by which differences between individuals are reduced to their DNA codes, with most disorders, behaviours and physiological variations defined, at least in part, as genetic in origin' (Arribas-Ayllon, 2016).

The concept of 'geneticization' has been introduced in the scholarly literature to describe the various interlocking and imperceptible mechanisms of interaction between medicine, genetics, society and culture. It is argued that Western culture currently is deeply involved in a process of geneticization. This process implies a redefinition of individuals in terms of DNA codes, a new language to describe and interpret human life and behavior in a

genomic vocabulary of codes, blueprints, traits, dispositions, genetic mapping, and a genotechnological approach to disease, health and the body (Mannette, 2021).

Participatory health research (PHR) is receiving increasing attention internationally as an approach for generating scientific knowledge to promote health equity for marginalized groups. The primary characteristic of PHR is the direct participation of those people in the research process whose work or living conditions are the subject of the research (Wright, 2021).

Participatory health research is closely related to the geneticization of society. In our opinion, the level of geneticization is determined by multi-level education, the development of molecular - genetic technologies and their practical applications in the sectors of the economy, and, undeniably, information support for new knowledge.

Apparently, that the level of genetic knowledge will depend on the professional skills of different sections of the country's population. There is no doubt that the objectivity of the assessment depends on the coverage of sections of the population. However, the first attempts to obtain data on people's awareness using the National University of Technologies and Design as an example, we hope, are worth attention. The objective of the questionnaire study is to analyze the level of awareness of Kyiv National University of Technologies and Design students, their relatives and teachers in the field of genetics.

METHODS

Questionnaires were completed by 173 adults. Participants were asked about their views on the nature of gene ("What do you know about DNA?"), gene localization ("Where are the genes?"), historical aspects of genetics ("What scientists' achievements, in your opinion, are related to genetics?"), future prospects of genetics ("Do you support human genetic editing?", ("In what cases do you think it is necessary to edit the genome?"), resources of genetic information ("How would it be more convenient for you to receive information about the achievements of genetics?").

The questionnaire was carried out using the Google-form. Analyses were adjusted for age, education and sphere of professional activity. Statistical processing of the results was carried out using Fisher's criterion.

RESULTS

Results revealed that 90% of respondents gave a correct definition of the term "gene". Paradoxically, when asked about gene localization, the number of correct answers from respondents who gave a correct understanding of the definition of a gene dropped to 80%.

The answers to the history of genetics turned out to be quite varied.

Only 51% of participants answered correctly that "What scientists' achievements, in your opinion, are related to genetics?". Most often, participants named Gregor Mendel, Bohemian monk who discovered laws of Mendelian inheritance, Thomas Hunt Morgan author who won the Nobel Prize in Physiology or Medicine for discoveries elucidating the role that the chromosome plays in heredity, James Watson and Francis Crick, authors of the double helix structure of the DNA molecule.

Unfortunately, only an insignificant number of participants are familiar with the name of Hryhori Levitskyi, only 8% indicated his name. While he is a Ukrainian botanist, a geneticist, a cytologist, a karyologist, the founder of cytogenetics, author of the first monograph on cytogenetics, *The Material Basis of Heredity*, which was published in Kyiv in 1924 (Kunakh, 2008).

We have to admit that none of the respondents indicated the name Theodosius Dobzhansky (1910-1975), Ukrainian-US geneticist and evolutionary biologist. The scientist from Nemyriv (Vinnytsia region, Ukraine) made history as a super-effective and extremely fruitful scientist. He made more than three hundred discoveries, many of which have been prominent in the further development of genetics and biology. During the lifetime of Theodosius Grygorovych, he became known as the “new Darwin”: he studied the problems of the gene pool, the synthetic theory of evolution, and chromosomal mutations. However, his most fundamental work that went down in history is the four editions of his book *Genetics and the Origin of Species* (the name of the last is *Genetics of the Evolutionary Process*). Each subsequent edition had significant additions (Palamarchuk, 2023).

T. Dobzhansky not only met G.A. Levitsky and listened to his lectures, but also lived with him in the same apartment in Kyiv. His first teacher in genetics was, of course, G.A. Levitsky, who had a significant influence on the young entomologist, “infecting” him with genetics (Zakharov, 2020). Theodosius Dobzhansky made more than three hundred discoveries, many of which have been prominent in the further development of genetics and biology.

9% of participants reported in the questionnaires that Jennifer Doudna lab’s research into RNA biology led to the discovery of CRISPR-Cas9 as a tool for making targeted changes to the genome. It is likely that such detailed awareness of respondents about the field of modern scientific achievements is connected with the fact that the event of the awarding of the Nobel Prize for the discovery of genome editing was recently covered.

Despite the fact that only 9% know the author of the genome editing method, 70% of respondents support the introduction of this latest technology into medical practice (Fig. 1).

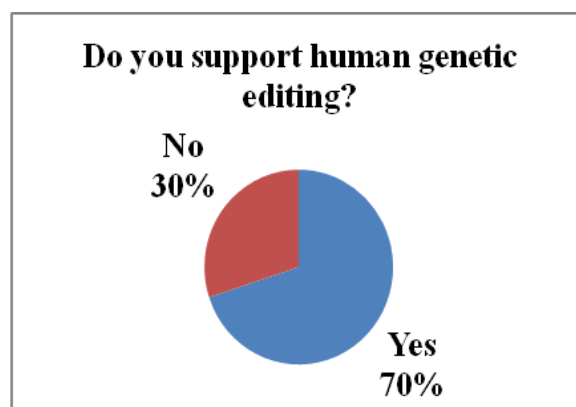


Figure 1. Distribution of opinions in answering the question “Do you support human genetic editing?”

The respondents’ opinions on the purpose of using genome editing were quite different. 90% supporters of human genome editing believe that this is necessary if preventing children from inheriting a genetic disease that leads to disability and death.

To the question “What cases do you think it is necessary to edit the genome?” 88% of participants answered that this is necessary for “preventing children from inheriting a genetic disease, which leads to diseases that affect the quality of life”.

22% of participants hope that genome editing will help to improve human intelligence.

An important purpose of the genomic technology, which was introduced by Jennifer Doudna and Emmanuelle Charpentier, 18% of participants of questionnaire study considered is the possibility to improve a person’s athletic abilities.

And finally, 15% of questionnaire study participants believe that genome editing should be used to improve a person's appearance.

The last question we planned to ask was to find out the most acceptable form for receiving information. The question was formulated as follows: "How would it be more convenient for you to receive information about the achievements of genetics?".

The dominant majority of participants preferred Lectures on YouTube (82%).

Kyiv National University of Technologies and Design students were almost equally divided between classics and ultra-modernism. So, 33% of questionnaire study participants chose books, and 30% chose art objects.

24% and 21% of participants are ready to perceive information about the achievements of genetics through lectures on TV and advertising in the subway & transport, respectively.

Only 10% of respondents prefer to receive information via radio.

Our first results of monitoring knowledge of genetics in the student and student-related community of the Kyiv National University of Technologies and Design provide grounds for continuing the study with expanding the audience to obtain statistically significant results in different professional groups and ages.

CONCLUSION

The advent of 'omics' era and innovations in genetic and protein engineering approaches, application in industries besides food, pharmaceuticals, like leather, textiles, requires a creative approach to the study of genetics at the department of biotechnology, leather and fur.

The questionnaire study revealed a generally correct understanding of the nature of the gene, as well as a good knowledge of the most significant and famous scientists - geneticists.

Gaps in the history of the contribution of scientists of Ukrainian origin can be explained by the silence of names that in one way or another challenged the totalitarian regime of the Soviet past, as well as insufficient educational work on this issue over the past 30 years.

The heritage of Ukrainian science is fantastically rich, but it is still underestimated and completely unknown to the world community.

In connection with this, the department staff held the DNA day in 2024 dedicated to Hryhori Levitskyi, the founder of world cytogenetics, the author of the term "karyotype". For students of Ukraine, especially now, when the country is fighting for its independence, it is important to remind that the scientist Hryhori Levitskyi was born, studied and worked in Ukraine and died in soviet gulag. Hryhori Levitskyi's book *Material Basis of Heredity* was published in Kyiv 100 years ago. The flowering of human cytogenetics led the way to the establishment of clinical genetics as one of the most important developments in medicine in the twentieth century (Jacobs, 2014). We consider it logical to continue the historical relay race and, in this regard, we are planning to dedicate DNA Day in 2025 to Theodosius Dobzhansky, timed to coincide with the 125th anniversary of his birth.

The 30% preference for receiving information through art objects stimulates the involvement of designers and artists to create original, modern and understandable objects dedicated to genetic research and its authors.

Arts in education are largely related to forming and questioning human relations with the world. Such practices and discourses are concerned with the development of meaning; they provoke and challenge our understanding of skill and technique; they query the idea of the artist and the art object; they problematize the relations between the artist, the object and the spectator (or the person who responds to the work); they encourage us to think about who we are, how our identities are formed and about our relations with others; they call us to

address issues of social awareness. Generally speaking, such practices and their attendant discourses provide new encounters for learning and how learning can be understood and through such encounters new subjectivities emerge. This produces a new ontological order within the pedagogic space, which is not about reproduction but one that is focused upon challenging and rearticulating subjectivities (Atkinson, 2007).

Looking forward, the improvements in the science and genetic knowledge in the information field of various layers of society can develop a sustainable process of geneticization of the country's population.

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PROCEDURE FOR THE CIRCULATION OF DOCUMENTS TO SUPPORT THE DIGITALIZATION PROCESS IN PUBLIC INSTITUTIONS

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The digitization of public institutions in Romania is a crucial aspect of the country's global digital transformation efforts. Digitization involves the transition from traditional, paper-based processes to digital systems to increase efficiency, transparency and accessibility. There are many initiatives related to the digitization of public institutions in Romania, such as the E-government services. Romania aimed at the digitization of public services through various e-government initiatives. They usually involved providing online platforms and portals where citizens and organizations can access a wide range of government services and information. The main objective of the paper is to propose a unique document identification system (DIS) in a Romanian public institution (Directorate for Social Assistance and Child Protection - D.G.A.S.P.C.) and to introduce a specialized software program to implement this system. The proposed solutions offer significant benefits, particularly in terms of efficiency, accuracy, and security. Before the development of the software program, a new work procedure regarding the document circuit was created, using the principle of the unique registration number. The benefits include the simplification of internal work procedures of D.G.A.S.P.C., shorter time to complete the tasks, reflecting in shorter time to answer citizens' requests, better transparency and traceability of documents, and so on. The paper also advances recommendations for public institutions to implement more easily the new unique document identification system and the associated software program, such as training sessions for employees, to ensure that officials feel comfortable with the new digital tools.

Keywords: digitalization, public institutions, E-government.

INTRODUCTION

The impact of digitalization in Romania was significant, transforming various aspects of society, economy and government. Digitalization will lead us to increase the efficiency of business operations, improve access to information and fast services for citizens and improve connectivity and communication. It also created new opportunities for innovation, entrepreneurship and economic growth. However, digitalization has also brought challenges, such as the need to improve and reskill the workforce, address the issues of the digital gap, and ensure data privacy and cyber security. In general, digitalization in Romania has the potential to stimulate sustainable development and improve the quality of life for its citizens.

DIGITALIZATION IN PUBLIC INSTITUTIONS

In general, the digitalization of public institutions in Romania remains a complex and continuous process, which requires collaboration, investment and commitment from government authorities, public sector employees and other interested parties. By adopting digital technologies and modernizing government operations, Romania can increase the efficiency, transparency, and involvement of citizens in the provision of public services.

The main objective of this paper is to create a procedure for introducing a unique document identification system (DIS) in a public institution in Romania (Directorate of Social Assistance and Child Protection – D.G.A.S.P.C.), which will be used to develop a specialized

software program for the implementation of this system. The proposed solutions provide significant benefits, especially with respect to efficiency, accuracy and security.

Before the development of the software program, the document circuit working procedure using the principle of the unique registration number was created. By implementing these strategies and promoting an environment proper to digitalization, Romania can accelerate its journey to becoming a digitally empowered nation.

Working Procedure for Introducing a Unique Document Identification System (DIS) in D.G.A.S.P.C.

The authors created the working procedure for implementing a unique number for the identification of each document, entitled document identification system (DIS). This procedure will then be used for the development of our software program that will digitalize the document circuit in this public institution. The procedure is described below:

A.1. Paper documents that enter the institution from outside by fax, post, courier or submitted directly by the applicant are scanned and registered at the Registry and then distributed, in a digital system.

Documents that enter the institution from the outside via official emails assigned to the domain office@dgaspc5.ro will be redirected by the designated official to the address of the Registry Service, which will register and distribute them on the circuit. In the case of documents submitted personally by the petitioners to the Registry, at the time of registration, the petitioner will be compulsorily issued a receipt with the document's unique registration number.

Documents in a language other than Romanian will be registered and distributed to the General Director, respectively to departmental directors, after their translation into Romanian by the department to which the document is intended, in no more than 72 hours after receipt.

Civil status documents, transcripts, school certificates, and medical certificates will not be registered, but the forwarding address that accompanies them.

The registration and circulation of documents in and from the Registry will be carried out considering one of the situations provided below:

A.2. After registration, the documents entered into the institution, which are responses to requests made to the functional structures of the D.G.A.S.P.C., are distributed directly to their designated department.

When registering the document, the department that receives it can connect the received document to the previously sent one and give it the same number, if the received document is the same as the previous version.

A.2.1. The documents entered into the institution that are not responses to requests from the functional structures of the D.G.A.S.P.C. and represent requests according to the law GEO 27/2002 (Romanian Government, 2002), are then distributed to the designated person by the Registry Department. along with the registration and monitoring of the petition resolution activity. After their registration in the Registry, the documents are sent by the Department's Director to the General Director.

A.2.2. The documents entered into the institution that are not responses to requests from the functional structures of D.G.A.S.P.C. and are not petitions according to the law GEO 27/2002 (Romanian Government, 2002), are distributed by the General Director's Registry.

A.2.3. The following documents constitute exceptions to point A.2.2.:

- Documents provided for in point A.1.;
- The subpoenas, communications, and court decisions (which are then scanned, recorded, and distributed directly to the Director of the Litigation Department).

- Utility bills (which are scanned, recorded and distributed directly to the receiving accountant or the Director of Administrative - Supply Department, as appropriate;
- Invoices submitted to the D.G.A.S.P.C. headquarters by third parties, other than utilities. These are scanned, recorded, and distributed directly to the accounting person as the recipient, and the Director of the Administrative - Supply Department. For invoices addressed to the internal departments, they will be also distributed to the Budget - Budget Execution Department. The same procedure applies to invoices with unknown recipients).
- The invoices that accompany the goods handed over in the centers/complexes. They are scanned, uploaded into the centralized system, and are then registered in the RXX - Registry Department. They must be registered within a maximum of 24 hours from receiving them, they are categorized in the group of administrative and accounting documents;
- Offers received for procurement procedures – they are scanned, recorded, and distributed directly to the head of the Public Procurement Department.
- Requests based on Law 544/2001 - they are scanned, recorded, and distributed directly to the person in charge of the respective law within the Communication and Public Relations Department;
- Requests based on Regulation (EU) 2016/679 regarding the transmission of documents containing personal data - they are scanned, recorded, and distributed directly to the Human Resources Department;
- Documents regarding persons sentenced to community service from the Probation Service or Prosecutor's Office – they are scanned, recorded and distributed directly to the person in charge.

A.2.4. If the document entered in the D.G.A.S.P.C. by fax, post, or courier refers to different annexes, which are missing at the time of registration, the staff at the Registry who received it mentions this aspect in the internal system, otherwise being responsible for their loss.

A.2.5. If the document entered in the D.G.A.S.P.C. by email refers to some annexes that are missing from the received email, the sender is requested to submit all the documents prior to the registration of the main document in the unique register. If, within 24 hours, the sender does not submit the requested documents, the registration will be made according to the notes in point A.2.4.

A.2.6. If the document entered in D.G.A.S.P.C. by submission by the petitioner refers to different annexes that are missing at the time of submission, the Registry staff requests the petitioner to complete it with all nominated documents. In the situation when the petitioner insists on registering the document in the absence of all annexes, the petitioner will have to cut the missing documents from the document and sign next to the cuts, prior to the registration of the document.

By registering the document submitted at the counter, without fulfilling the previously presented formality, the Registry staff will be responsible for the lack of unattached documents.

Here is an algorithm for implementing the document processing procedure presented in sections A.2. to A.2.6. This algorithm deals with the registration and distribution of different types of documents within the D.G.A.S.P.C.

Document processing algorithm:

- Document reception
- Input: document (with receipt method, type, timestamp and language)
- Document registration

- Register (document) call
- Determining document type

If document is response to request:

- Call Distribute-to designated department (document)

Otherwise, if document is request under law GEO 27/2002:

- Call Distribute-request to designated person

Otherwise:

- Call Distribute-other documents

Distribute to designated department:

- Connect to previous document if applicable, if is same as previous version (document): connect to previous document
- Distribute the document directly to the designated department – **Direct**

Distribution to department

A.3. Documents coming from outside the institution will be distributed electronically in compliance with the following terms and conditions:

A.3.1. The documents will be distributed by the Registry Department, on the day of their receipt, as follows:

a) on Monday, Tuesday, Wednesday and Thursday, all documents that enter the Registry will be scanned, registered and distributed until 4:30 p.m. Documents entered after this time will be scanned/registered and distributed, with speed, on the next working day, until 10.00 a.m. at the latest;

b) on Friday, all documents that enter the Registry will be scanned, registered and distributed by 2:00 p.m. Documents that enter the institution after this time will be scanned, registered and distributed quickly, on the next working day, until 10:00 a.m. at the latest.

A.3.2. The documents will be distributed by the directors to the heads of subordinate departments or their substitutes as follows:

a) Documents received by 15.30 on Monday, Tuesday, Wednesday and Thursday working days will, as a rule, be distributed on the same day. When this is not possible for objective reasons, the distribution of these documents, together with the distribution of documents received after 3:30 p.m., will be done, expeditiously, on the next working day, until 12:00 p.m. at the latest.

b) Documents received on working days from Friday, until 13.00, will be distributed, as a rule, on the same day. When this is not possible for objective reasons, the assignment of these documents, together with the assignment of documents received after 1:00 p.m., will be done, expeditiously, on the next working day, by 10:00 a.m. at the latest.

A.3.3. The documents reached at the level of the structures will be distributed by the heads of the structures, or their substitutes, to the subordinate staff as follows:

a) Documents received by 3:30 p.m., on Monday, Tuesday, Wednesday and Thursday working days, will be distributed, as a rule, on the same day. When this is not possible for objective reasons, the assignment of these documents, together with the assignment of documents received after 3:30 p.m., will be done, expeditiously, on the next working day, by 12:00 p.m. at the latest.

b) Documents received on working days from Friday, until 1:00 p.m., will be distributed, as a rule, on the same day. When this is not possible for objective reasons, the assignment of these documents, together with the assignment of documents received after 1:00 p.m., will be done, expeditiously, on the next working day, by 11:00 a.m. at the latest.

A.3.4. The documents in physical format are handed over by the persons from the Communication and Public Relations Department, which are designated for this purpose, on

document handover/receipt registers, to the structures that have already received them in electronic format, twice a week (Tuesday and Friday). The exceptions are the documents mentioned at point A.1., as well as those whose original written format is necessary for carrying out the activities (e.g. subpoenas, communications, court decisions, invoices, offers for procurement procedures, tender applications, files from Probation/Prosecution, etc.). These documents will be handed over daily, or whenever needed, to the relevant services.

A.3.5. The documents in physical format are handed over to the departments that have already received them in electronic format, using a document submission/receipt report, twice a week, except for those documents whose original format is necessary for carrying out the activities.

A.3.6. The Director of a department or the person who picks up documents from the Registry, must write in the Register his/her full name, signature, and the number of the documents he/she picked up.

Here is an algorithm for implementing the document distribution procedure described in sections A.3.1. to A.3.6. This algorithm will handle the scanning, registration and distribution of documents received by the registry service.

Document distribution algorithm:

- Document reception
 - Input: document (with receipt method, timestamp and format)
- Determination of current day and time
- Current day = Get the current day (Monday, Tuesday, etc.)
- Current time = Get the current time
- Scan and register the document
- Call Scan and Register (document)
- Check distribution time
- If current day is in ["Monday", "Tuesday", "Wednesday", "Thursday"]:
- If current time <= "16:30":

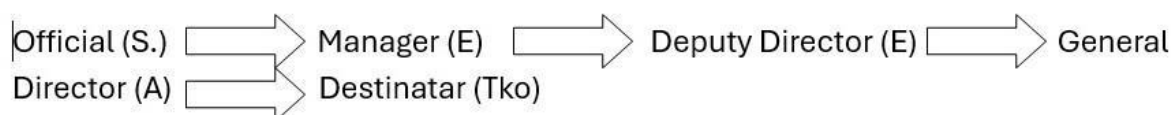
Distribute the document

Otherwise:

- Schedule for the next day (10:00 a.m.)
- Else if current day = "Friday" = If current time <= "14:00":
- **Distribute document**
- Else: Schedule for next day (10:00 a.m.)

The route of an internal document within the public institution

The route of an internal document in the public institution D.G.A.S.P.C. is presented in Figure 1:



Legend: S – Sender, E - Endorsed, A-Approved, Tko - Taking over the task.

Figure 1. Route of an internal document in D.G.A.S.P.C.

The unique registration number will be issued for a document after all the signatures are obtained for that document. Only under these circumstances, the unique number will be visible to all the people involved.

The digital system will automatically record all requests and assign a unique identification number to each case. The employees of the institution working on solving a certain request receive notifications about new developments, while the applicant also receives notifications and confirmations as the work unfolds.

The beneficiary can monitor the progress and communicate with the public institution employee through the envisaged digital software. The platform will offer the public institution employee the possibility to connect both as a natural person and as a civil servant.

The digital system sends automatic notifications for periodic revisions of intervention plans and updates on the beneficiaries' situation. All records, reports, and documents are stored securely in the digital system and are accessible only to authorized personnel.

Reports and statistics required for internal and external reporting are automatically generated, reducing the time and effort required for documentation.

The digital platform is secured to protect the confidentiality of the beneficiaries' data, with policies and security measures in accordance with legal regulations. The access to information is restricted and controlled based on roles and permissions assigned to staff, and activities are audited to ensure compliance and transparency.

The staff is trained in the use of the digital platform through a customized training program and has access to online documentation and ongoing technical support. It is preferable to organize mandatory training sessions for all employees, focusing on the basics of the new system. These should cover how to create, retrieve, and manage documents using the new system. Also, customize training modules based on employee roles, providing advanced training for those who will use the system more intensively.

Feedback from users is collected to identify and fix possible problems or deficiencies in the digital system, contributing to continuous improvements and optimizing the user experience.

CONCLUSIONS

The main objective of this paper is to create a procedure for introducing a unique document identification system (DIS) in a public institution in Romania (Directorate of Social Assistance and Child Protection – D.G.A.S.P.C. Sector 5), which will be used to develop a specialized software program for the implementation of this system.

The authors created the aforementioned procedure, described it in detail for all types of documents available in the public institution, and provided clear instructions for dealing with them in an efficient and organized manner.

Implementing a new unique document identification system and associated software requires careful planning to ensure a smooth transition to digitalization in public institutions. The proposed solutions provide significant benefits, especially with respect to efficiency, accuracy and security.

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POTENTIAL OF SILVER NANOPARTICLES IN IMPARTING ANTIMICROBIAL PROPERTIES TO LEATHER

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To protect natural leather from microorganisms, substances with antimicrobial properties are used. The rapid adaptation of microorganisms to antimicrobial agents requires a constant search for fundamentally new materials. When choosing antimicrobials, attention should be paid to their safety. An increase in the antimicrobial activity of a drug leads to an increase in its toxicity. Traditional antimicrobial agents are based on cationic surfactants, guanidine or polyalkylene guanidine compounds. However, given the possibility of antimicrobial resistance, research should be aimed at finding new effective biocidal products. Today, one such product is metal nanoparticles, whose properties can be successfully used in leather technology. The aim of this work is to summarize antimicrobial properties of silver nanoparticles and to study the advantages of biosynthetic production of silver nanoparticles. Biosynthesis of silver nanoparticles involves the reduction of silver ions to form silver nanoparticles. This process is environmentally friendly, and silver nanoparticles have potential to be used to impart antimicrobial and antifungal properties to leathers and leather products.

Keywords: silver nanoparticles, antimicrobial properties, leather.

INTRODUCTION

Leather is a material of biogenic origin. Its production involves the multi-stage and sequential processing of raw hides and skins with chemicals to create the required set of quality indicators. Leather production is based on the processing of canned hides and skins during preparatory, tanning and finishing processes. This process affects the collagen structure of the dermis with salts, acids, alkalis, enzymes, tannings, surfactants, fats, etc.

Raw hide contains proteins, water, lipids, carbohydrates, etc., which makes it susceptible to microorganisms. The challenge in leather processing lies in achieving strong antimicrobial protection without compromising the natural properties of the material.

At different stages of leather production, the nature of biodamage and the development of microorganisms is different. Gram-positive (*Bacillus*, *Staphylococcus*, *Clostridium*) and gram-negative (*Pseudomonas*, *Proteus*, *E.coli*) bacteria and fungi (*Penicillium*, *Aspergillus*, *Mucor*) have been identified as microorganisms that can adversely affect the structure and properties of the dermis. In order to avoid bioinjury, technological measures have been developed, the use of which is aimed at not inhibiting the action of microorganisms and protecting dermal collagen from damage to preserve the integrity of the dermal structure and form the necessary set of functional properties of the genuine leather.

Aerobic bacteria are the most active at the stage of hide curing. Once the hide has been removed, the metabolic processes that were previously occurring cease and the hide begins to decay (Andreieva, 2012). Autolysis leads to an increase in the looseness of the leather and a decrease in its strength. Bacteria of the genus *Bacillus*, *Pseudomonas* and *Proteus* damage the proteins in the hide and the grain of the leather. With further decay, bacteria of the genus *Clostridium* multiply in the hide (Demkevych *et al.*, 2013). As a result of storage at high humidity in poorly ventilated rooms, hides may be affected by fungi of the genus *Penicillium*, *Aspergillus*, *Mucor*. The damage consists in the appearance of spots on the surface of the hide, which then spread to the hide. The effects of molds can lead to a loss of leather strength.

That is why antiseptic materials of different nature are traditionally used for hide curing. Namely: formalin, sodium hexafluorosilicate, zinc chloride, etc.

At the preparatory stage of liming, non-spore-forming and spore-forming bacteria are active. The use of alkaline materials for dermis liming reduces the risk of bioinjury.

At the stage of tanning, bacteria of the *Bacillus mesentericus* species, fungi *Aspergillus niger*, *Penicillium chrisogenum*, *P. ceclopium* can be agents of biodamage. Their effect on semi-finished products and leather is to damage the grain and reduce the strength of the leather structure. At the same time, the risk of biological damage to the dermis can be reduced by using mineral and organic tannins for tanning. The risk of biodamage occurs when the semi-finished leather product is stored or transported in a wet state under plastic wrap.

The use of synthetic dyes and tannins, as well as fat-liquoring materials, in the subsequent after-tanning processes of leather production further increases the biostability of leather. But despite this, genuine leather remains susceptible to biological damage.

If the proper storage conditions for leather and leather products are not met, for example, in humid conditions at a temperature of 22-25°C, or if leather products get wet or are not dried sufficiently, or if products are used in harsh climatic or living conditions, the occurrence of biological damage is quite likely.

Production must ensure the safety of genuine leather at different stages of the life cycle. The market offers a limited range of antimicrobial products to protect the structure of the leather dermis that would be environmentally tolerant, not pollute the environment, be technologically available and cost-effective, and have a wide range of antimicrobial effects on microorganisms of different groups (bacteria, fungi, etc.) with a long protective effect. To impart antimicrobial properties to natural leathers, the following are used: quaternary ammonium compound derivatives, phenol derivatives, chloroactive compound derivatives, aldehyde-based preparations, preparations based on high molecular weight cationic surfactants, polymeric guanidine or polyalkylene guanidine compounds (Kozar *et al.*, 2017).

Currently, the most widely used and relatively safe drug is polyhexamethylene guanidine hydrochloride. This is a cationic polyelectrolyte, a biocide with a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, viruses, fungi, etc. When treated with this polyelectrolyte, a biostable film is formed on the surface of the material. After treatment, the materials can retain their bactericidal effect for 3 days to 8 months. The polyelectrolyte is classified as a class 3 moderately hazardous substance.

Therefore, to protect natural leather from microorganisms at various stages of animal hide processing, substances with broad-spectrum antimicrobial properties should be actively used. The goal of leather treatment is to provide powerful antimicrobial protection while maintaining the material's natural properties. However, given the possibility of antimicrobial resistance, research should be aimed at finding new effective biocidal products. Today, one such product is metal nanoparticles, whose properties can be successfully used in leather technology.

SUBSTANCES WITH ANTIMICROBIAL PROPERTIES AND REQUIREMENTS FOR THEM

Antimicrobial agents are used to prevent biodamage of various materials. From the point of view of use, these drugs must meet two main requirements: penetrate the surface or inside the microorganism's cell and, accumulating in it, disrupt at least one vital process of the microorganism.

Drugs with antimicrobial properties are called biocides. They should have a broad spectrum of antibacterial action against pathogenic microflora and be produced from available raw materials for industrial production. The range of such drugs is quite large. At the same time, the use of some drugs is limited due to the safety of their use.

The possibility of using biocidal products is regulated by the requirements of Regulation (EU) No. 528/2012 and a number of other EU legislative acts (Document 32012R0528, 2012). The Regulation stipulates the application of precautionary measures to

protect against harmful effects of biocides on human or animal health or unacceptable environmental impact. The Regulation prohibits the use in a biocidal product of a substance that is classified as dangerous in accordance with Regulation (EC) No 1272/2008 or in a dangerous concentration (Document 32008R1272, 2008); a substance that meets the criteria for being a persistent organic pollutant or meets the criteria for being persistent, bioaccumulative and toxic or very persistent and very bioaccumulative in accordance with Regulation (EC) No 1907/2006 (Document 32006R1907, 2006).

Microorganisms often exhibit natural resistance or rapid adaptation to antimicrobial drugs. However, an increase in the antimicrobial activity of a drug often leads to an increase in its toxicity. Thus, every year dozens of drugs are discontinued due to their low antimicrobial activity, high toxicity, low environmental safety, and the ability to cause dermatitis or allergic reactions. These characteristics are unacceptable when using such products for natural leather. The active ingredient of a biocidal product may not contain carcinogens, mutagens, toxic substances that affect the reproductive or endocrine system of humans or animals, environmental pollutants, and substances with high bioaccumulation.

In order to improve the safety of biocides, alternative materials with antimicrobial properties are constantly being sought. Research is mainly focused on finding polyfunctional or hybrid materials. For example, the antimicrobial effectiveness of polyhexamethylene guanidine hydrochloride can be increased by combining it with natural silicates (Kozar *et al.*, 2017). Oil obtained from *Origanum vulgare* is also considered as a preparation with antimicrobial properties for the treatment of leather or leather materials (Bayramoglu, 2007; Bielak *et al.*, 2020). The ability of chitosan to impart antimicrobial properties to leather materials is being studied (Ocak *et al.*, 2015). The effectiveness of the use of hybrid organic-mineral nanomaterials based on essential oils is being investigated (Bacardit, 2016).

The development of nanotechnology and functional features of nanomaterials have opened up new opportunities for the production of drugs with antimicrobial properties. The synthesis of nanoparticles of various metals (copper, silver, titanium, zinc, etc.) and their use as biocides for the treatment of leather materials makes it possible to expand the range of safe materials with antimicrobial properties (Carvalho *et al.*, 2018; Maestre-Lopez *et al.*, 2015).

Metal nanoparticles are often used to combat multidrug resistance in microorganisms. Silver nanoparticles are most often considered because of their broad spectrum of action and reliable antimicrobial properties (Mijnendonckx *et al.*, 2013; Morones *et al.*, 2005). Moreover, researchers have noted (Dakal *et al.*, 2016; Skiba *et al.*, 2019) that the stability of nanoparticles, their size, shape, and surface chemistry play an important role in their antibacterial activity.

Thus, the prospects for the use of metal nanoparticles, especially silver, can be considered as a unified approach to solving the problems of bio-damage to the skin dermis structure at different stages of production.

ANTIMICROBIAL POTENTIAL OF SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) are clusters of silver atoms. A cluster contains about 20-15000 silver atoms, and its size ranges from 1 to 100 nm. AgNPs are characterized by high surface sorption activity and are of great interest to researchers in various fields (Siddiqui *et al.*, 2023).

AgNPs are part of packaging materials, consumer products made of polymeric and composite materials, and mixtures designed to protect materials from external influences. AgNPs are used in the textile, electroplating, paper, perfumery, and food industries. Another area of application for AgNPs is water purification: the use of nanoparticles in filters and wastewater treatment plants.

The use of AgNPs as components of antimicrobial agents in the healthcare industry is considered effective. AgNPs are proposed to be used as carriers for the effective delivery of active pharmaceutical ingredients or to improve the solubility of drugs (Farjadian *et al.*,

2019). Dressings with silver nanoparticles significantly reduce wound healing time and increase the rate of cleaning of infected wounds (Ronavari *et al.*, 2021).

The effectiveness of AgNPs has been observed against a number of gram-positive and gram-negative bacteria, fungi, and viruses (Carvalho *et al.*, 2022; Gajbhiye *et al.*, 2009; Roy *et al.*, 2013; Skiba *et al.*, 2019). Numerous studies have proven the antimicrobial effect of AgNPs against *Escherichia coli*, *Salmonella typhimurium*, *Bacillus anthracis*, *B. cereus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, etc. AgNPs also have antifungal potential against *Aspergillus niger*, *A. foetidus*, *A. flavus*, *A. oryzae*, *A. parasiticus*, *Phoma glomerata*, *P. herbarum*, *Fusarium semitectum*, *Trichoderma sp.* *Oxyasporum*, etc.

Considering the tasks of tanning and after-tanning processes, it should be noted that AgNPs are also capable of interacting with lipids and proteins. AgNPs have a high affinity for sulfo-, amino-, and carbonyl groups, which are widely present on the membrane or proteins. AgNPs can bind to protein groups to form stable bonds that can change the three-dimensional structure of proteins and block their active sites (Klueh *et al.*, 2002).

The antibacterial activity of AgNPs is associated with the release of silver ions, which can be created and introduced by oxidative dissolution of AgNPs in the presence of oxygen (Reidy *et al.*, 2013). It is assumed that the antibacterial effect is also associated with the interaction of AgNPs with the bacterial cell walls. As a result of this interaction, the permeability of the cell membrane changes, which causes cell death (Ivask *et al.*, 2014).

The size of nanoparticles has a particular impact on their antibacterial activity. Studies have shown that for the formation of a bond between the membrane and AgNPs and further penetration into the membrane, the size of nanoparticles should be 1-10 nm (Morones *et al.*, 2005). Considering the wide range of antimicrobial activity of silver nanoparticles against microorganisms of different nature, it is possible to predict the effective effect of nanomaterials at different stages of leather production.

SYNTHESIS OF SILVER NANOPARTICLES

The main methods of synthesizing silver nanoparticles are chemical, physical, and biological (Almatroudi *et al.*, 2024). Chemical and physical methods of nanoparticle synthesis are resource-intensive and involve the use of strong reducing agents or organic solvents, various types of irradiation, ultrasonic treatment, etc. The implementation of these technologies produces toxic emissions, pollutes industrial wastewater, and worsens the environmental aspect of production. The most promising is the method of producing metal nanoparticles through green biosynthesis, or “green” technology (Ahmad *et al.*, 2019; Bawskar *et al.*, 2015).

The “green” technology for producing AgNPs is based on the environmentally friendly synthesis of nanoparticles by using the ability of biological systems (yeast, fungi, bacteria, plants) to synthesize metal nanoparticles through the enzymatic reduction of metal ions (Peiris *et al.*, 2017; Murali Krishna *et al.*, 2016; Voloshyna *et al.*, 2023). “Green” biosynthesis of nanoparticles occurs in the absence of toxic chemical materials.

Biosynthesized AgNPs for the leather industry should exhibit strong antimicrobial properties and reduce biofilm formation or destruction. To ensure the diffusion of AgNPs into leather materials, a spherical or elliptical shape of the nanoparticles is desirable.

Nowadays, plant extracts are most often used to implement the technology of green biosynthesis of AgNPs. Extracts from various plant parts can be used for synthesis. Synthesis of AgNPs using plant extracts is interesting due to cheap raw materials and the possibility of obtaining stable nanoparticles (Akhter *et al.*, 2024; Rehman *et al.*, 2023; Mohanta *et al.*, 2020). The biological safety of the synthesis AgNPs is ensured by the absence of a biological agent cultivation stage.

In general, plant-based biosynthesis involves the extraction of plant material with water, alcohol or a mixture of both, the addition of argentum nitrate to the extract, the biosynthesis stage, centrifugation and drying.

Biosynthesis based on *Aloe vera* (Yadav *et al.*, 2016) allows to obtain spherical AgNPs with a size of 30-40 nm.

The biosynthesis based on *Coffea arabica* (Dhand *et al.*, 2016) allows to obtain AgNPs agglomerates of spherical and ellipsoidal shape with a size of 30-40 nm. The minimum inhibitory concentration of the synthesised AgNPs is 0.2675 mg/l against *E. coli* and *S. aureus*.

The biosynthesis based on *Murraya Koenigii* (Philip *et al.*, 2011) allows to obtain AgNPs agglomerates of spherical and ellipsoidal shape with a size of 10 nm.

The biosynthesis based on *Coriandrum sativum* (Ahmad *et al.*, 2024) allows to obtain cubes AgNPs with a size of 100 nm. The researchers found that the mechanism of antibacterial action is morphological damage, metabolic disruption and respiratory inhibition, leading to bacterial death.

Different classes of algae are used for the synthesis of AgNPs: *Cyanophyceae*, *Phaeophyceae*, *Chlorophyceae*, *Rhodophyceae* (Fawcett *et al.*, 2017). When using *Codium capitatum*, we obtain AgNPs with a size of 3-44 nm of mixed spherical and cubic shapes; when using *Spirogyra insignis*, spherical AgNPs with a size of 30 nm; when using *Padina tetrastrum*, spherical AgNPs with a size of 5-35 nm.

Microbial biofilms are often resistant to various treatments, but biosynthesized AgNPs from biopolymers like chitosan have demonstrated significant potential in disrupting these biofilms (Regiel-Futyr *et al.*, 2017). The biosynthesis involves adding a chitosan solution to a solution of silver nitrate and ascorbic acid, subsequent stirring for 15 hours, evaporation, neutralisation, and drying. The technology allows the synthesis of spherical AgNPs with a size of less than 10 nm.

In the technology of “green” biosynthesis of AgNPs, bacterial cultures are most often used. Microbial biosynthesis of nanoparticles can occur intracellularly or extracellularly.

The biosynthesis of AgNPs includes a biological agent cultivation step, an argentum nitrate injection step, an inoculation step for 24 hours, a centrifugation step, and a drying step.

The first biosynthesis of metal nanoparticles using the bacterial culture *Pseudomonas stutzeri* was carried out in 2000. Another interesting area is the use of bacteria of the genus *Lactobacillus*, as they are inhabitants of normal human microflora. The green synthesis of AgNPs involves the reduction of silver ions to form silver nanoparticles under the action of bacterial cells and their metabolites. It is well known that silver nanoparticles are synthesized by the interaction of *Lactobacillus mindensis* biomass and their metabolites with silver ions. A safe and rapid method for the biosynthesis of AgNPs using *Lactobacillus acidophilus* in Mann-Rogosa-Sharp broth has been developed (De Man *et al.*, 1960). It has been proved that cultures of other microorganisms can also be used for the green biosynthesis of silver nanoparticles, namely: *Bacillus licheniformis*, *B. subtilis*, *Brevibacterium frigoritolerans*, *Aspergillus flavus*, *Pseudomonas aeruginosa*, *Pediococcus pentosaceus*, *Enterococcus faecium*, *Lactococcus garvieae*, *Citrobacter freundii* etc (Al-Asbahi *et al.*, 2024; Bharose *et al.*, 2024; Xia *et al.*, 2023; Shakhathreh *et al.*, 2021; Sulaiman *et al.*, 2015).

Lactobacillus sp. allows the synthesis of spherical AgNPs with a size of 7.97-14.3 nm, and *Bacillus sp.* – 11-22.8 nm (Al-Asbahi *et al.*, 2024). The synthesised AgNPs showed high antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antifungal activity of spherical AgNPs produced by *Bacillus subtilis* against *Aspergillus flavus* was determined (Bharose *et al.*, 2024).

Citrobacter freundii can be used for biosynthesis to produce stable spherical AgNPs with a size of 15-30 nm (Shakhathreh *et al.*, 2021). The synthesised nanoparticles are characterised by antibiofilm activity against *Staphylococcus aureus* and high antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa*.

AgNPs obtained by microbial biosynthesis can have different shapes and thus can exhibit high antimicrobial efficiency. The green biosynthesis technology is environmentally friendly, resource-efficient and has a wide range of applications.

Nanosynthesis technologies involve the production of AgNPs of various shapes: spherical, nanocubes, nanoplatelets, nanowires, etc. (Helmlinger *et al.*, 2005). The shape and size of nanoparticles affects the efficiency of surface binding to the cell membrane, cellular uptake, and effective bacterial killing. Researchers believe that the properties of AgNPs are directly related to the methods of their synthesis (de Souza *et al.*, 2019; Kesharwani *et al.*, 2018; Oves *et al.*, 2018; Saravanan *et al.*, 2018). The antibacterial activity of AgNPs also depends on their concentration in the drug.

Given the environmentally friendly nature of green biosynthesis and the antimicrobial properties of AgNPs, it would be beneficial to focus research on their potential in leather production, particularly during tanning and post-tanning processes, to improve both product durability and safety.

CONCLUSIONS

The prospect of using silver nanoparticles to impart antimicrobial properties to materials is growing. The safety and undisputed antimicrobial properties of silver nanoparticles can be a prerequisite for their successful use in the treatment of leather. The replacement of traditional biocidal agents with silver nanoparticles will contribute to resource efficiency of the technology, increase the safety of leather and leather products, and reduce the environmental impact. Future research should focus on optimizing the biosynthesis of silver nanoparticles to further reduce costs and environmental impacts, while expanding their application in various industries.

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PRODUCTION AND PERFORMANCE ENHANCEMENT OF TRANSPARENT AND SHADOW PUPPET SHOW LEATHERS

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Transparent leathers have attracted significant attention in the fashion industry due to their unique aesthetic appeal. Over recent decades, various companies have intermittently produced these materials; however, inherent performance limitations from the chemicals used remain unresolved. Despite their commercial appeal, the mechanical properties and durability of these leathers are often compromised; limiting widespread adoption. This study involved collaboration with international companies experienced in producing transparent leathers. A detailed exchange of technical knowledge aimed to address performance issues through modifications of existing production recipes. This approach focused on enhancing physico-mechanical properties such as tensile strength, elasticity, and durability while ensuring that alterations did not compromise transparency or visual appeal. Additionally, the dyeability of both leather types was thoroughly investigated, emphasizing the need for uniform and aesthetically pleasing coloration without affecting transparency for high-end fashion applications. The findings provide valuable insights into producing more durable and versatile transparent leathers, aligning with both performance and aesthetic requirements of the fashion industry. This study contributes to ongoing efforts to optimize the production of transparent and shadow puppet show leathers, making them more viable options for widespread use.

Keywords: transparent leathers, shadow puppet show leathers, transmittance

INTRODUCTION

Transparent leathers, appreciated for their aesthetic appeal, have gained attention in the fashion industry, although performance limitations have restricted widespread use (Yiying *et al.*, 2024). Today, transparent leathers are used in various applications, including the production of garments, footwear, bags, belts, wallets, watch straps, shoelaces, lampshades, drum coverings, and decorative household items, typically for accessories (China Arts, 2019). Also, transparent leathers have a longstanding tradition in shadow puppetry, serving as a medium for visually engaging representations. Shadow puppets, made from flat, transparent cut-outs of leather or paper, are affixed to a supporting stick and manipulated with at least two slender rods, allowing for intricate movements and storytelling (Orr, 1974). To ensure the quality of raw hides/skins intended for transparent leather production, specific characteristics must be present. These include low fat content, the absence of defects such as scars or irregularities, a tight structural composition, a high inter-fibrillar angle, and a predominance of the reticular layer compared to the papillary layer (Bayraktar *et al.*, 2005). Over the past two decades, companies have experimented with production, but challenges related to chemical treatments persist, particularly affecting durability and mechanical strength.

This study seeks to refine the production recipes of transparent and shadow puppet show leathers to mitigate current performance deficiencies. By optimizing essential properties, including mechanical strength and aesthetic clarity, this research endeavors to enhance both the functional and visual characteristics of these leathers. The ultimate aim is to render them more suitable for a range of applications within the fashion industry and other sectors where durability and visual appeal are of utmost importance.

EXPERIMENTAL

Materials and Methods

The production of transparent leather involved the use of six pickle (pH 2.5) goat skins and two pickle kid skins as raw materials. These skins were processed using six distinct recipes (Table 1). All recipes are clearly detailed below (Tables 2-7), and information regarding the commercial chemical products used in this study, which were sourced from Güvener Company (Türkiye), is provided in this section.

Table 1. Recipes and their codes

Recipes	Code
1. Glycerin	A
2. Fatliquor with Special Tanning Effect	B
3. Fatliquor with Special Tanning Effect + Dyeing	C
4. Bleached and Glycerin	D
5. Bleached and Glycerin+ Dyeing	E
6. Shadow Puppet Show Leather	F
Shadow Puppet Show Leather (Yellow)	F1
Shadow Puppet Show Leather (Red)	F2

In Table 2, the skins underwent depickle, washing, and pickle, followed by controlled drying to form transparent leather. Despite treatment with “Transparent Leather Tanning Agent” without auxiliary chemicals after depickle, moisture retention issues persisted, leaving the skins vulnerable to ambient humidity.

Table 2. Recipe A, Glycerin

Material Weight	Pickle Goat Skin Pickle + %50						
Process	%	Material	Time (min)	Speed (rpm)	Temp (°C)	pH	Description
Depickle	300	Water	15	18	20	5	
	1.5	NaCOOH	3 × 15				
	1.5	Microbial Protease	60				Drain, cold water wash
	4	Ecological Degreasing Agent	60				Drain, wash
	4	Ecological Degreasing Agent	60				Drain
Washing	150	Water	30				Drain
	400	Water					Salt-free float
Pickle	1.5	Formic acid	5 × 25				3h + overnight rest
	40	Trnsp. Leat. Tan. Agent	120			3.5	Drain, buck
Drying	Controlled toggle drying						

In Table 3, skins undergo depickle, washing, pickling, and tanning with specialized fatliquor and tanning agents to enhance flexibility and durability. Controlled drying on a toggle drying ensures the desired texture and transparency. Formic acid is used in the pickle stage for controlled swelling, followed by “Transparent Leather Tanning Oil”. To prevent moisture absorption and achieve non-hygroscopic properties, “Moisture Stabilizer (Polyalcholes)” and “Moisture Stabilizer (Silicone Base)” are added. Finally, “Anti-Adhesive Agent” is applied to eliminate the sticky texture common in transparent leathers.

Table 3. Recipe B, Fatliquor with Special Tanning Effect

Material Weight	Pickle Goat Skin Pickle + 50%						
Process	%	Material	Time (min)	Speed (rpm)	Temp (°C)	pH	Description
Depickle	300	Water	15	16	20		
	1	NaCOOH	3 × 15			4.2	
	1.5	Microbial Protease	60				Drain, cold water wash
	1.5	Microbial Protease	60				Drain, cold water wash
	4	Ecological Degreasing Agent	120				Drain, wash
	3	Ecological Degreasing Agent	120				Drain
	1	NaCOOH	3 × 15			4.2	
Washing	150	Water	30		20		Drain
Pickle	400	Water			20		Salt-free float
	1.2	Formic Acid	4 × 30			3.5	3 hours + overnight rest Drain in the morning
Tanning	40	Trnsp. Leat. Tan. Oil					
	4	Moisture Stab. (Polyalcholes)					
	0.4	Moisture Stab. (Silicone Base)					
	0.01	Mold Preventive Fungicide	180	8			Drain, wash
	300	Water		20			
	2	Anti-Adhesive Agent	20				Drain, buck
Drying	Controlled toggle drying						

In Table 4, the skins processed according to Recipe B were treated with an acid dye, which was precipitated onto the surface using formic acid. After swelling the skins with formic acid, they were subjected to fleshing, washed, and dried to achieve the desired dyed appearance. This method not only enhanced the visual characteristics of the leather but also ensured a uniform distribution of color throughout the surface.

Table 4. Recipe C, Fatliquor with Special Tanning Effect + Dyeing

Material Weight	Pickle Goat Skin Pickle + 50%						
Process	%	Material	Time (min)	Speed (rpm)	Temp (°C)	pH	Description
Depickle	300	Water	15	16	20		7 Be °
	1	NaCOOH	3x15			4.2	
	1.5	Microbial Protease	60				Drain, cold water wash
	1.5	Microbial Protease	60				Drain, cold water wash
	4	Ecological Degreasing Agent	120				Drain, wash
	3	Ecological Degreasing Agent	120				Drain
Washing	150	Water	30		20		Drain
Pickle	400	Water			20		Salt-free float
	1.2	Formic Acid	4 × 30			3.5	3 hours+overnight rest, drain
Tanning	40	Trnsp. Leat. Tan. Oil					
	4	Moisture Stabilizer (Polyalcholes)					
	0.4	Moisture Stabilizer (Silicone Base)					
	0.01	Mold Preventive Fungicide	180	8			Drain, wash
	300	Su			20		
	2	Anti-Adhesive Agent	20				Drain, wash
Dyeing	300	Water	20				
	0.01	Dyestuff	30				
	1	Formic Acid	2x15				Check for swelling, if there's swelling, drain it. Fleshing (tight), wash, drain, buck
Drying	Controlled toggle drying						

In Table 5, pickled goat leather was bleached with hydrogen peroxide and treated with “Transparent Leather Tanning Agent”. However, without auxiliary agents, the leather failed to retain moisture after drying, leaving it sensitive to humidity and persistently damp. This underscores the need for appropriate auxiliary agents in leather processing.

Table 5. Recipe D: Bleached and Glycerin

Material Weight	Pickle Kid Skin Pickle + 50%						
Process	%	Material	Time (min)	Speed (rpm)	Temp (°C)	pH	Description
Depickle	300	Water	15	16	20		7 Be °
	1	NaCOOH	3x15			5.5	
	2	Microbial Protease	60				Drain, cold water wash
	3	Ecological Degreasing Agent	60				Drain, wash
	4	Ecological Degreasing Agent	60				Drain
Washing	150	Water	30		20		Drain
Pickle	100	Water			20		Salt-free float
	1	H ₂ O ₂	60				
	1.5	H ₂ SO ₄	5x15			3.5	Drain
Washing	150	Water	3x15		20		Drain, Fleshing
	30	Trnsp. Leat. Tan. Agent					Anhydrous float
			6h+overnight				Automatic. Remove in the morning.
Drying					35°C		Buck, toggle drying

Recipe E in Table 6 is derived from Recipe D with the addition of an acid dye. The dye was precipitated formic acid, followed by swelling, rinsing, and fleshing to tight setting on the surface. This process gave the leather a dyed appearance while eliminating moisture retention issues and imparting a rigid, plastic-like texture.

Table 6. Recipe E, Bleached and Glycerin+ Dyeing

Material Weight	Pickle Kid Skin Pickle + 50%						
Process	%	Material	Time (min)	Speed (rpm)	Temp (°C)	pH	Description
Depickle	300	Water	15	16	20		7 Be °
	1	NaCOOH	3x15			5.5	
	2	Microbial Protease	60				Drain, cold water wash
	3	Ecological Degreasing Agent	60				Drain, wash
	4	Ecological Degreasing Agent	60				Drain
Washing	150	Water	30		20		Drain
Pickle	100	Water					Salt-free float
	1	H ₂ O ₂	60				
	1.5	H ₂ SO ₄	5x15			3.5	Drain
Washing	150	Water	3x15		20		Drain, Fleshing (tight)
	30	Trnsp. Leat. Tan. Agent					Anhydrous float, 6 hours+overnight
							Automatic. Remove in the morning.
Drying		Buck, toggle drying					
	300	Water					
	0.01	Acid Dyestuff	30				
	1	Formic Acid	2x15				Check for swelling, if there's swelling, drain it. Fleshing (tight), wash

In Table 7, glycerin was omitted as soft leathers were not desired. Instead, semi-transparent, rigid leathers for shadow puppet shows were produced by swelling the leather

with acid and toggle drying to bond collagen fibers. Yellow (F1) and red (F2) dyes were added to create colored shadow puppet show leathers.

Table 7. Recipe F, Shadow Puppet Show Leather

Material	Pickle Goat Skin						
Weight	Pickle + 50%						
Process	%	Material	Time (min)	Speed (rpm)	Temp (°C)	pH	Description
Depickle	300	Water		16	25		
	1	NaCOOH	60			4.5	
	1.5	Microbial Protease	60				Drain, cold water wash
	4	Ecological Degreasing Agent	60				Drain, wash
	3	Ecological Degreasing Agent	60				Drain
Washing	150	Water	30				Drain
	100	Water			20		Salt-free float
	1	HCL	2x10				
	0.01	Bactericide	100				Drain
Drying	Controlled toggle drying						

Spectrophotometric Color Measurement and Light Transmittance Determination

To accurately evaluate the colorimetric properties and light transmittance characteristics of leather specimens, a Konica Minolta CM 3600D spherical spectrophotometer, interfaced with a computer, was used. This instrument facilitates precise measurements across a wide spectrum, ensuring reliable data for subsequent analysis.

Physical Tests

All physical tests followed TS EN ISO 2418, 2006 standards for sample preparation. Samples were conditioned as per TS EN ISO 2419, 2006 at $23 \pm 2^\circ\text{C}$ and $50\% \pm 5\%$ relative humidity. Leather thickness was measured using a Satra Thickness gauge according to TS 4117 EN ISO 2589, 2006. Tensile strength, percentage of elongation and double edge tear strength of samples were determined by using Shimadzu AG-IS Tensile Tester and Trapezium-2 software according to TS EN ISO 3376, 2012, TS EN ISO 3377-2, respectively. The distension and strength of surface tests were done according to TS EN ISO 3379, 2016.

RESULTS AND DISCUSSIONS

The Lab* color values measured using a spectrophotometer are shown in Table 8. The “L*” value ranges from near black (0) to white (100), while the “a*” and “b*” values indicate red-green and yellow-blue tendencies, respectively. Upon examining the “L*” values, it is observed that leathers treated with glycerin (A) exhibit a darker coloration, whereas the shadow puppet show leather samples (F, F1, F2) display a lighter appearance.

Table 8. Spectrophotometer color measurements of leathers

Code	L*	a*	b*
A	33.22	1.49	6.63
B	41.06	1.49	8.54
C	44.58	2.85	2.13
D	40.02	1	5.46
E	36.32	-16.29	1.44
F	78.79	1.3	8.51
F1	66.22	1.49	40.57
F2	67.32	13.99	6.61

The photographs depicting the leathers processed in accordance with the specified recipes are presented in Figure 1. Each image is accompanied by its corresponding identification code, which serves to enhance the clarity of reference and facilitate comprehensive analysis of the visual characteristics associated with each treatment method.

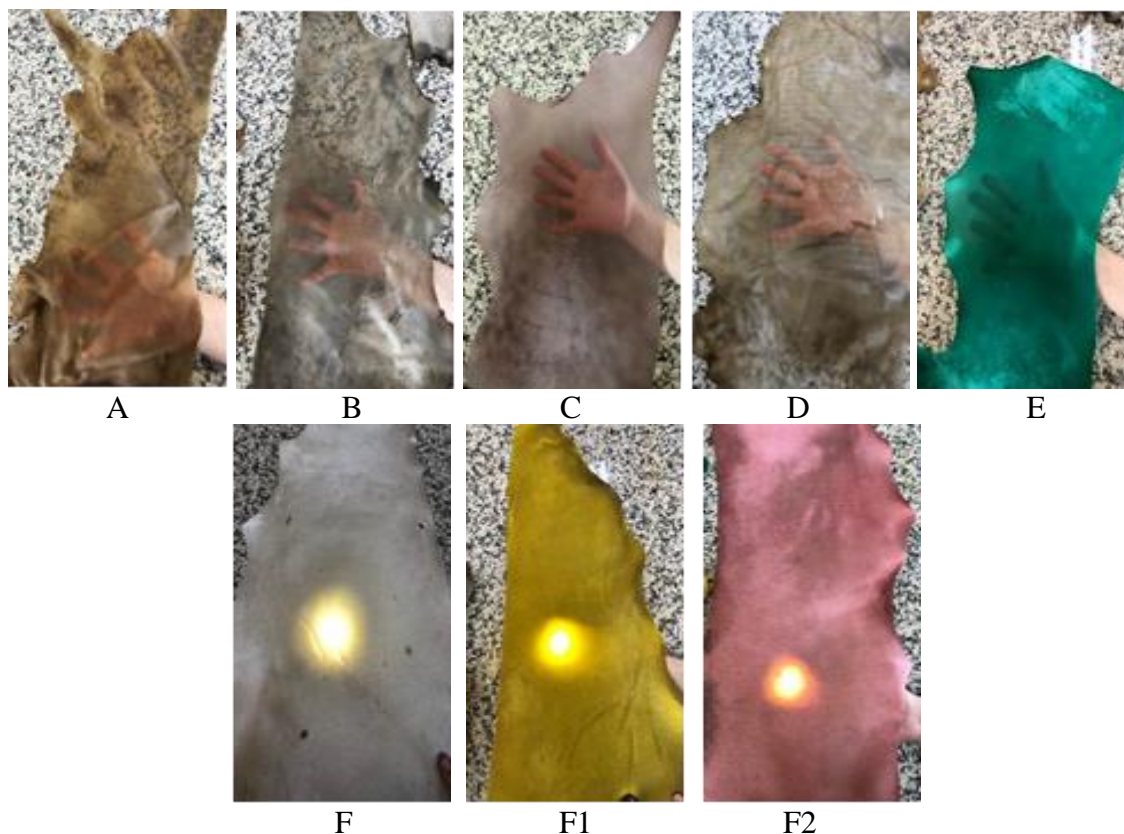


Figure 1. Photographs of the transparent and shadow puppet show leathers

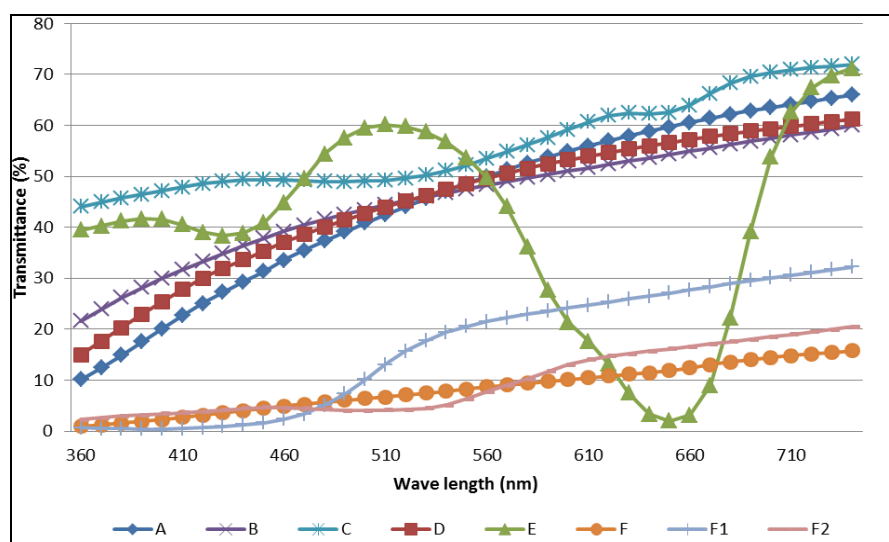


Figure 2. Transmittance spectra of transparent leathers

The percentage transmittance values measured by the spectrophotometer are illustrated in Figure 2. Upon analyzing the graph, Recipe C demonstrates the highest transparency in the

visible spectrum, followed by B, D, and A. Leathers processed with E show relatively high transparency, but a decline is seen between 510 and 710 nm due to the light-reflective properties of the green dye. The lowest transparency values were recorded for F1, F2, and F. The reduced light transmittance of these leathers is attributed to the intended semi-transparency. Furthermore, these leathers require rigidity due to their use as show curtains, a property achieved by omitting glycerin treatment, which preserves their semi-transparent nature. Similar trends were reported about some materials by An *et al.* (2017), Alam *et al.* (2016), and Qiang *et al.* (2019). Based on the reviewed literature, the transparency characteristics of leathers processed with recipes C, A, D, and B in this study can be deemed satisfactory.

One key factor in determining leather suitability is its tensile and tear strength, which are influenced by the three-dimensional structure of the fibers and the treatments they undergo. Table 9 shows that leather treated with recipe E had the highest tensile strength (83.87 N/mm²), followed by recipes C, F1, F2, and F, with strengths ranging from 82.82 to 71.6 N/mm². Recipes D and B showed moderate values, while recipe A had the lowest tensile strength at 5.38 N/mm², falling below the United Nations Industrial Development Organization (UNIDO), (1996), minimum of 20 N/mm² for goat leathers. Recipe A also had the highest elongation (115%), while recipe F showed the lowest. The highest tear strength was recorded for recipe D (73.14 N/mm), and recipe A again had the lowest value (18.20 N/mm), below the UNIDO standard of 40 N/mm.

Table 9. Mean values some mechanical tests of leathers

Code	Thickness (mm)	Tensile strength (N/mm ²)	Elongation (%)	Tear Load (N)	Tear strength (N/mm)
A	1.53	5.38	115	26.02	18.20
B	0.89	20.33	104	46.62	52.98
C	0.24	82.82	18	14.92	57.39
D	0.71	25.35	83	49.73	73.14
E	0.27	83.87	20	15.73	65.58
F	0.42	71.6	17	29.94	69.64
F1	0.32	82.53	21	22.61	68.52
F2	0.34	81.4	20	25.57	71.03

In the leather production process, all materials come into contact with grain side, which can lead to a loss of flexibility in over-tanned leather sections. According to the data obtained from Table 10, leathers from recipes E, F, F1, and F2 burst without cracking in the grain layer. The highest bursting load values were seen in recipes F2, F, and F1, with minimal variation, while recipe A had the lowest. The longest bursting length was 15.63 mm for recipe B, and the shortest was 6.61 mm for recipe E.

Table 10. Mean values of distension and strength of surface test of leathers

Code	Thickness mm	Cracking		Bursting	
		kgf	mm	kgf	mm
A	1.60	7.80	10.91	4.72	11.27
B	0.88	14.00	13.33	20.37	15.63
C	0.24	7.00	5.76	29.50	7.29
D	0.68	14.25	11.58	23.00	13.50
E	0.25	-	-	25.00	6.61
F	0.34	-	-	31.75	7.02
F1	0.31	-	-	31.25	7.15
F2	0.38	-	-	33.00	7.29

CONCLUSION

This study provides a comprehensive examination of leather produced according to formulated recipes, focusing on their dyeability and transparency. The findings indicate that the use of direct acid dyes and specific mechanical processes effectively enhances the dyeing capabilities of transparent leathers. Notably, Recipe C, which employed a pink dye with the special tanning effect fatliquor, exhibited the highest transmittance percentage, as visually corroborated by captured photographs. In contrast, leather samples processed under Recipes F, F1, and F2 showed significantly lower transmittance values, aligning with their intended application in shadow puppet shows, where semi-transparency is obscured while allowing for shadow projection.

Furthermore, physical testing results indicate that dyed leather samples generally possess higher strength values, although those treated with glycerin exhibited lower performance metrics. It is crucial to emphasize the importance of researches aimed at enhancing the strength and performance characteristics of transparent leathers, facilitating the development of innovative techniques that improve the quality and utility of leather in various applications.

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REVIEW ON SOUNDBOARD IN REBAB-MAKING. PART I: STRUCTURE OF REBAB, SKIN, AND BOVINE PERICARDIUM

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The rebab is a stringed, bowed instrument, coated in leather, with a resonance box made from calabash or wood. Leather or bovine pericardium, a skin-like material, serves as the soundboard for the instrument. The leather's structure should be homogenous, compact, and robust. The dermis layer of the skin and its fibrillar structure are significant. The angles of the fibers must be acute, and the packing density must be high. The crystalline structures of collagen provide high efficiency. When making a rebab, the strength and viscoelasticity of the materials used must be meticulously assessed. Consideration must be given to the thickness and characteristics of the leather. The bovine pericardium comprises collagen. It is a significant and distinctive tissue characterized by a heterogeneous collagen fiber structure. It demonstrates regionally varied physical limitation behavior. The predominant materials utilized in rebab fabrication are bovine pericardium and fish leather. Nevertheless, the acoustic output generated by fish leather is substantial relative to the instrument's size. The membrane's slender, robust, and flexible composition provides excellent resonance. Simultaneously, in conjunction with the calabash, it produces a distinctive sound reminiscent of leather. The production of rebabs can be standardized through leather processing, utilizing it as high-value recyclable waste. This review made assumptions by concentrating on the bovine pericardium, which has not yet been examined via an academic lens in the context of rebab-making.

Keywords: Rebab-making, skin histology, fibrillar architecture of the dermis, bovine pericardium.

INTRODUCTION

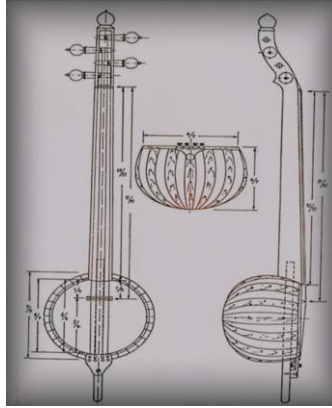
Human beings, having been perpetually enveloped by sound since their inception, have conceptualized sound as a phenomenon through their experiences and observations. Sound holds greater significance for humanity than thought. Sound serves as a mechanism for detecting threats essential for survival, constituting a fundamental instinct (Degirmenli, 2023). Individuals have constructed instruments from pole to pole utilizing the natural resources available to them (Akyol, 2017). Animal hide has been utilized for the creation of instruments since antiquity. In antiquity, the saz (rebab) was made by tautly stretching leather over a turtle shell or a calabash. The leather-covered turtle shell is exhibited at the Cairo Museum (Sonmez, 2008). Historically, individuals began producing noises by rubbing their hunting arrows against their bows (okluğ), then experimenting with bows crafted from horsehair, which were enhanced by affixing a calabash to the tip (ıklığ). Subsequently, they affixed handles to the calabash, upon which they extended thin leather. To provide a clearer sound, they extended stringer wires over the leather. Consequently, the rebab, an elongated string instrument, originated (Alaskan, 2013).

Rebab

The rebab is a traditional stringed instrument from Turkey (Ozdemir, 2020). It is a stringed instrument featuring a leather covering and a hemispherical body, played by positioning the string upon it. It is among the oldest folk instruments of the Turks that has had

minimal structural alteration (Sener, 2019). The *iklig* was performed by the Uyghur Turks in Central Asia during the 7th century (Acin, 1998).

The rebab comprises the following components: resonance box, neck (fingerboard), soundboard, post, threshold, pegbox, tuning pegs, string, bow, and spike. Polishing is used to achieve a smooth surface. The instrument originated with two strings, subsequently a third string was incorporated, and eventually a fourth string was added to enhance the tonal range. It is an instrument devoid of frets. The sound breadth spans 2.5 to 3 octaves. Consideration must be given to the equilibrium and proportions among the components of the instrument (Yegin, 1994; Kaya, 1998; Acin, 1998; Sezer, 2016; Altiparmak, 2007).



with soap. Rebabs with hardwood soundboards in lieu of leather have also been manufactured (Sener, 2019; Celik, 2023). A composite body constructed from glass, carbon fiber, and polyester resin has been developed, replacing the traditional handmade body with a synthetic construction (Acet & Saati, 2015). Notwithstanding these factors, the rebab continues to be crafted using traditional processes and techniques. It is an instrument that perpetuates the master-apprentice connection, with its soundboard crafted from amateur leatherwork (Majid *et al.*, 2023). When bovine pericardium serves as a soundboard, no preparation is required beyond the aforementioned procedures.



Figure 2. Rebab (Ofnaz, 2008)

Below are shared photographs of rebabs, which were personally captured and utilize calf pericardium as a soundboard.



Figure 3. Rebabs at the Instrument Sales Unit of Ege University State Turkish Music Conservatory (EUSTMCIS)

To comprehend the interactions between the instrument body and the leather, it is essential to first grasp the structure of the skin and the bovine pericardium.

Histological Composition of the Skin

The skin comprises the epidermis, dermis, and hypodermis layers. The epidermis and hypodermis layers are excised throughout the process. Amorphous proteins, lipids, and other substances in the skin structure are eliminated (Harmancioglu & Dikmelik, 1993). The central region of the corium comprises a composite architecture of type I collagen and an elastin fibril network, which contributes to the leather's strength. Following all elimination processes,

the dermis comprises 90-95% of the skin's mass. The upper dermis layer is referred to as the epidermis, whereas the lower dermis layer is termed corium (Covington, 2009).

The dermis layer comprises tightly interlaced collagen bundles. In the central region of the dermis, the fibers increase in coarseness and strength, forming distinct angles that influence the final characteristics of the leather (Sharphouse, 1971). The dermis comprises two layers. The stratum papillare constitutes the upper layer of the dermis, whereas the stratum reticulare comprises the bottom layer (Harmancioglu & Dikmelik, 1993).

Fibrillar Architecture of the Dermis

Protein-based fibrous formations manifest as elongated polypeptide chains within the dermal layer. They vary from one another owing to distinctions in their peptide structures. This structure has three types of fibers: collagen fibers, elastic fibers, and reticular fibers (Harmancioglu & Dikmelik, 1993). Leather chemist Gerngross categorized unit fibrils into two classifications: crystallized and non-crystallized. Thus, power loss resulting from improper orientation can be mitigated. There exists a disparity in reactivity between crystalline and non-crystallized regions. In crystalline regions, molecular chains are interconnected by many hydrogen bonds (Phillips, 1954). The integrity of the skin's structure and surface relies on the fiber bundles remaining complete and erect. Orientation of skin fiber bundles: They may exhibit oblique angles (0° - 20°), low angles (20° - 45°), normal angles (45° - 70°), and straight angles (70° - 90°). Skin exhibiting angles of 45° or less is lax and fragile. Fiber bundles can be arranged in a dense, moderate, or sparse configuration. These characteristics influence the skin's management, mechanical attributes, and level of water absorption (Harmancioglu & Dikmelik, 1993).

Histological Composition and Mechanical Characteristics of Bovine Pericardium

The pericardium is a fibrous membrane that encases and safeguards the heart of mammals, facilitates blood circulation, and houses pericardial fluid (Oswal, 2007). The pericardium comprises three layers: serosa, fibrosa, and epipericardial connective tissue (Ishihara *et al.*, 1981). The three fiber layers are oriented at an angle of roughly 60° relative to one another (Elias & Boyd, 1960). The serosa comprises a delicate glass-like covering, flattened mesothelial cells situated adjacent to the pericardial cavities, and a slender submesothelial space. The fibrosa layer contains overlapping collagen bundles that exhibit a flattened, undulating appearance and diverse orientations. Elastic fibers are present in this location as well. Elastic fibers converting to mesothelium are smaller than those in fibrosa. Findings from light microscopy, scanning electron microscopy, and transmission electron microscopy elucidate the histology of bovine pericardium (Ishihara *et al.*, 1981).

The multi-directional arrangement of collagen stacks in pericardial fibrosa and the undulating collagen fibrils inside each stack substantially influence the mechanical properties of pericardial tissue. The pericardium's capacity for expansion is contingent upon the interplay of three elements, given that collagen fibrils are inextensible. 1- Orientation of collagen stacks relative to the unidirectional extension axis; 2- mobility of elastic fibers inside the tissue; 3- extent of collagen fluctuation that ceases upon tissue extension (Ishihara *et al.*, 1980; Ishihara *et al.*, 1981). The fibrous layer of the mature calf pericardium exhibits a collagen fiber structure that is distinctly heterogeneous. The pericardium thickness of newborn calves exhibits minimal variability internally. The mean thickness of the adult calf pericardium is 0.36 mm, while the mean thickness of the newborn calf pericardium is 0.12 mm (Sizeland *et al.*, 2014).

Matrix metalloproteinases (MMP) 2 and 9 were identified in bovine and porcine pericardium via the zymography technique. The activity of MMP-9/MMP-2 in bovine pericardium is 8.5 times greater (Calero *et al.*, 2002). MMP, primarily responsible for tissue rearrangement and repair, also significantly contributes to embryonic development, morphogenesis, and the management of various disorders (Bode *et al.*, 1999; Bode and Maskos, 2001; Celentano and Frishman, 1997; Massova *et al.*, 1998).

Prior research indicates that tensile strength and stiffness correlate with the anatomical orientation of the membrane (Zioupou & Barbenel, 1994a; Zioupou & Barbenel, 1994b). Nonetheless, it is recognized that the selection of a certain membrane region partially elucidates mechanical behavior. Regional variability in structure may exist (Hiester & Sacks, 1997b). Additionally, previous research (Simionescu *et al.*, 1993) corroborates the findings that tissue structure significantly differs according to the orientation of the fibers, as verified by other research (Hiester & Sacks, 1997a). This illustrates the spatially variable physical restriction properties of the pericardium (Belenkie *et al.*, 1992; Chien & Chang, 1972; Grant *et al.*, 1994; Grant *et al.*, 1992; Smiseth *et al.*, 1994).

The skin possesses a non-linear, anisotropic, and viscoelastic structure as a result of collagen orientation. Nonetheless, there exists no correlation between these two (Wong *et al.*, 2015). Skin and pericardium, both collagen-rich connective tissues, exhibit analogous characteristics.

RESULTS AND CONCLUSIONS

To produce a melodious tone and ensure durability, the instrument must be constructed from suitable materials and adhere to balanced proportions and dimensions. The thickness, type, and characteristics of the leather utilized in the instrument are crucial. The selection of leather for rebab-making considers its superior strength and acoustic qualities. To achieve this, the collagen fiber bundles are complete, the weaving angles of the bundles are acute, and the bundle density is elevated, resulting in the desirable compact structure. The influence of intercellular molecules on the mechanical characteristics of leather is significant. The interfiber material, specifically globular proteins, between the bundles must be eliminated. Simultaneously, eliminating secondary forms adhered to the skin as much as feasible yields a uniform structure, hence enhancing and streamlining sound transmission within the structure. Furthermore, it is essential that the crystalline structure is maintained in collagen. Consequently, a compact structure is achieved, enhancing its strength, and the sound vibration within the leather can be standardized by diminishing its water absorption capacity. Furthermore, with advancing age, cellularity and water content diminish, fibril alignment becomes more uniform, and cross-links intensify. These advancements enhance the leather's alignment with the intended characteristics.

The calabash and leather employed in its fabrication impart a distinctive tone to the instrument. Porous materials, like leather, typically possess characteristics such as inherent sound absorption and noise attenuation. The formation of pores due to the progressive arrangement of fiber bundles diminishes the mechanical strength of the leather while enhancing its softness. The inherent composition of leather facilitates air permeability and the absorption and buffering of thermal and acoustic energy (Chen & Shan, 2022). Bovine pericardium, which reinforces the intricate arrangement of collagen fibers and elastic fibers inside its fibrosa layer, is a more appropriate option for the instrument regarding its acoustic qualities. Regional membrane selection may be conducted by considering its heterogeneous composition.

While several leathers and membranes serve as soundboards in rebab-making, calf pericardium and fish leather are the most prevalent. Capricorn leather is typically favored due

to its availability. Fish leather, conversely, produces a rich and vibrant sound; yet, the instrument's volume is limited, generally inadequate for this auditory output (Altıparmak, 2007). The bovine pericardium, particularly that of calves, is the most often utilized membrane. The bovine pericardium produces a high-pitched, resonant sound due to its thin and flexible composition (Sezer, 2016).

Thick leather should be avoided. Alternatively, the instrument's sound gets muted. The utilization of thin or low-strength leather is readily compromised by environmental conditions including temperature, moisture, humidity, and inadvertent physical harm. Furthermore, as the instrument's threshold contacts the soundboard from above, it may lead to complications, particularly when utilizing membranes. Nonetheless, the membrane's elastic, robust, and slender composition generates significant resonance power (Rai *et al.*, 2019). It generates a vibrant and high-decibel auditory output. Simultaneously, it is essential to note that the leather utilized for tenor and bass rebabs must be substantial in thickness to provide adequate mechanical volume due to their big dimensions. Utilizing pericardium in this instance may yield suboptimal outcomes.

The inferences drawn did not account for leather processing. Today, sustainability has gained significant importance in the leather sector (Bayramoğlu *et al.*, 2024; Bayramoğlu, 2012). This phenomenon is also observed in other domains. In the context of technological and environmental considerations, high efficiency and minimizing waste by enhancing added value are crucial principles. The academic application of bovine pericardium in rebab-making can be advanced by standardization. In the context of standardizing rebab production, bovine pericardium may undergo rigorous testing and leather treatment to enhance its durability and acoustic qualities. Additionally, the characteristics of collagen, including hygroscopic qualities, uneven alkaline swelling, and swelling in response to acid and salt, must be considered (Harmancıoğlu & Dikmelik, 1993; Dikmelik, 2013). Consequently, durable rebabs can be developed over time, hence broadening the application scope of this material. This may foster the advancement of intercultural connections in the long run, as the instrument has disseminated throughout a broad geography.

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REVIEW ON SOUNDBOARD IN REBAB-MAKING. PART II: RELATIONSHIP BETWEEN THE MECHANICAL BEHAVIOR OF BOVINE PERICARDIUM AND SOUND

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Collagen fibrils, which may already possess a compact configuration in the absence of stress, realign and adopt a more rigid structure when subjected to tension. Microfeatures, including the D-period in collagen's molecular structure, variations among collagen types, the presence of glycosaminoglycans, intercellular substances, and collagen linkages, along with macrofeatures, provide strength through their mechanical properties and influence sound mechanics. The nonlinear, heterogeneous, and anisotropic nature of most collagens complicates the assessment of the mechanical properties of skin. As sound traverses' porous structures, it is attenuated, resulting in energy loss. Bovine pericardium has regional variation; yet, its features, including fiber density and orientation, remain consistent throughout the tissue. The instrument conveys the sound produced by the vibrating bow and wood into the air. The front and rear plates alternately expel air via the openings in the front plate, facilitated by vibration. Simultaneously, the membrane typically decreases the modal frequency under air loading conditions. The scaffold is characterized by the textural angles of the bovine pericardium, orthotropic anisotropy, thinness, strength, viscoelasticity, and the acoustic properties it produces, along with sound mechanics. This review made assumptions by concentrating on bovine pericardium, which has not yet been examined through an academic lens in the context of rebab-making.

Keywords: Collagen structure, mechanical characteristics of bovine pericardium, sound mechanics, instrumental acoustics.

INTRODUCTION

Collagen is a polypeptide chain characterized by a segmental structure. It lacks flexibility; however, it can extend by 5% due to the fibrils interlocking in a helical configuration. In the absence of the force inducing elongation, they can revert to their original state due to the elastic fibers (Harmancioglu & Dikmelik, 1993) and are encased in type I collagen, an extracellular matrix protein prevalent in various tissues including skin, tendons, bones, and blood vessels (Francis-Sedlak *et al.*, 2009; Avery & Bailey, 2006). Dry skin comprises almost 70% of its mass (Naffa *et al.*, 2019). Cellular behavior influenced by collagen and the extracellular matrix is determined by the substrate's chemical, mechanical, and physical characteristics (Kuzuya *et al.*, 1998). The consistency of the solid extracellular matrix, migration (Hartog *et al.*, 2005), and proliferation (Francis *et al.*, 2008) affect cellular activity, including its features (Francis-Sedlak *et al.*, 2009). The integrity of the skin is conferred by the embedded tropocollagen molecules and collagen glycosylation within the fibers and fibrils, as well as the cross-covalent bond architecture facilitated by glycosaminoglycan (Fratzl, 2008). Elastin cross-links: deH-LNL and Des; Collagen cross-covalent linkages include deH-HLNL, deH-DHLNL, HHL, and HHMD (Fratzl, 2008; Naffa *et al.*, 2016). Drawing inferences based solely on the association between collagen fibril diameter and skin strength would be inappropriate. The intricate and varied structure of

collagen can alter its characteristics. Nonetheless, cross-covalent connections, many hydrogen bonds, or hydrophobic interactions in collagen might enhance its strength (Wells *et al.*, 2013).

Acoustic mechanics and tissue scaffolding are two interrelated ideas. The quantity of collagen, its molecular configuration (D-period of collagen, collagen type), its capacity for self-crosslinking, fiber dimensions, and orientation are the determinants of skin strength (Sizeland *et al.*, 2013). D-period pertains to the fibril length and the mean angular orientation inside collagen bundles. Collagen fibrils exhibit a D-period amplitude distribution of up to 10 nm in the dermis, bone, and tendon. The D-period value of collagen in the tendon is around 67 nm. The D-period along the fibril axis represents the projection of the actual D-period, $\alpha=17-18^\circ$ (Fang *et al.*, 2012; Ottani *et al.*, 2001).

$$64 \approx 67 \cdot \cos(\alpha) \quad (1)$$

The molecules constituting the linear fibrils of collagen stretch at an angle of less than 5° relative to the fibril axis, whereas the same molecules in the helical configuration maintain a stable angle of 17° (Ottani *et al.*, 2001). This property is congruent with the partially crystalline hexagonal configuration (Yamauchi *et al.*, 1996). Measurements of collagen orientation and D-period indicate that fibrils under tension reorient and adopt a more compact form (Basil-Jones *et al.*, 2012). Research suggests that collagen fibrils can be categorized into two distinct forms: The first kind is T-type collagen, characterized by its heterogeneous composition, tightly packed in parallel arrangements, exhibiting exceptional tensile strength; the second type is C-type collagen, distinguished by its helical structure, comprising small homogeneous fibrils, and demonstrating resistance to multidirectional strain (Ottani *et al.*, 2001). Collagen orientation can be quantified through reflection anisotropy (Kronick & Buechler, 1986; Schofield *et al.*, 2011), small-angle scattering (Friedrichs *et al.*, 2007), confocal laser scanning microscopy (Billiar *et al.*, 1997), Raman scattering (Jor *et al.*, 2011), anisotropic Raman scattering (Falgayrac *et al.*, 2010), multiphoton microscopy (Janko *et al.*, 2010), and small-angle X-ray scattering (Lilledahl *et al.*, 2011; Basil-Jones *et al.*, 2011).

Mechanical Characteristics of the Bovine Pericardium

The quality and quantity of collagen dictate the mechanical strength of the contained pericardium (Sacks *et al.*, 1994; Paez *et al.*, 2003). The bovine pericardium exhibits an anisotropic structure, with its strength and mechanical properties demonstrating predictable variation based on orientation angles. This guarantees superior membrane functionality, longevity, and efficacy (Zioupos & Barbenel, 1994a; Zioupos & Barbenel, 1994b).

Bovine pericardium can be characterized as mechanically (Gabbay *et al.*, 1984) and structurally (Clark, 1973) anisotropic (Zioupos & Barbenel, 1994a). Despite the inability to establish a correlation between mechanics and tissue, this structure is characterized as uniaxial (Clark, 1973; Lee *et al.*, 1989) and biaxial (Choi & Vito, 1990) according to test methodologies (Zioupos & Barbenel, 1994a). Despite the difficulty in detecting, it within collagenous tissues like the pericardium, the expansion capability of the asymmetric axis surface further corroborates this characteristic (Zioupos *et al.*, 1992). Alterations in the orientation of collagen fibrils induce anisotropic behavior (Paez *et al.*, 2000; Paez *et al.*, 2001; Sacks *et al.*, 1994), but reorientation loadings do not influence anisotropy (Zioupos *et al.*, 1994).

Three possible fibril distributions were analyzed to comprehend the material symmetry. The elongation results indicated by the modulus of elasticity demonstrate that the pericardium is orthotropic. Despite being a nonlinear anisotropic material, it is important to note that soft tissue fibrils are realigned in the direction of maximal tensile force (Zioupos & Barbenel, 1994a).

Biaxial mechanical tests were conducted on 12-18-month-old cow pericardium samples to ascertain the link between collagen structure and mechanical anisotropy (Sacks *et al.*, 1994).

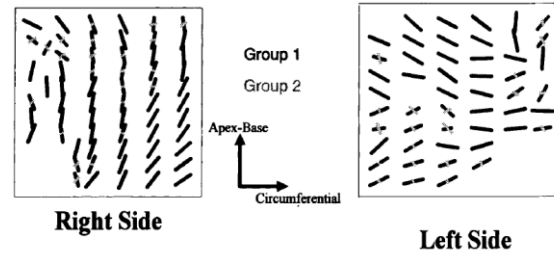


Figure 1. Vectorial condition of collagen fibrils in the anterior right and left regions of the bovine pericardium (Sacks *et al.*, 1994)

The orientation of collagen fibrils and their volumetric ratio exhibit partial consistency across several samples. The alteration of collagen fibril orientations in connective membrane tissues influences mechanical anisotropy. Additional histological characteristics, like fiber density and orientation degree, also maintain stability within the tissue. The material has considerable stability in its mechanical behavior owing to its collagen fiber architecture. Once more, the stability of outstanding strength, maximum strength, and complete stretchability qualities is contingent upon the collagen structure. The right section of the example exhibits greater instability and displays a broad spectrum of fiber orientations. The section on the left exhibits greater homogeneity (Sacks *et al.*, 1994).

The thickness measurements revealed that bovine pericardium measures 0.42 ± 0.01 mm, sheep pericardium measures 0.32 ± 0.02 mm, porcine pericardium measures 0.20 ± 0.01 mm, and canine pericardium measures 0.19 ± 0.01 mm. Less elastic pericardiums are found in thinner canines and porcines. A robust association was observed between thickness and mechanical behavior. Stress values are defined by viscoelasticity (Sacks *et al.*, 1994). The pericardium of canines and porcines is both viscoelastic and more rigid compared to the thicker pericardium of cattle and sheep. Biochemical investigations indicate that thin tissues possess elevated levels of strongly cross-linked type III collagen (Naimark *et al.*, 1992).

By analyzing the mechanical properties of bovine pericardium, conclusions can be drawn concerning the appropriateness of these structures for the fabrication of rebabs.

Acoustic Physics; Instrumental Acoustics and Architecture

Sound is a perceptual phenomenon that arises in the brain when the periodic effects generated by a vibrational source in the environment are detected by the auditory system (Degirmenli, 2023). Sound constitutes a form of energy. Vibrations produced by a material system provide stimulus. The sound source arrives at the receiver via the transmitting medium. Movement in any section of the material environment also stimulates other sections of the material due to its inherent flexibility. Sound is conveyed through the cyclical conversion of kinetic and potential energy in the transmitting medium (Zeren, 1997). The formation and propagation of sound within the skin arise when the molecules in its structure become unstable due to mechanical forces, contingent upon the skin's structural features (Alaskan, 2013).

Wavelengths are the recurring equal intervals when a wave propagates. Sound waves can propagate both transversely and longitudinally in solid materials. Amplitude refers to the extent to which a structure in equilibrium deviates from its existing position in response to a

stimulus. The wave speed is the distance covered by the wave per unit of time. The frequency of vibrations per unit time is referred to as the period. The frequency is defined as the rate of expansion and compression (vibration) occurring per unit of time (Degirmenli, 2023; Oflaz, 2008; Mazlum, 2011; Alaskan, 2012; Alaskan, 2013).

$$v = \lambda f \quad (2)$$

$$x = v t \quad (3)$$

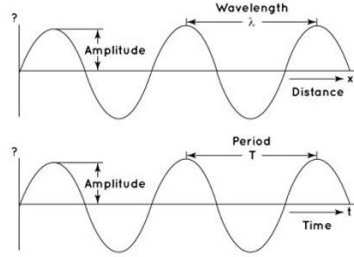


Figure 2. Sine wave equations (Degirmenli, 2023)

Waves exhibit distinct phenomena including reflection, refraction, diffraction, and interference. The reflected wave returns from the surface at an equivalent angle. A wave entering a different medium alters its direction and refracts due to a change in velocity, unless it approaches at a perpendicular angle. When the crest of the wave in the same medium encounters an impediment, it decelerates, alters its trajectory, and undergoes refraction. The velocity of sound in a given environment fluctuates due to variables including temperature, humidity, and pollution (Zeren, 1997).

The operational basis of membranes is that air loading, bending resistance, and shear resistance influence their modal frequencies. Primarily, air loading generally reduces modal frequencies, whereas the other two components typically augment them. In thin membranes, air loading typically exerts a predominant influence (Fletcher & Rossing, 1998).

The scientific understanding of vibrating strings was initiated by Galileo Galilei (1564-1642) and Marin Mersenne (1588-1648) (Fletcher & Rossing, 1998).

In the rebab, vibrations generated by the bow are conveyed to the resonance box and the rear of the instrument via a bridge. The broad plates of the body are often constructed from wood to generate frequency-induced oscillations in the vibrating bow. The instrument conveys the sound produced by the vibrating bow and wood into the air. The front and back plates sequentially expel air through the apertures in the front plate, facilitated by vibration (Berg & Strok, 1982). The materials chosen for each component of the rebab must be modified based on the material's flexibility and strength (Rai *et al.*, 2019).

RESULTS AND CONCLUSIONS

Sound is a basic mechanical phenomenon resulting from the vibrating of molecules within a medium (Alaskan, 2012; Alaskan, 2013). The instrument's strings vibrate due to the bow, and as the waves propagate along the string at a consistent speed, they experience a reduction in velocity upon reaching the threshold, subsequently transitioning to the membrane with a change in direction. Energy conversions within the membrane will likely occur concurrently.

Each leather kind possesses distinct strength and acoustic characteristics attributable to its unique collagen composition. Molecular characteristics, including collagen type and D-period structure, significantly influence sound transmission within the membrane or leather. If

the impediments posed by the partially crystallized hexagonal structure and the variable angular configurations along the fibril axis exceed the wavelength, it will result in wave refraction, altering the trajectory and direction of the sound wave. Density variations or humidity zones inside the structure induce wave breaking, resulting in the absorption and conversion of some sound energy into thermal energy. Additional losses will transpire in viscous areas.

The parameters of strength, tightness, thickness, and viscoelastic properties of the structure collectively influence the sound behavior and determine its acoustic characteristics. The diverse alignment of collagen bundles is a crucial aspect. The skin exhibits anisotropy due to the volumetric expansion of the animal, leading to inconsistent mechanical performance (Sizeland *et al.*, 2013). In Part I, we noted that the fibrous structure of aged animal skin strengthens and exhibits favorable properties. This condition may vary due to variances in internal structure and mechanical behavior tendencies. Regional heterogeneous anisotropy can result in both scenarios (Hiester & Sacks, 1997b). The angular ratio and orthotropic anisotropy of the pericardial tissues confer remarkable strength. Its stable structure is in harmonious alignment with the sound mechanics.

The vibrational characteristics within the instrument's resonance box may vary based on the dimensions and wavelengths of the apertures in the box. Concurrently, the membrane often decreases the modal frequency under air loading conditions.

The mechanical properties and density of the extracellular matrix, along with the presence of glycosaminoglycans, will influence minimal wave orientations. Hooke's Law was employed to elucidate the structure of the collagen pair (Atanackovic & Guran, 2000); analogous straightforward mathematical modeling techniques were devised (Berg & Stork, 1982). The hypothesis is as follows: The greatest number of oligomers in a glycosaminoglycan chain of maximum length can be identified, and the activity of this chain can be transmitted to the force between collagen fibrils, thereby preserving the stabilization of the collagen pair (Chan *et al.*, 2009).

Due to the significant variation in rawhide structure, the characteristics of different sections of the skin also differ markedly. This applies not only to physical properties but also to chemical properties. The investigation considered hide samples and adhered to the standards outlined in (EN ISO 3376:2008). Tensile strength, bulk density, scanning electron microscope examination, and acoustic signal analyses were conducted (Alaskan, 2012; Alaskan, 2013). The steps undertaken in this study may serve as a guide for informed academic action. To acquire empirical data, the propagation of sound waves under mechanical influences must be associated with the results of physical-chemical tests on leather, analyses of interior microscopic structure, and sound assessments, followed by statistical evaluation.

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MORPHOLOGICAL CHARACTERISTICS AND VOC CONTENT OF AGRICULTURAL SUBSTRATES

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The physical-chemical analysis of agricultural substrates is essential for optimizing fungal growth in the production of mycelium-based biomaterials. Such analyses study the metabolism efficiency of inoculated agricultural residues, enhancing fungal biomass production and the development of biomaterials used in industries like packaging, construction and even textile applications. The research paper investigated the physical morphology and Volatile Organic Compounds (VOCs) content of five agricultural substrates that will be used in future studies for manufacturing of microbial biomaterials, consisted of 2 sources of barley straw, 2 sources of wheat straw and one source of maize cobs. Microscopical investigations highlighted the specific substrates morphology and can also provide detailed insights into fungal hyphae interactions, substrate colonization, and degradation of key components like lignin and cellulose. VOC analysis highlighted the existence of hexanal compound in all samples. Hexanal in agricultural substrates acts as a volatile organic compound with antifungal properties, helping control fungal growth and post-harvest diseases. These studies help optimize conditions for fungal growth and ensure the structural properties of the final biomaterials. VOCs emitted during fungal cultivation play a significant role in substrate quality monitoring. This combined approach is especially useful in large-scale industrial applications, where efficiency, sustainability, and safety are paramount.

Keywords: biomaterials, fungi, agricultural waste

INTRODUCTION

The physical-chemical analysis of agricultural substrates for obtaining fungal biomaterials is crucial for ensuring optimal growth conditions and substrate performance in the cultivation of fungi. These analyses help to understand substrate properties and adjust them for efficient fungal metabolism, biomass production, and, ultimately, the formation of biomaterials (Yang *et al.*, 2021). By optimizing the physical and chemical properties of the substrate through the above analyses, fungi can efficiently metabolize the agricultural residues to produce mycelium-based biomaterials. These biomaterials have applications in: packaging materials, construction (e.g., mycelium bricks), textiles and leather alternatives, bio-composites etc. (Picco *et al.*, 2023). Microscopical investigation of agricultural substrates is an important step in the production of fungal biomaterials. It provides insights into the interaction between fungi and the substrate, helping optimize conditions for fungal growth and biomaterial formation. There are some key reasons why this investigation method is useful in the field of biomaterial: 1) Microscopic analysis allows for detailed observation of fungal hyphae (filamentous structures of the mycelium) as they colonize and degrade the substrate. It helps assess the extent of colonization, the direction of growth, and how well the fungi penetrate the substrate (Li, 2013). 2) Agricultural substrates, such as straw, wood chips, or husks, are typically composed of lignin, cellulose, and hemicellulose. Microscopic investigation can reveal how effectively the fungal enzymes are breaking down these components (Andlar *et al.*, 2018). 3) Fungi can modify the microstructure of the substrate as they grow. Microscopical techniques can reveal changes in porosity, pore size, and overall

structural arrangement as fungal colonization progresses. 4) The fungal cell wall is composed of chitin, glucans, and other polysaccharides that contribute to the mechanical properties of the fungal mycelium. Microscopic techniques like fluorescence microscopy can help visualize the accumulation of fungal biomass and the composition of cell walls (Sydor *et al.*, 2022). The analysis of volatile organic compounds (VOCs) in agricultural substrates plays a significant role in the production of fungal biomaterials. VOCs are small, volatile molecules that are emitted by both the substrate and the fungal cultures because of biological and chemical processes (Kaddes *et al.*, 2019). Monitoring and understanding these compounds provide insights into the substrate's quality, fungal growth, and overall biomaterial production process. Agricultural substrates used for fungal growth undergo microbial decomposition, leading to the release of various VOCs such as alcohols, aldehydes, esters, and acids. These VOCs are by-products of the breakdown of lignin, cellulose, hemicellulose, and other organic materials. Agricultural substrates can be prone to contamination by bacteria, molds, or other microorganisms that can compete with the fungi for resources. Different substrates emit varying VOCs as they decompose. By analyzing the VOCs from different agricultural residues (e.g., straw, corn stover, sawdust, or rice husk), it is possible to understand how suitable a substrate is for fungal growth (Ventura-Aguilar *et al.*, 2024). The VOC profile of fungal cultures can serve as a biomarker for the quality of the fungal biomaterial. Differences in VOC emissions can reflect variations in fungal growth rates, substrate degradation efficiency, or the presence of contaminants.

MATERIALS AND METHODS

Five types of agricultural substrates were subjected to both microscopy analysis and Volatile Organic Compounds (VOC) analysis: two types of barley straws (Laverda and Smarald varieties from Ilfov county), two types of wheat straws (Glosa C1 variety from Ilfov county and Anapurna C1 variety from Giurgiu County) and one type of maize cobs (Golden West GW 2122 (FAO 330) hybrid corn from Giurgiu County) (Fig. 1).



Figure 1. Photos of agricultural substrate samples

The agricultural samples were mechanically cleaned of impurities (by shaking), then manually cut in small pieces of 5-10cm, for better handling. The samples were analyzed as they were, without any other preparations, at room temperature (25°C +/- 2°C; RH between 40%-50%), without sterilization (to avoid influencing the VOC concentration in the samples).

Optical Microscopy

Microscopic analysis refers to optical, opto-electronic or electronic methods of structural analysis and surface microtopography. Morphological modifications of the morphological components of agricultural substrates as well as the interaction between biological and cellulosic and polymeric materials can be characterized by optical and electron microscopic methods, which can provide important structural information. An Olympus SZ61-TR stereomicroscope (Fig. 2.a), with an additional trinocular module (for mounting the

DSLR camera for image acquisition) and “Off the Bench” illuminator (Fig. 2.b), was used for the characterization of the substrates by optical microscopy, at 0.67x and 3x magnification, with a zoom ratio of 6.7:1 (range 0.67-4.5x).



Figure 2. SZ61-TR stereomicroscope

The surface morphology of the substrates was analyzed without prior preparation of the samples, and the visualization of the samples was done at 0.67x and 3x magnification using WHSZ10X-H/22 eyepieces. Image acquisition was done through a Canon EOS 1200D DSLR (18 megapixels, CMOS sensor, DIGIC image processor) and QuickPhoto Camera 3.1 image acquisition software.

Volatile Organic Compounds (VOC) Analysis

The analysis for the evaluation of volatile organic compounds was performed on the GC-MSD Agilent 6890N/ 5973 N Gas Chromatograph, headspace (HS) mode. The analysis was performed on Phenomenex Zebron™ ZB-5MSi capillary column, suitable for the analysis of pesticides, drugs, EPA methods, nitrosamines, phenols, with high selectivity, like that of 5% phenyl columns. The process parameters of the apparatus were as follows: HS parameters: vial temperature: 100°C; loop temperature: 120°C; transfer line temperature: 125°C. GC-MS parameters: capillary column: Phenomenex Zebron™ ZB-5MSi; length: 30 m; inner diameter 0.25 mm; layer thickness: 0.25 µm; injection system: splitless; injector temperature: 200°C; constant flow rate: 1 mL/min; carrier gas: helium; temperature program: 60°C to 280°C (5°C/min); injection volume: 1.0 µL; detector: MS, scan mode: 20-350 amu; auxiliary: 280°C.

RESULTS AND DISCUSSION

Microscopic analysis of the selected substrates allowed visualization of the surface morphology of each agricultural sample (Fig. 3-7).



0.67x (exterior)



3x (exterior detail)

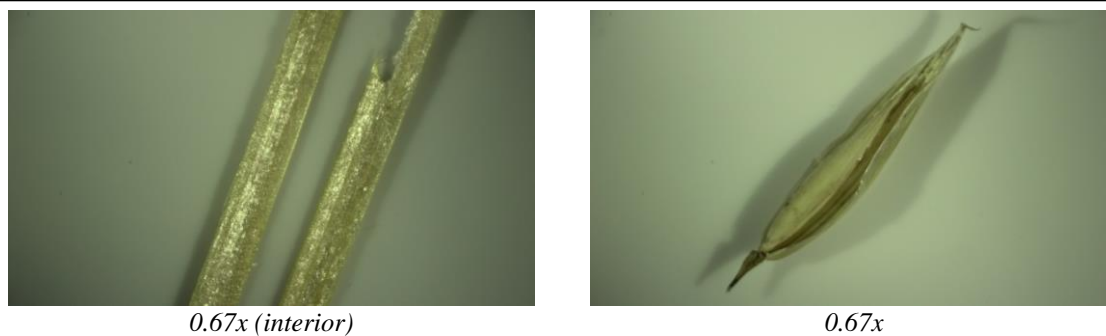


Figure 3. Optical microscopy barley straw (source 1)

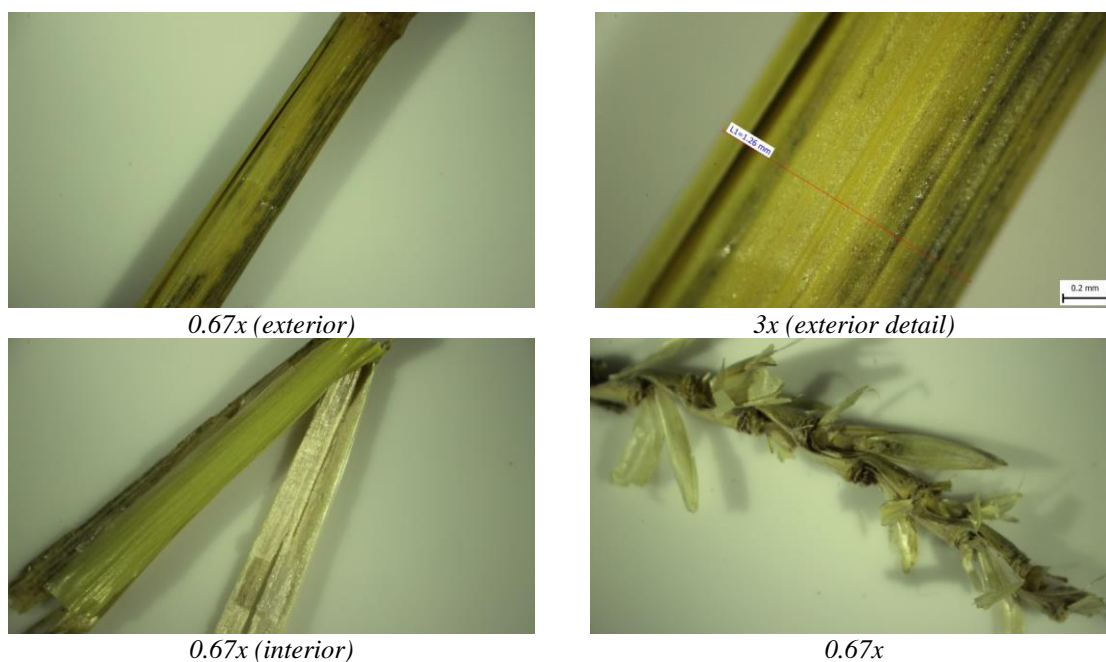


Figure 4. Optical microscopy barley straw (source 2)

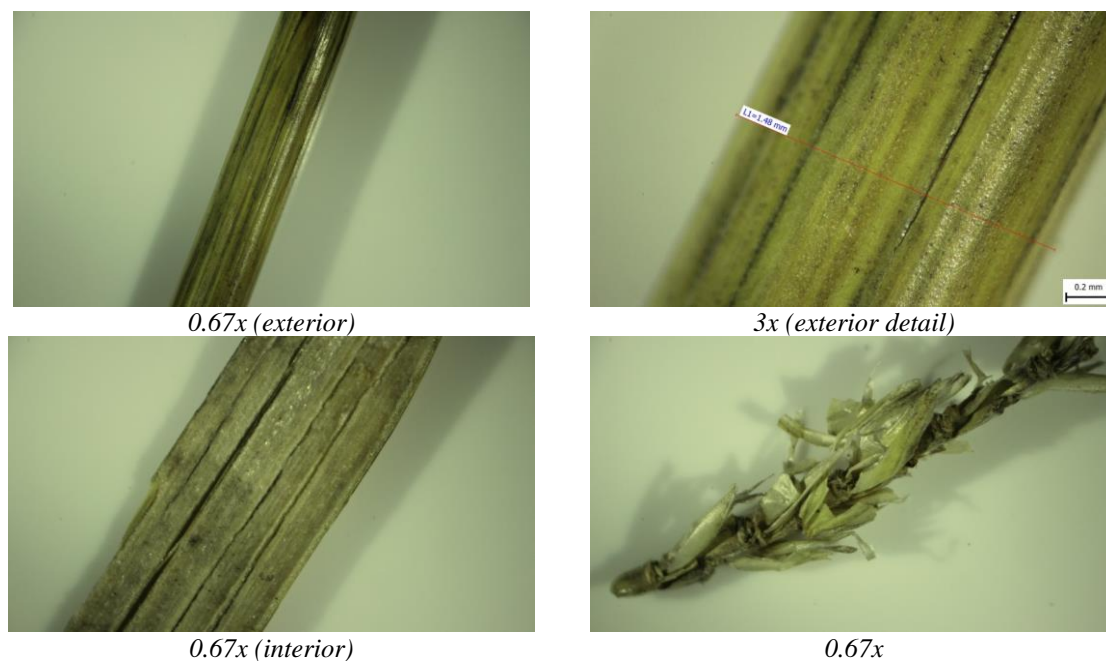


Figure 5. Optical microscopy wheat straw (source 1)

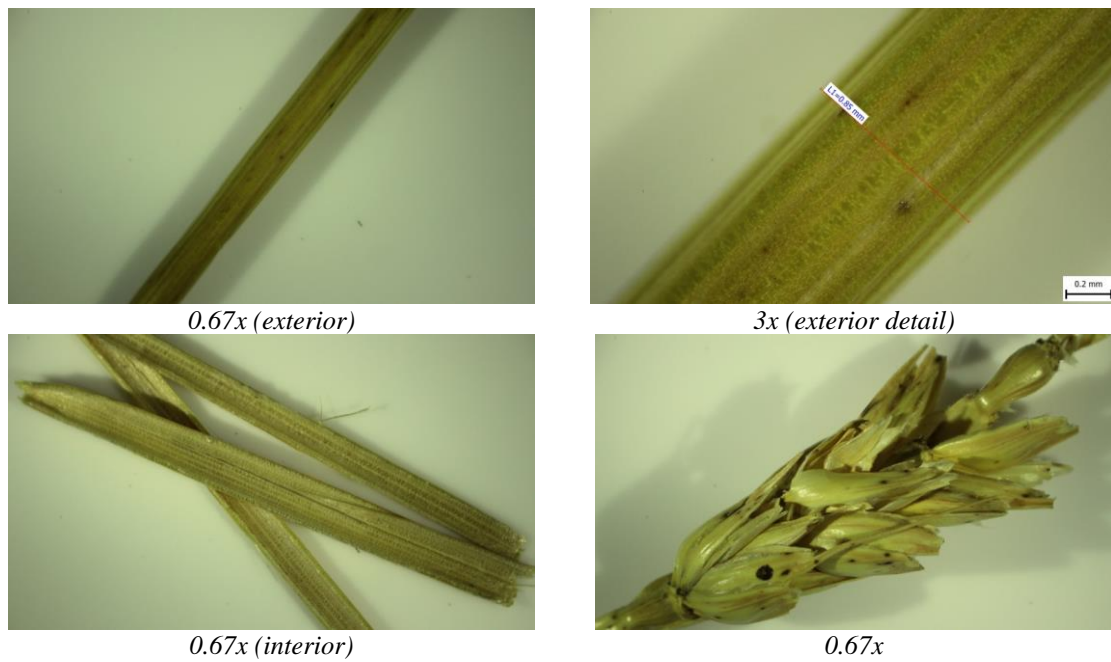


Figure 6. Optical microscopy wheat straw (source 2)

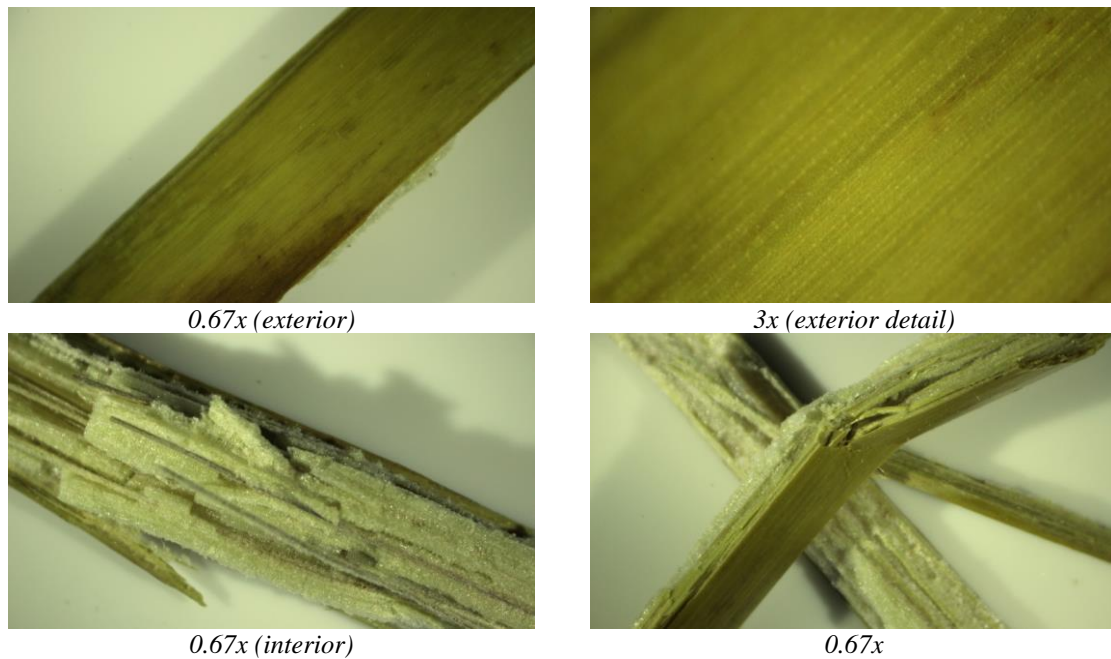


Figure 7. Optical microscopy maize cobs

The optical microscopy analyses performed on the five variants of agricultural substrates highlighted their specific surface morphology and can be used as a control tool for future experiments of inoculation of the substrates with macroscopical fungal strains. Optical microscopy shows a smooth exterior surface of the samples, which may indicate the need of a proper enzymatic digestion by the colonization strains. The substrates can also be pre-treated via physical chemical methods (treatment with Ca(OH)_2 , cold fermentation, pre-treatment with hot water etc.), in order to increase their bio-availability, thus shorting the period needed for optimal colonization of the substrate. Also, optical microscopy is a fast and accurate

method to check the development stage of the strain on the substrate, allowing to fine-tune the process of obtaining the final biomaterial demonstrator.

For future research, that will aim at using the tested substrates in obtaining microbial biomaterials, several key parameters must be considered. The substrate must be tailored to the specific type of biomaterial being produced, but the following general parameters can help ensure high productivity and efficiency: organic matter (ideal range: 40-60% dry matter); C/N ratio (ideal range: 20:1 to 30:1); acidity/alkalinity (ideal range: pH 6.0 – 7.5); moisture content (ideal range: 50-70%); water holding capacity (ideal range: high water retention with rapid drainage); nutrient availability (good traces of nitrogen, phosphorus, potassium, calcium, magnesium etc.); contaminant free (preferably, absence of heavy metals, pesticides, herbicides, pathogens – which can be removed by thermal sterilization of the substrate); thermal stability (ideal range: resistance to extreme temperature fluctuations). Another key factor is the texture and particle size, which has the purpose to support fungal growth by providing adequate surface area for nutrient exchange while maintaining good aeration and water retention.

Volatile organic compounds are a broad category of organic chemicals that have a high volatility, which means that they evaporate easily at room temperature. These compounds contain carbon and hydrogen atoms and may also include other atoms such as oxygen, nitrogen, sulphur and halogens (such as chlorine, fluorine or bromine). Among the most common examples of VOCs are well-known substances such as benzene, formaldehyde, toluene, acetone, ethanol, hexane etc. The overlapped chromatograms of the five agricultural biomass samples are showcased in Figure 8.

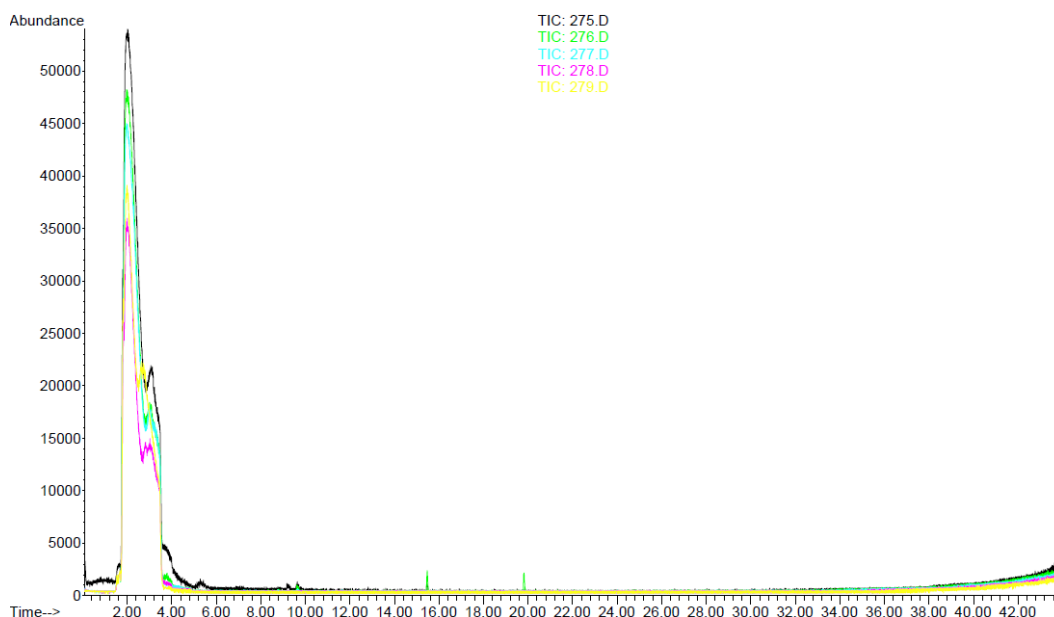


Figure 8. 275.D-barley straw #1; 276.D-barley straw #2; 277.D-wheat straw #1; 278.D-wheat straw #2; 279.D-maize cobs

The hexanal compound (Figure 9) was found in all samples, at retention time 3.80 min. It is an alkyl aldehyde, also called hexanaldehyde or caproaldehyde and is a volatile organic compound that imparts an odor similar to freshly cut grass as *cis*-3-hexenal (Gardini *et al.*, 1997).

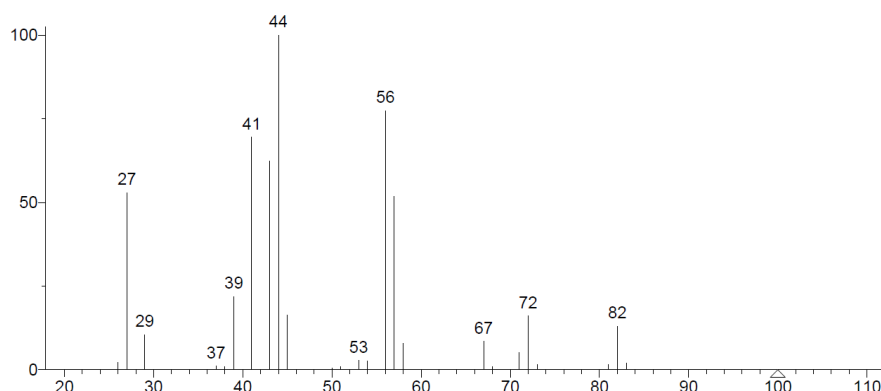


Figure 9. Hexanal mass spectra

In the literature, hexanal is described as a volatile organic compound with antifungal properties, being responsible for the reduction of post-harvest diseases (Song *et al.*, 1996). The effect of hexanal on the germination of spores of *Botrytis cinerea*, *Monilinia fructicola*, *Penicillium expansum*, and on the mycelial growth of *Sclerotinia sclerotiorum*, *Alternaria alternata* and *Colletotrichum gloeosporioides* has been studied (Song *et al.*, 2007). The effectiveness of hexanal in control methods is dependent on its concentration, the duration of treatment and the sensitivity of the fungal strain to hexanal vapors. Hexanal, an organic volatile agent naturally produced by plants, is commonly used as a food flavoring, and is generally known to be a safe compound. However, hexanal content does not pose an inhibitory threat to the colonization process, since the compound may be efficient against normal concentration airborne pathogens, when compared against the higher microbial concentration used in the manufacturing of biomaterials, in microbial inoculation phases. Also, one of the main steps in producing biomaterials is heat sterilization of the substrate, which helps with volatilization of parts of the compound. In previous research, the authors of the papers also obtained several prototypes based on barley, wheat and cobs substrates, using various fungal strains, and hexanal content did not limit the colonization process.

CONCLUSIONS

Microscopical investigation is a vital tool in understanding and optimizing the production of fungal biomaterials from agricultural substrates. It allows researchers and producers to monitor fungal growth, substrate degradation, hyphal interactions, and microbial contamination. By providing insights into the micro-level processes occurring during fungal cultivation, microscopical analysis enables the refinement of substrates and growth conditions, leading to high-quality, consistent biomaterials for industrial applications. VOCs play a critical role in the monitoring, optimization, and quality control of agricultural substrates used for fungal biomaterial production. Through VOC analysis, researchers and producers can ensure that the substrate is properly degraded, the fungal growth is healthy, contamination is controlled, and the final biomaterial meets the desired standards. This analysis provides a valuable tool for enhancing the efficiency and sustainability of the fungal biomaterial production process, particularly in large-scale or industrial applications (Attias *et al.*, 2020). Volatile organic compounds can have adverse effects on human health, such as irritation of the eyes, nose and throat, headaches, dizziness and in severe cases, can cause damage to the liver, kidneys or central nervous system. Some VOCs are also known carcinogens. This is why it is important to determine these compounds in the selected

agricultural substrates, as they are destined for composite biomaterials that must have the highest degree of biocompatibility.

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THE IMPACT OF LASER FINISHING ON LEATHER PROPERTIES

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According to the results of the study of the influence of laser engraving on the hygienic properties of natural leather for shoe uppers, the nature of the change in the micro- and macrostructure of the dermis under the action of laser engraving and the permissible limit values of the depth of laser ablation, which do not impair their hygienic properties, were determined. With an increase in the ablation depth to 0.7 mm (50% of the total thickness of the sample), the relative vapour permeability increases in all samples of the studied skins. At the same time, the relative vapour permeability for the skin of Crust increased by 5%, Flotar by 13.5%, and Nappa by 9.5%. On the front surface, the structure features are revealed, which are characteristic only for the area of the direct action of the laser beam; namely, the samples of Flotar and Nappa skins have clear signs of welding collagen fibres. In the zone of laser ablation, an increase in interstructural distances between bundles of collagen fibres was also found, resulting from the thermophysical processes of laser radiation's action.

Keywords: laser finishing, leather products, properties of leather.

INTRODUCTION

The hygienic properties of shoes are mainly determined by the properties of the materials from which they are made and their construction. To ensure comfortable operating conditions, materials for the top of shoes must have vapour permeability and the ability to absorb water vapour to provide the necessary microclimate in the inner space of the shoe. If the removal of steam in shoes is difficult due to low vapour permeability and moisture removal, then in the process of wearing, when the foot rubs, micro-areas of increased temperature are created and, as a result, unpleasant sensations of overheating and burning of the foot. The authors believe that improving the hygienic properties of shoes can be realised by decorating the upper leather parts with laser engraving.

Finishing leather products with laser engraving requires compliance with rational processing parameters, taking into account the individual characteristics of the leather, namely its raw material origin, tanning method, degree of treatment of the front surface, etc. The laser beam can heat the product's irradiated area to high temperatures in a very short time, during which the heat does not dissipate, and the treated area may be softened, recrystallised, melted, or even partially evaporated. The destructive nature of the process directly affects the facial surface of the skin and its structure, creating a unique three-dimensional pattern, the meaning of which is based on the visual effect and aesthetic features of the product.

The results of previous studies of the impact of laser processing on physical and mechanical properties (Pervaia *et al.*, 2022) became the basis for determining the rational technological parameters of laser engraving and its impact on the hygienic properties of natural leather for footwear.

MATERIALS

The different samples of chrome-tanned leather from raw cattle (Cattle) used for manufacturing shoes and leather goods, namely Krust, Flotar and Nappa, were taken to research the laser effect. Crust leather was produced using a new unified resource-saving technology using polymer compounds at the stage of tanning and liquid finishing (Andreyeva *et al.*, 2019).

Leather samples with a 1.40-1.45 mm thickness were subjected to laser engraving at a laser ablation depth from 0.1 mm to 0.7 mm in steps of 0.1 mm.

METHODS

The effect of laser radiation on the microstructure of dermal collagen was examined using scanning electron microscopy (SEM) on a Tescan Mira 3 LMU (Czech Republic). For more complete information, a first layer of conductive material in the form of platinum was sprayed on the front surface of the leather sample after laser finishing, which covered the surface of the sample and the area of the direct action of the laser beam (Krishtal *et al.*, 2009).

The leather finishing was performed on a CO₂ Comelz CZ/M (Italy) laser machine.

The study of the hygienic properties of Crust, Flotar, and Nappa leathers was carried out by ISO 14268:2008 by determining the vapour permeability, which characterises the ability of the leather to pass water vapour. Previously, engraved elements were applied to the working areas of the samples, making up 1%, 25%, and 50% of the working area of the tested sample, with different depths of laser ablation from 0.1 mm to 0.7 mm in steps of 0.1 mm (Fig. 1).

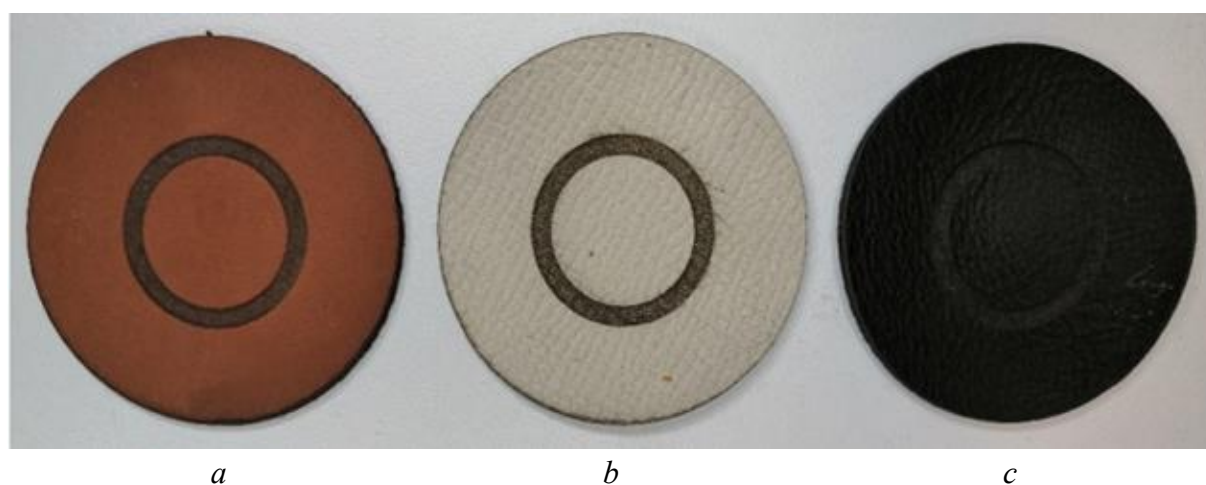


Figure 1. Leather samples for testing: a – Crust; b – Flotar; c – Nappa

Table 1 shows the technological parameters of laser engraving, in which the engraving of the studied samples of Crust, Flotar and Nappa leathers was performed.

Table 1. Technological parameters of laser engraving of leather for shoe uppers and leather goods

Leather type	Depth of ablation, mm	Beam power, W	Speed of the laser head, mm/s
Crust	0.1	11	300-350
	0.4	17	
	0.7	28	
Flotar	0.1	11	270-300
	0.4	17-19	
	0.7	30	
Nappa	0.1	11	270-300
	0.4	15-17	
	0.7	28	

RESULTS

After laser treatment, all types of leather retained their natural histological structure, collagen bundles were not deformed, and the layers were evenly spaced without increasing the density of the structure. The microphotographs of the sections revealed features that are typical only for the area of direct laser beam exposure, namely, the samples of Flotar and Nappa leathers show clear signs of collagen fibre welding. In contrast, the sample's surface does not have a layer of residual coating film or other chemical components/fillers. The removed layer of leather is converted to amorphous carbon and removed from the surface. Further analysis of microphotographs revealed an increase in the separation of collagen fibre bundles in the laser ablation zone (on the surface).

The results of electron microscopy of leather samples (Fig. 2) indicate that laser treatment has no negative effect on the dermis structure and opens the capillary-porous structure of the leather, thereby increasing its vapour permeability.

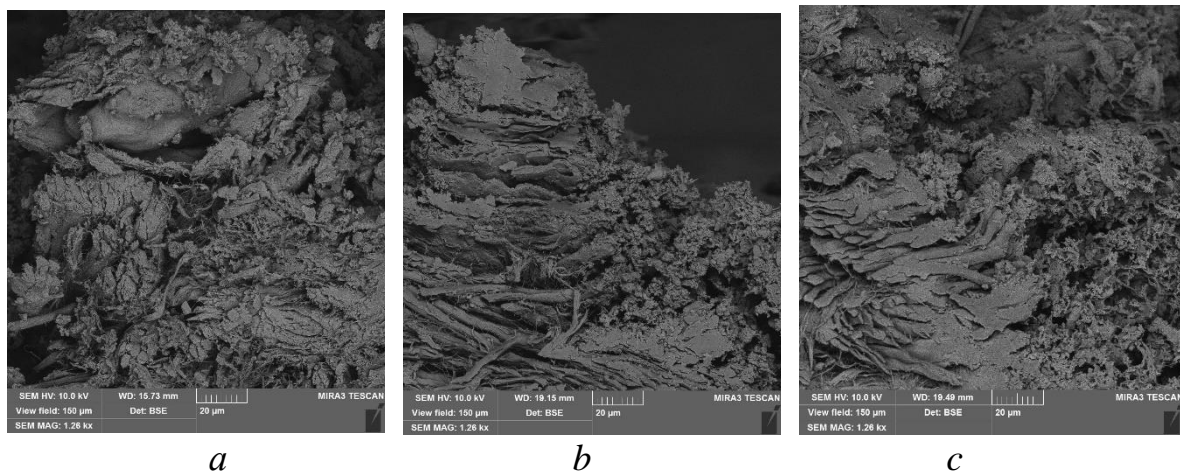


Figure 2. Electron microscopic images of cross-sections of leather samples in the ablation zone: a – Crust leather; b – Flotar leather; c – Nappa leather

The results of electron microscopy show that the structure of the dermis under laser radiation has not undergone morphological changes, which confirms the correct choice of technological parameters for the laser engraving operation. All types of leather have retained their natural histological structure, collagen bundles are not deformed, and the layers are evenly

spaced without increasing the density of the structure. Microphotographs (top view) of the sections revealed features that are characteristic only of the area of direct laser beam exposure, namely, samples of Flotar and Nappa leathers have obvious signs of welding of collagen fibres, while the surface of the sample does not have a layer of residues of the covering film or other chemical components/fillers of the leather (Fig. 3 b, c).

The leather layer removed by the laser beam is converted into amorphous carbon and removed from the surface. Further analysis of microphotographs revealed an increase in the separation of collagen fibre bundles in the laser ablation zone (on the surface).

The results of electron microscopy of skin samples indicate that laser treatment has no negative effect on the dermis structure and opens the capillary-porous structure of the skin, thereby increasing its vapour permeability (Fig. 4).

The study of the hygienic properties of Crust, Flotar, and Nappa leathers was carried out by ISO 14268:2008 by determining the vapour permeability, which characterises the ability of the leather to pass water vapour.

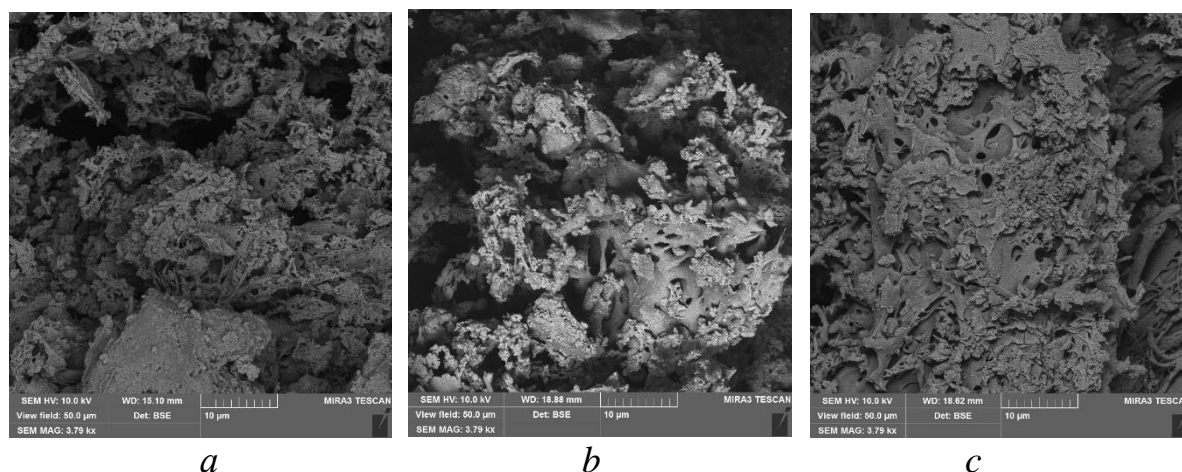


Figure 3. Electron microscopic image of the top view of the surface layer of leather samples in the laser ablation zone: a – Crust; b – Flotar; c – Nappa

The constructed diagrams demonstrate (Fig. 3) that with an increase in the ablation depth up to 0.7 mm (50% of the total thickness of the sample), the relative vapour permeability increases in all samples of the studied leathers. Compared to the control samples, where there is no ablation, the relative vapour permeability for the skin of Crast increased by 5%, Flotar by 13.5%, and by 9.5% for Nappa. When the ablation area is increased to 50% of the working area of the samples, there is a sharp increase in the relative vapour permeability of Flotar leather by 25% compared to the control sample. Correspondingly, the indicator's increase is within 8.5% for Crust's leather and for Nappa – 11.6%. Thus, the dependence of vapour permeability on the depth and area of laser ablation has been proven, and the influence on the hygienic properties of the leather decorated with laser engraving has been established.

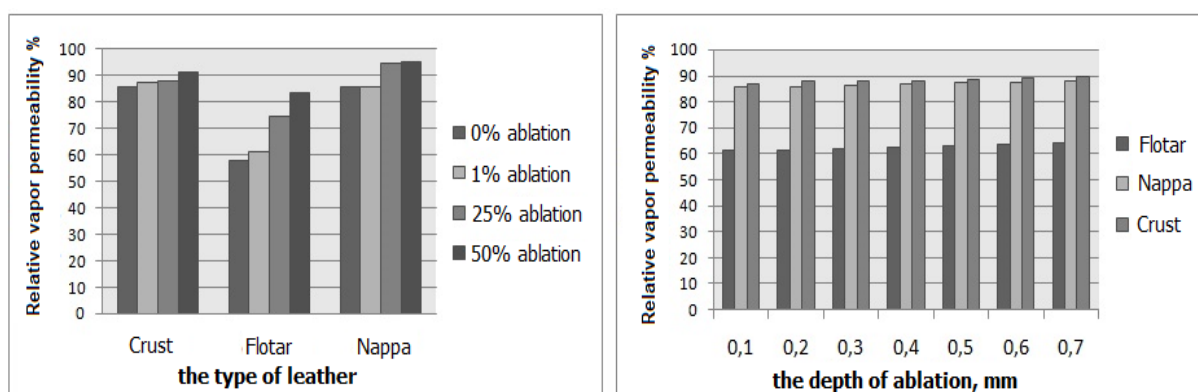


Figure 4. Dependence of vapour permeability on the area and depth of ablation

Thus, it is proved that the increase in vapour permeability is directly proportional to the increase in the depth and area of laser ablation, which significantly affects the hygienic properties of leather products, especially footwear.

CONCLUSION

It has been proven that the increase in vapour permeability is directly proportional to the increase in the depth and area of laser ablation. This significantly affects the hygienic properties of leather products, especially footwear.

It was established that the state of the structure of the skin dermis under the influence of laser radiation does not undergo fundamental morphological changes in structure, and the laser engraving process itself does not hurt its macrostructure. The changes observed in the ablation zone, namely in the Flotar and Nappa skin samples, indicate clear signs of welding of collagen fibres.

It has been proven that increasing the ablation depth to 50–52% of the sample thickness and the ablation area to 50% of the total area significantly increases its vapour permeability in all leather samples, which occurs due to the opening of the capillary-porous structure of the leather. Compared to the control samples, where there is no ablation, the relative vapour permeability for Crust increased by 8.5%, Flotar by 25%, and Nappa by 11.6%.

The depth of laser ablation, equal to 25–30% of the leather thickness, and the area of engraved elements less than 50% of the total area of the part are rational technological parameters for laser treatment of leather, which will ensure good hygienic properties and will not impair the operational characteristics of the products.

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MODIFICATION OF CHEMICAL FIBERS WITH PLANT POLYPHENOLS TO IMPROVE THEIR SORPTION PROPERTIES

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The purpose of the research is to create new combined composite fibrous materials for the sorption of heavy metal ions from waste water of industrial enterprises. To study the sorption capacity of the sorbent, the content of Fe^{3+} in model solutions was determined by analytical methods before and after sorption, the maximum recovery ratio for Fe^{3+} was calculated, %; chemical fibers were studied before and after treatment with tara/quebracho tannins and iron (III) compounds to determine the mechanism of interaction with plant polyphenols by the FTIR spectroscopy. It was established that interaction of Fe^{3+} with chemical fibers treated by polyphenols probably occurs as a result of the formation of coordination bonds between neighboring hydroxyl groups of the aromatic ring one molecule and the C=O group of the unsaturated aromatic ring of another polyphenolic molecule or due to the formation of chemical bonds with CO groups of modified chemical fibers. Sorption of Fe^{3+} ions after treatment of the fibrous sorbent with tara tannins at a temperature of 40 °C is higher than after treatment with quebracho tannins under similar conditions. Treatment during the first four hours is most effective. At the same time, the maximum recovery ratio for Fe^{3+} reaches 90%. The advantages of the obtained sorbent include high sorption activity and the ability for further modification, the methods of preparation are quite simple and cheap, and the possibility of producing the sorbent from secondary raw materials helps to solve the problem of utilization of fibrous waste.

Keywords: sorption properties, chemical fibers, plant polyphenols

INTRODUCTION

Waste water from textile or leather industry enterprises, electroplating shops is mostly contaminated with salts of copper, chromium, iron, nickel and other metals. The sorption extraction of metals from wastewater has become quite widespread due to its high efficiency and the absence of secondary pollution. The group of synthetic polymer sorbents includes polypropylene, polyethylene, polyacrylamide (Manju *et al.*, 2002), polystyrene, and polyurethane, which are used to manufacture special sleeves, mats, and pillows for sorption of hazardous liquids. Polymer adsorbents have hydrophobic properties, low volume density; sorption capacity can exceed 100 g/g (Li *et al.*, 2012; Matveeva *et al.*, 2012). Due to their low density and hydrophobicity, they are mainly used in water environments. Mineral adsorbents have such advantages as non-flammability, chemical inertness, relatively low cost and availability. Most mineral adsorbents are used in the form of powder or granules. Mineral sorbents are effective in eliminating oil spills (Bandura *et al.*, 2017). Organic adsorbents include peat, moss, dry leaves, straw, sawdust, bark and waste from the processing of cellulosic raw materials (Yu *et al.*, 2001; Palma *et al.*, 2003; Wahi *et al.*, 2013; Hoang *et al.*, 2018). Natural organic sorbents are inexpensive, available, environmentally friendly, but their low bulk density limits their use in the aquatic environment.

Fibrous sorption-active materials are increasingly used due to their characteristics, in particular, compared to granular sorbents, they have higher chemical and thermal stability, a homogeneous porous structure and a large active surface area, a significant volume of

micropores and a high mass transfer coefficient, and installations, in which fibrous materials are used, occupy a much smaller area (Adam *et al.*, 2019). In addition, the possibility of production of fibrous sorbents from secondary raw materials allows solving the problem of utilization of leather, textile and polymer production waste.

The authors of the article developed a method of obtaining an environmentally safe polymer composite material with sorption properties from fibrous waste of the textile industry based on high-volume combined loop threads consisting of two components (Fig. 1a). Fibrous textile wastes containing 70% polyurethane fibers 162C (linear density 4.4 tex) and 30% polyamide fibers 6.6 f20/1 (linear density 3.3 tex) were used to obtain a polymeric composite material with sorption properties (Tarasenko *et al.*, 2019). Polyurethane fibers are similar in their chemical properties to polyamides, as they equally contain amide groups --NH--CO-- , which participate in the formation of hydrogen bonds. However, the additional oxygen atom included in the polyurethane chain --NH--CO--O-- gives it greater flexibility, so polyurethanes have a lower melting point compared to polyamides of a similar structure ($\sim 178^\circ\text{C}$ for polyurethane versus 255°C for polyamide 6.6).

Bicomponent adhesive fibers (BAF) Acebon 4/51 black, 0.44 tex (20 mass. %) were added to the original composition. Acebon 4/51 black are adhesive fibers of the “core-sheath” type, which consist of polyethylene terephthalate (core, T melting point = 265°C) and PET copolymer (the upper low-melting component T melting point = 110°C). Fig. 1b shows a microphotograph of the surface of the non-woven material in reflected light after the thermobonding process. It is noticeable that the heat treatment of the non-woven fabric leads to the melting of the surface layer of bicomponent fibers. At the same time, cohesive bonds are formed at the intersections of such fibers, marked by red arrows on the photomicrograph. At the same time, it is clearly visible that in the places of contact of bicomponent fibers with the main fibrous material (PU/PA-6,6), the formation of adhesive bonds between them takes place (indicated in the photomicrograph by green arrows).

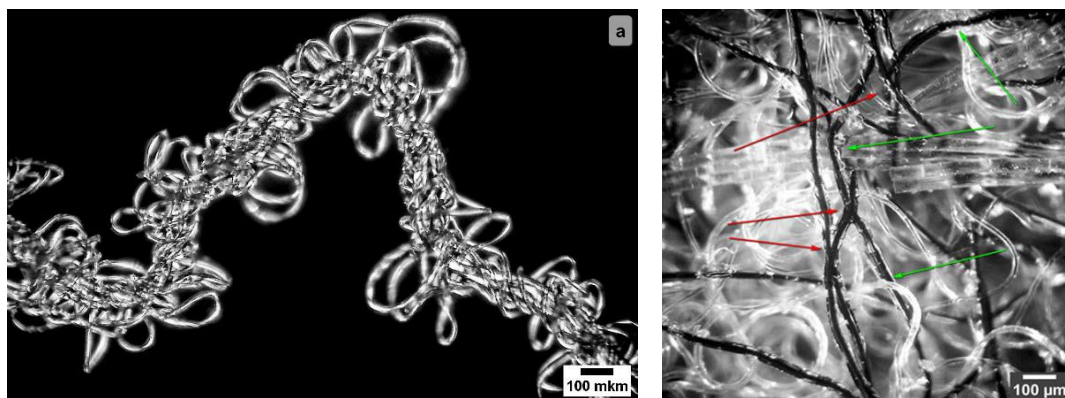


Figure 1. Microphotographs of the fibrous textile wastes: a) high-volume combined loop threads consisting of two components; b) the surface of the non-woven material in reflected light after the thermobonding process

It is of interest to determine an effective method of modifying the natural and synthetic fibers to increase the activity of their functional groups, in particular by the method of controlled chemical destruction, taking into account that the effectiveness of the sorbent depends on the presence of active functional groups that are capable of irreversibly binding heavy metal ions. Chemical destruction of both polyurethanes and polyamides can occur, in particular, under the influence of organic solvents alcohols, acids, alkalis, phenols and their derivatives, and is accompanied by the cleavage of C-N bonds (Nelson, 1976). As a result of

this rupture, two macromolecules containing an amino group and a carboxyl group are formed (Fig. 2).

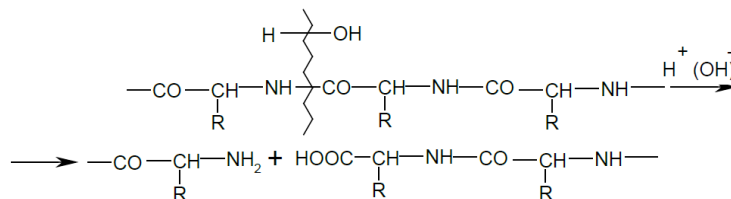


Figure 2. Chemical destruction of both polyurethanes and polyamides

The purpose of the research is to obtain new ecologically safe combined composite fibrous materials based on polyurethane-polyamide chemical fiber waste and to study their sorption properties for water purification from Fe^{3+} ions.

EXPERIMENTAL SECTION

To increase the sorption capacity of fibrous sorbents to iron compounds, the initial fibers were pre-treated with extracts of quebracho (KB) and tara (Tara) tannins, which belong to different classes and differ in the nature of the functional groups of polyphenols in the structure of tannins (Faber, 1990). Tara tannins are obtained from pods of small trees of *Caesalpinia spinosa* or *Caesalpinia tinctoria* species (Bertnet, 2001). Tara tannins belong to the group of pyrogallols, that is, they are hydrolyzed with the formation of a mixture of gallic and ellagic acids (Fig. 3) and have carboxyl and hydroxyl groups in the structure of polyphenols (Vitolo *et al.*, 2003).

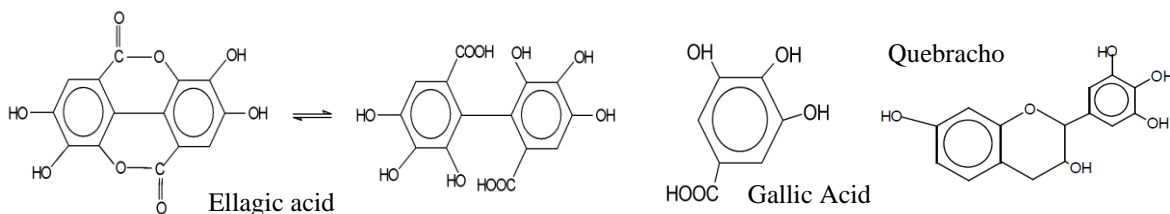


Figure 3. Polyphenols of quebracho and tara tanids

Quebracho is the general name of various tree species (*Quebrachia lorentzii* syn. *Schinopsis balansae*) of the families *Anacardiaceae* and *Apocunacea*. Quebracho tannins belong to the pyrocatechin group. Quebracho tannins have only hydroxyl groups (Fig. 3) in the structure of polyphenols (Covington and Shi, 1998), and therefore the process of sorption of heavy metals will occur differently.

Pretreatment of Fibrous Sorbents with Plant Extracts

Quebracho and Tara tannins solutions with a concentration of 10 g/l of tannins were used for fiber pretreatment. To prepare a solution of quebracho tannins, a portion of dry extract of quebracho was weighed on an analytical balance (it was taken into account that the content of tannins in the dry extract is 68%), placed in a heat-resistant round-bottomed flask with a volume of 500 ml, 200-250 ml of warm water was added there, the solution was adjusted to boiling to completely dissolve the quebracho extract, then the solution was cooled and quantitatively transferred to a 1000 ml volumetric flask and made up to the mark with distilled water. Similarly, a solution of tara tannins was prepared (the content of tara tannins in the dry extract was 38%).

Fibers were pre-treated for 4 hours at a temperature of 40 °C. The volume of the tannin solution for treating the fibrous sorbent was 10 times greater than the mass of the fibrous material. At the end of the treatment, the tannin-filled fibrous material was wrung out, dried, and later used to determine its sorption efficiency to Fe^{3+} ions (Table 1). A solution of ferric ammonium alum $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 10\text{H}_2\text{O}$ with a concentration of Fe^{3+} ions ~10 g/l was used as a model of Fe-containing wastewater. Treatment was carried out at temperatures of 20 and 40 °C, the concentration of Fe compounds in the spent solution was determined after 1, 2, 4 and 24 hours of treatment (Table 1) by the titrimetric method in the presence of sulfosalicylic acid (State Standard of Ukraine 7262:2012). The maximum recovery ratio (RR_{max}) for Fe^{3+} was calculated according to the formula:

$$\text{RR}_{\text{max}} = \frac{C_{\text{Fe}^{3+} \text{ initial}} - C_{\text{Fe}^{3+} \text{ finish}}}{C_{\text{Fe}^{3+} \text{ initial}}} \cdot 100\% \quad (1)$$

Table 1. Parameters of treatment of fibrous material with Fe^{3+} compounds

Duration of the fibrous material treatment in Fe^{3+} solution (h) after treatment with tannins	t, °C	A type of tannins for pre-treatment of fibrous material	Content of Fe^{3+} in the initial/spent solution, g/l	The maximum recovery ratio for Fe^{3+} , %
0	20	–	9,97	–
0,5	20	Quebracho	3,89	61,0
1,0	20	Quebracho	3,72	62,7
2,0	20	Quebracho	3,85	61,5
4,0	20	Quebracho	3,80	61,9
24,0	20	Quebracho	3,53	64,6
0	40	–	9,99	–
0,5	40	Quebracho	4,89	51,0
1,0	40	Quebracho	4,76	52,4
2,0	40	Quebracho	3,21	67,8
24,0	40	Quebracho	3,14	68,5
0	40	–	9,97	–
0,5	20	Tara	3,95	60,4
1,0	20	Tara	3,64	63,6
2,0	20	Tara	1,93	80,6
4,0	20	Tara	1,53	84,6
24,0	20	Tara	2,53	75,6
0	40	–	9,91	–
0,5	40	Tara	1,14	88,5
1,0	40	Tara	1,08	89,1
2,0	40	Tara	1,06	89,3
4,0	40	Tara	0,96	90,3
24,0	40	Tara	1,14	88,5

It was determined that the sorption of Fe^{3+} ions after treatment of the fibrous sorbent with tara tannins at a temperature of 40 °C is higher than after treatment with quebracho tannins under similar conditions (Table 1). Treatment during the first four hours is most effective. Then the treatment efficiency decreases significantly, and after a day the reverse desorption process begins.

The interaction features of Fe^{3+} compounds with fibrous sorbent were determined by the method of IR spectroscopy. IR spectroscopic studies were performed on a universal Fourier–IR spectrometer TENSOR-37 (BRUKER, Germany). The test samples were thoroughly crushed and pressed into tablets with KBr. IR absorption spectra were studied in the frequency range of 400...3800 cm^{-1} . The infrared spectra of the studied samples were characterized using the following indicators: A – Gaussian peak area, relative units (r.u.); W – peak half-width, r.u.; ν –

wave number, cm^{-1} (maximum peak). IR spectra were interpreted using tables of characteristic frequencies. The results of IR spectroscopic studies are shown in Tables 2-3, Fig. 4.

Noticeable peaks at 3430, 2920, 2850 and 1640 cm^{-1} observed for all fiber samples are considered by the authors (Huang *et al.*, 2019) to be characteristic absorption peaks for polyamide PA 6 (Tables 2-3). The authors (Liang *et al.*, 2020) explain the strong absorption peak with a maximum at 3430 cm^{-1} by valence asymmetric vibrations of the N-H group of amides. A strong absorption peak at 1640 cm^{-1} is associated with bending vibrations of amide and water (Yu *et al.*, 2019). Symmetrical and asymmetric stretching of $-\text{NH}_2$ in both bands shifted to a low-frequency region from the position of the free amino group (3500 and 3400 cm^{-1}), which makes it possible to conclude that the $-\text{NH}_2$ groups are involved in the formation of a hydrogen bonds due to the influence of OH groups of polyphenols.

Table 2. Results of IR spectroscopic studies of the fibrous materials samples treated with quebracho polyphenols

Functional groups	Samples								
	A, r.u.	PU-PA v, cm^{-1}	W, r.u.	A, r.u.	PU-PA+KB v, cm^{-1}	W, r.u.	A, r.u.	PU-PA +KB+Fe v, cm^{-1}	W, r.u.
$\delta\text{N}-\text{H}$ (Longitudinal direction, wagging and twisting vibrations at $\text{R}-\text{NH}_2$ $\text{Ar}-\text{NH}_2$)	141	687	190	24	697	66	3	683	23
$\nu\text{C}-\text{O}$	16	1072	59	30	1062	75	1	1073	14
$\delta_{\text{as}}\text{N}-\text{H}$ (Latitudinal direction, scissoring vibrations at $\text{R}-\text{NH}_2$ $\text{Ar}-\text{NH}_2$) amide II	119	1544	103	114	1532	90	111	1531	86
$\nu_{\text{s}}\text{C}=\text{O}$ (amide I)	14	1739	56	120	1675	133	133	1670	135
$\nu_{\text{s}}\text{N}-\text{H}$ ($\text{R}-\text{NH}_2$)	69	3056	121	42	3069	87	54	3069	97
$\nu_{\text{as}}\text{N}-\text{H}$ ($\text{R}-\text{NH}_2$)	344	3307	274	230	3303	220	236	3301	214

Table 3. Results of FTIR spectroscopic studies of the fibrous materials samples treated with tara polyphenols

Functional groups	Samples								
	A, r.u.	PU-PA v, cm^{-1}	W, r.u.	A, r.u.	PU-PA+tara v, cm^{-1}	W, r.u.	A, r.u.	PU-PA +tara+Fe v, cm^{-1}	W, r.u.
$\delta\text{N}-\text{H}$ (Longitudinal direction, wagging and twisting vibrations at $\text{R}-\text{NH}_2$ $\text{Ar}-\text{NH}_2$)	141	687	190	66	704	92	35	700	74
$\nu\text{C}-\text{O}$	16	1072	59	50	1058	97	28	1055	76
$\delta_{\text{as}}\text{N}-\text{H}$ (Latitudinal direction, scissoring vibrations at $\text{R}-\text{NH}_2$ $\text{Ar}-\text{NH}_2$) amide II	119	1544	103	132	1539	87	121	1533	93
$\nu_{\text{s}}\text{C}=\text{O}$ (amide I)	14	1739	56	33	1733	67	88	1675	132
$\nu_{\text{s}}\text{N}-\text{H}$ ($\text{R}-\text{NH}_2$)	69	3056	121	53	3067	96	55	3069	99
$\nu_{\text{as}}\text{N}-\text{H}$ ($\text{R}-\text{NH}_2$)	344	3307	274	253	3302	220	219	3298	199

In the FTIR spectra of polymeric materials treated with quebracho and tara polyphenols and Fe(III) compounds, a change in the shape, height, and half-width of a rather broad absorption band in the region of 3200-3600 cm^{-1} is observed. In this interval, there is an overlap of absorption bands with a frequency of 3300-3500 cm^{-1} , which correspond to N-H bonds of amino groups; 3200-3400 cm^{-1} , which correspond to intra- and intermolecular hydrogen bonds

of phenolic compounds and the absorption band of the bound OH group in the region of 3200-3300 cm^{-1} . This confirms the interaction of polyphenols with polyamide-polyurethane fibers and Fe(III) compounds due to hydrogen bonds with the participation of phenolic hydroxyl groups and amino groups of polymeric materials.

Attention is drawn to changes in the low-frequency region of the spectrum (Tables 2-3). The area of the peak, which belongs to $\delta(\text{N-H})$ with a maximum of 687 cm^{-1} gradually decreases: 141 r.u. for starting polymer material, 66 r.u. after processing the polymer material with tara polyphenols, 35 r.u. after treatment with Fe^{+3} compounds. This is accompanied by a decrease in the half-width of the peak from 190 to 92 and 74 r.u., respectively. A similar situation is observed after processing the polymer fiber with Quebracho tannins. 190, 66, 23 r.u. – the half-width decreases; 141, 24, 3 r.u. – the area of the Gaussian peak decreases.

There is a shift of the frequency corresponding to $\nu \text{C=O}$ vibrations to the low-frequency region (1739, 1675, 1670 cm^{-1} after processing the polymer material with quebracho polyphenols and 1739, 1733, 1675 cm^{-1} after processing the polymer material with tara polyphenols). The shift of the absorption peak of the C=O carbonyl group to the low-frequency region indicates the presence of aromatic elements in the system. This is accompanied by increase in the half-width of the band (56 r.u. for starting polymer material, 67 r.u. after processing the polymer material with tara polyphenols, 132 r.u. after treatment with Fe(III) compounds; 133 r.u. after processing the polymer material with quebracho polyphenols, 135 r.u. after treatment with Fe^{+3} compounds).

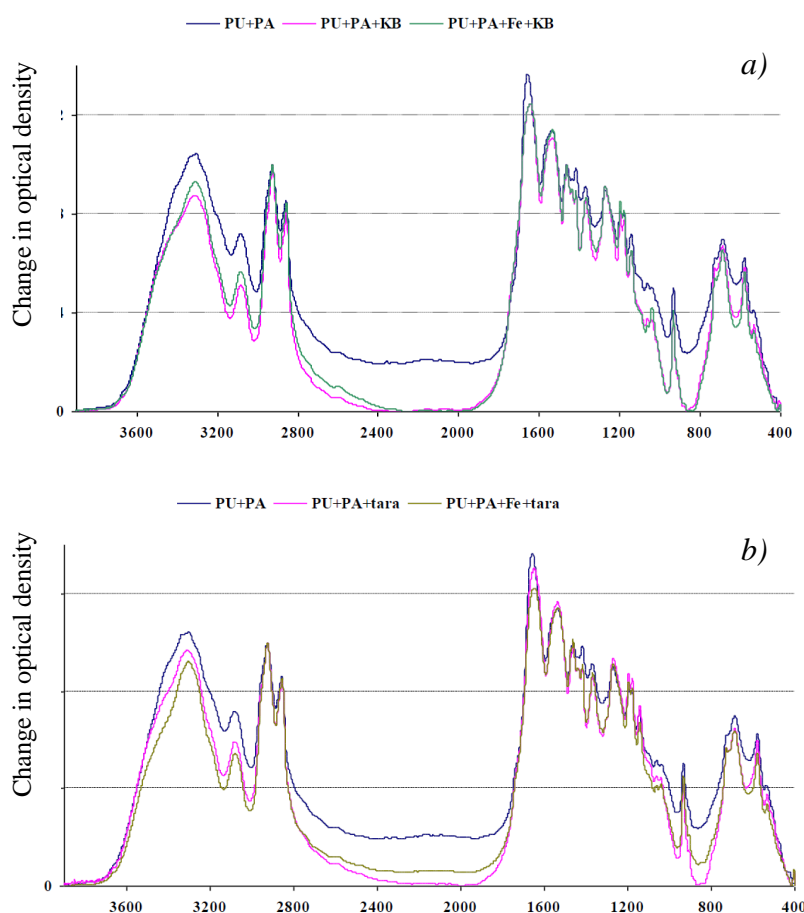


Figure 4. Results of FTIR spectroscopic studies of fibrous sorbent treated by Fe^{3+} and polyphenols Quebracho (a) / Tara (b)

According to the authors (Walenkcik *et al.*, 2024), metal–polyphenols networks are usually formed using coordination bonds during complexation. At the same time, coordination bonds between metal ions and polyphenol molecules are formed with the participation of neighboring hydroxyl groups of the aromatic ring one molecule and the C=O group of the unsaturated aromatic ring of another molecule (Fig. 5). This assumption is fully confirmed by the results of our research. In particular, a similar type of interaction is evidenced by the shift of the absorption band of valence vibrations of C–O bonds $\sim 1100\text{ cm}^{-1}$ to the low-frequency region ($1052\text{--}1073\text{ cm}^{-1}$), a decrease in the area of the peaks and their half-width as a result of the interaction with metal ions.

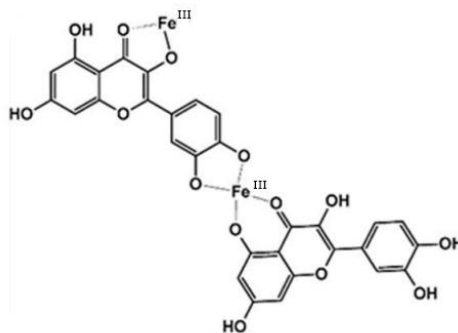


Figure 5. Schematic representation of the coordination interaction of Fe^{3+} ions and plant polyphenol molecules according to Walenkcik *et al.*, 2024

That is, the interaction of Fe^{3+} with polyamide and polyurethane fibers treated by polyphenols probably occurs as a result of the formation of coordination bonds between neighboring hydroxyl groups of the aromatic ring one molecule and the C=O group of the unsaturated aromatic ring of another polyphenolic molecule. Perhaps the interaction of Fe^{3+} with the sorbent probably occurs as a result of the formation of chemical bonds with –CO groups of modified polyamide and polyurethane fibers. Prior blocking of amino groups of modified polyamide fibers can contribute to increasing the efficiency of such interaction, in particular as a result of binding with polyphenols and their derivatives, which are usually contained in the wastewater of industrial enterprises. In this way, the problem of complex wastewater treatment may be solved.

CONCLUSIONS

The developed method of the fibrous materials modification is based on the material treatment with tannin solutions of the different chemical structure, the main component of which are plant polyphenols. It was established that the sorption capacity of the fibrous sorbent for Fe^{3+} ions after treatment with tara tannins at a temperature of $40\text{ }^{\circ}\text{C}$ is higher than after treatment with quebracho tannins under similar conditions. Treatment within the first four hours is most effective. At the same time, the maximum extraction rate of iron-ammonium alum solution reaches 90%. It was established by FTIR spectroscopy that the interaction of Fe^{3+} with polyamide and polyurethane fibers treated by polyphenols probably occurs as a result of the formation of coordination bonds between neighboring hydroxyl groups of the aromatic ring one molecule and the C=O group of the unsaturated aromatic ring of another polyphenolic molecule or due to the formation of chemical bonds with CO groups of modified polyamide and polyurethane fibers. Thus, the fibrous waste of the textile industry can be used to obtain an environmentally safe polymer composite material for the purification of wastewater from heavy

metal ions. The advantages of the obtained sorbent include high sorption activity and the possibility of further modification, and the production methods are quite simple and cheap.

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COMPARATIVE ASSESSMENT OF SURFACE PROPERTIES FOR SOME HYDROCOLLOID SYSTEMS BASED ON HYALURONIC ACID AND CARBOMER 940

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The objective of the study was to develop hydrocolloid systems using hyaluronic acid and Carbomer 940, intended for buccal application, and explain their superficial characteristics by applying two different methods. Starting from the formulation of two macromolecular solutions of hyaluronic acid 0.3% and Carbomer 0.3%, four different blends were prepared in different ratios of 1:1, 1:2, 1:3, and 1:4. The goniometric analysis guided by the pendant drop model was performed to evaluate the surface tension of the samples, while the wettability behavior was explored through sessile drop model. Comparatively, the superficial characteristics were described using force tensiometer and explained through the Wilhelmy plate model. During goniometric analysis, surface tension depended upon the samples composition and varied between 42.07-76.48 mN/m. The comparative evaluation of the wettability behavior at contact with solid surfaces emphasized the hydrophilic structure of polymeric blends. The analysis conducted using the sessile drop technique in static mode revealed angles of 35.70°-61.05°. The dynamic contact angle fitted during Wilhelmy plate measurement varied between 45.35°-49.46° on the immersion side, being diminished to 43.97°-15.043° during emersion, in the sense of increasing Carbomer 940 ratio. The contact angle hysteresis defined by the difference in advancing and receding angles was related to the presence of Carbomer 940, which is responsible for maintaining the mucoadhesive action. The results obtained showed that Wilhelmy plate-based tensiometry provides an accurate technique to assess the wettability of hydrocolloids on various surfaces. The surface properties of the analyzed hydrocolloids play a significant role in explaining their influence on buccal application as therapeutic treatments for managing oral diseases.

Keywords: hydrocolloids, surface tension, wettability, goniometry, force tensiometer

INTRODUCTION

Nowadays, an outstanding interest is given in developing polymer-based materials for drug delivery intended for multiple applications in both local and systemic administration. The biomaterials tailored from natural-derived polymers in their simple and complex reticulated state, or grafted with new chemical groups are studied for their regenerative properties and capacity to adhere and create protective films with healing activity (Thang *et al.*, 2023).

In oral disease management, the application of biocompatible supports is essential for the treatment of lesions produced by several affections like mouth sores (occurring in most cases as denture stomatitis or mucositis), periodontitis, oral lichen planus, or oral candidiasis, most of these pathologies being developed under individual exposure to mechanical stress induced by dentures, poor hygiene, immunological disorders, immunosuppressant drugs, antibiotics, or cancer chemotherapy (Chen *et al.*, 2023; Garcia *et al.*, 2024). Oral candidiasis is recognized as an oral fungi infection commonly triggered by pre-existent oral pathologies, such as the

previously mentioned ones (Anuța *et al.*, 2022). Cancer therapy and radiotherapy are tough interventions that increase the prevalence of oral infections with *Candida albicans* in cancer patients and seriously affect their life quality and forthcoming response to therapy (Sufiawati *et al.*, 2019). Its evolution through the development of resistant mixed biofilms depends on the immediate initiation of antifungal therapy, concentrated on administering local and systemic drugs (Anuța *et al.*, 2022).

Hydrocolloid carriers based on polymeric agents are used in the form of gels, hydrogels, mucoadhesive tablets or films, to assist an effective local therapy and regeneration of the mucosa (Ali *et al.*, 2022; Anuța *et al.*, 2022). Hyaluronic acid (HA), a biomacromolecule with powerful regenerative effects can be found in several commercial products for buccal application (Marques *et al.*, 2024). Recent evidence proposed the development of mouthwashes containing vitamin E 0.2%, triamcinolone acetonide 0.1%, and hyaluronic acid 0.2% (Agha-Hosseini *et al.*, 2021). The combination with a second polymer with mucoadhesive properties can be considered for the therapeutic valorization of HA.

Carbomer 940, a polyacrylic acid derivative with mucoadhesive attributes can enrich the physicochemical properties of polymeric-based systems with positive effects on viscosity, textural properties, surface characteristics and drug release. Mucoadhesive hydrogels tailored with Carbomer 940, carboxymethylcellulose, and different combinations of these were prepared to entrap a vegetable oil to increase the antifungal activity and reduce its volatilization (Nidhi *et al.*, 2023).

The limited data coverage for HA and Carbomer 940 hydrocolloid combination oriented the study through the development of some macromolecular solutions using both polymers and their research centered on superficial characteristics related to the surface tension and wettability behavior. The importance of surface properties assessment in characterizing buccal formulations consists of formulation performance to adhere and create a protective film on the mucosa. In this respect, the quantification of surface tension and contact angle is essential in demonstrating the formulation compatibility with the tissue environment, hydrophilicity degree and spreading capacity (Menzies and Jones, 2010; Talianu *et al.*, 2024)

The prior objective of the study was to develop hydrocolloid systems using hyaluronic acid and Carbomer 940, intended for buccal application, and explain their superficial characteristics by applying two methods based on goniometry and force tensiometry.

MATERIALS AND METHODS

Materials

All the ingredients were of analytical grade. Sodium hyaluronate 1200 kDa was acquired from Fagron, Carbomer 940 from Acrōs Organics, glycerin anhydrous from Chemical Company, Ultrapure Milli-Q water, with a specific resistance of 18.2 MΩ/cm, and a total organic carbon (TOC) of less than 5 µg/L was generated from a Milly-Q® Direct 8 Water Purification System (Merck Millipore), and was used as the aqueous phase.

Methods

Preparation of the Hydrocolloid Systems

To prepare the hydrocolloid systems, the two macromolecular solutions of HA 0.3% and Carbomer 940 0.3% were obtained. The two polymers were separately weighed and sprinkled on the aqueous phase previously mixed with a calculated amount of glycerin. The systems were

placed for 24 h in a cold environment to permit polymers swelling, and then appropriately homogenized. In the case of the Carbomer 940 solution, triethanolamine was added to adjust the pH. In the final step, four hydrocolloid systems resulted by mixing different proportions of the two stock solutions, as presented in Table 1.

Table 1. Composition of the hydrocolloid systems with hyaluronic acid and Carbomer 940

Ingredients (w/w, %)	HA 0.3%	HAC 1 1:1	HAC 2 1:2	HAC 3 1:3	HAC 4 1:4	Carbomer 940 0.3%
Sodium hyaluronate	0.3	0.15	0.09	0.075	0.06	-
Carbomer 940	-	0.15	0.20	0.225	0.24	0.3
Glycerin	5	8.50	9.66	10.25	10.6	12
Distilled water	94.7	91.20	89.99	89.44	89.12	88.3

Surface Tension Determination Using Goniometry

The surface tension (ST) of the hydrocolloids was tested with a CAM 101 goniometer (KSV Instruments), equipped with a Hamilton syringe and a C209-30 needle as reported (Popa *et al.*, 2021). Young-Laplace equation (eq.1) was applied to estimate the ST and the determinations were recorded in triplicate, at 24 ± 0.5 °C.

$$p_{\text{int}} - p_{\text{ext}} = \gamma_{\text{LG}} \left(\frac{1}{r_1} + \frac{1}{r_2} \right) \quad (1)$$

where $p_{\text{int}} - p_{\text{ext}}$ represents the Laplace pressure; γ_{LG} – superficial tension to liquid/gas (L/G) interface, and r_1, r_2 – the principal radii of curvature.

Contact Angle Determination Using Goniometry

As a first attempt on testing contact angle (CA), CAM 101 goniometer was used by applying the sessile drop model (Irimia *et al.*, 2019). CA was determined in triplicate, at 24 ± 0.5 °C using the Young equation (Talianu *et al.*, 2024).

$$\gamma_{\text{SG}} = \gamma_{\text{SL}} + \gamma_{\text{LG}} \cdot \cos(\theta) \quad (2)$$

where γ_{SG} is the interfacial tension to solid/gas (S/G) interface; γ_{SL} – interfacial tension to solid/liquid (S/L) interface; γ_{LG} – superficial tension to liquid/gas (L/G) interface, and θ – the contact angle made by the liquid drop with the solid surface.

Contact Angle Determination Using Force Tensiometry

Comparatively, the CA of the hydrocolloids was tested using a second equipment with a distinct methodology. Attension Sigma 700 force tensiometer (Biolin Scientific) was used in performing the measurement. A Wilhelmy plate was placed into the hook of the tensiometer, and used to analyze advancing and receding angles on the direction of plate immersion and emersion from the liquid samples (Daniel *et al.*, 2023). Wilhelmy equation was used to calculate the contact angle.

$$\cos(\theta) = \frac{F}{P \cdot \gamma_{\text{LG}}} \quad (3)$$

where θ represents the contact angle read at the solid surface, F – the buoyancy force, P – the plate perimeter, and γ_{LG} – the surface tension at the liquid/gas interface.

RESULTS AND DISCUSSION

The resulting hydrocolloid systems had a clear aspect and fluid structure, according to the composition. From Figure 1 the homogeneity of the two separate solutions of sodium hyaluronate 0.3% and Carbomer 940 0.3% can be seen, together with their macromolecular binary mixtures obtained in the four different ratios.

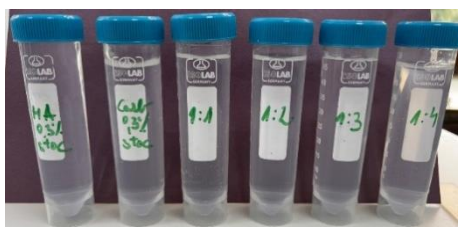


Figure 1. Hydrocolloid systems with hyaluronic acid, Carbomer 940, and their combinations resulted after preparation in the four ratios of 1:1, 1:2, 1:3, and 1:4

The variation in surface tension was dependent upon the composition of the macromolecular solutions, as can be noticed in Figure 2. The maximum value of the surface tension was specific for the hydrocolloid solution of hyaluronate 0.3%. With the addition of Carbomer solution, the ST diminished from 76.48 ± 0.39 to 42.07 ± 0.36 mN/m, being closer to the buccal surface tension.

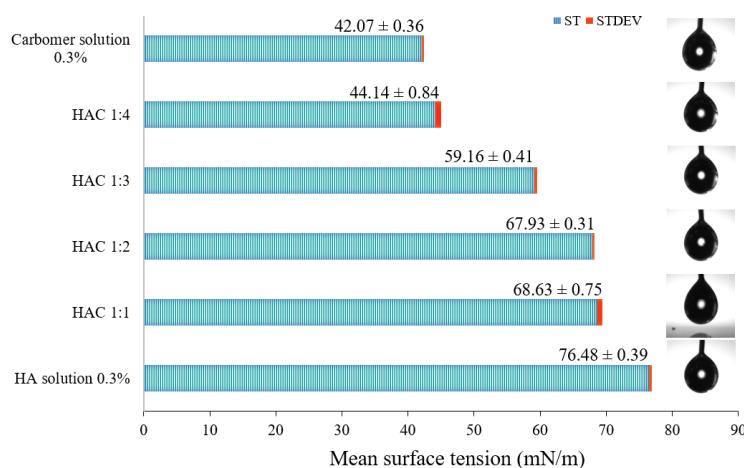


Figure 2. Graphical representation of the mean ST values for the hydrocolloid samples presented together with the corresponding drop shapes analyzed using the pendant drop model

Superficial tension represents a physical property affecting the release profile of a medicine from a pharmaceutical form. In this case, surface tension contributes to the understanding of the hydrocolloid spreadability to the mucosa and can be compared to the salivary fluid surface tension (Foglio-Bonda *et al.*, 2018). The obtained results confirmed the literature evidence. Human saliva was analyzed as a drug release media, and the surface tension values determined on unstimulated and stimulated saliva were similar, without significant differences between experimental data sets. The ST of the saliva samples was found to vary between 54 and 65 mN/m (Gittings *et al.*, 2015).

It is well known that the materials hydrophilicity is quantified by contact angle values of 0-90°. Over the analysis of the sessile drops fitted with the Young equation, the calculated

values of the CA varied between 37.33 ± 0.57 and $61.05 \pm 0.85^\circ$. The two edge values were specific for the solution of HA 0.3% and Carbomer 940 0.3%, respectively. As the Carbomer 940 ratio increased in the hydrocolloid solutions from HAC 1:1 to HAC 1:4, the calculated angles elevated from 44.47 ± 1.70 to $57.97 \pm 1.87^\circ$, as can be noticed in Figure 3 in the analyzed sessile drops captured with the digital camera. The mean values of the CA tested through goniometry are additionally presented comparatively in Table 2, together with the experimental data assessed from the tensiometry study. Knowing the mucoadhesive effect of the polymer, the formulations with a higher concentration in Carbomer 940, namely HAC 1:2, HAC 1:3, and HAC 1:4 can display adhesive hydrophilic films on the mucosa, maintaining at the same time the hydrating activity of the HA.

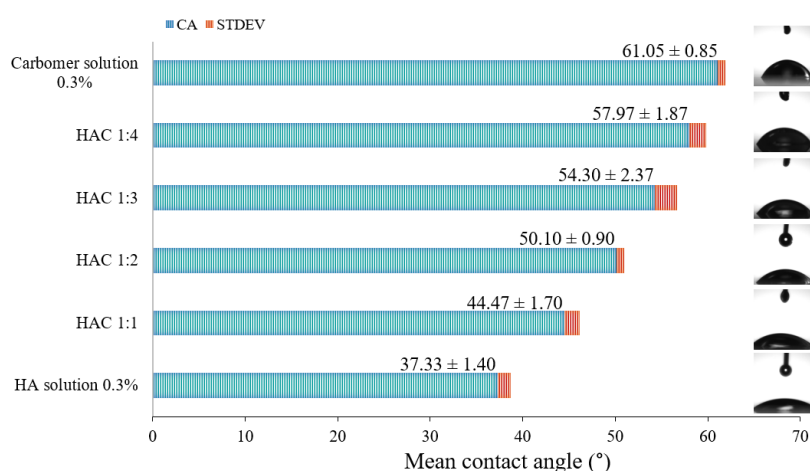


Figure 3. Graphical representation of the mean CA values for the hydrocolloid samples presented together with the corresponding drop shapes analyzed using the sessile drop model

The static goniometric analysis was emphasized in many studies, of which some important aspects were revealed. Thus, in a study conducted by Vorvolakos *et al.*, the wettability of HA ionically crosslinked with Fe^{3+} was studied to create a biodevice that can assure lubrication support in abdominal surgery. The CA values varied from 7 to 17° , relative to the increase in crosslinking grade (Vorvolakos *et al.*, 2011). Moreover, polymeric films containing hyaluronic acid, collagen, chitosan and their combinations were analysed following hydrophilicity, likewise, but at contact with glycerol or diiodomethane drops. Due to a marked hydrophilic character with CA values of 47° , HA contributed to the diminishing of collagen CA. In addition, the incorporation of chitosan into a polymeric blend made of collagen and HA (Col/HA) 80:20, respecting ascending weight fractions from 0.0 to 0.9, determined a decrease in CA of the Col/HA film from 72.3 to 46.6° , as the glycerol drop test suggested (Lewanodovska *et al.*, 2016).

It can be appreciated that the static CA analysis offered preliminary information concerning the initial contact of the sample with a surface, and can be followed by a dynamic CA analysis through the knowledge of intrinsic wettability behavior using the Wilhelmy plate model.

Following the tensiometry analysis of the hydrocolloid systems, the advancing and receding CAs were compared with the goniometric CA values. The CA results were obtained from graphical plots expressed as force versus the immersion depth calculated on each point of advancing and receding movement, as can be seen in the case of the four hydrocolloid binary mixtures of HAC 1:1- HAC 1:4 samples, and presented in Figure 4.

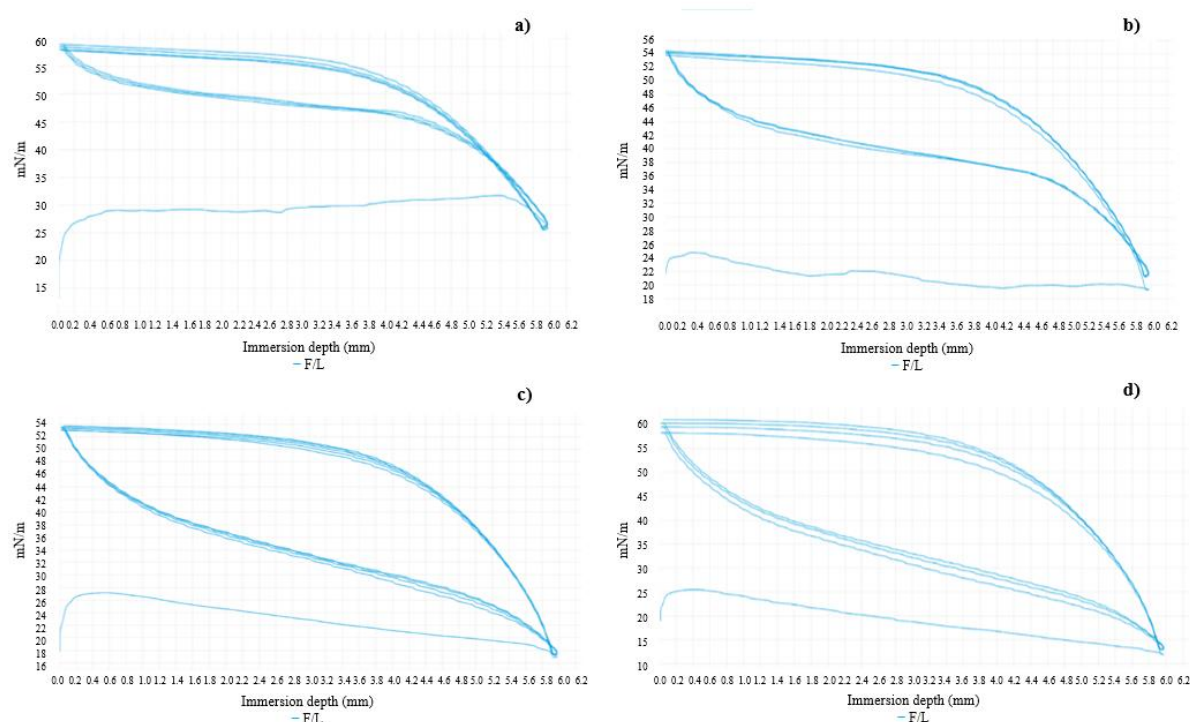


Figure 4. Advancing and receding profiles for the hydrocolloid samples tested using the Wilhelmy plate model: (a) HAC 1:1, (b) HAC 1:2, (c) HAC 1:3, and (d) HAC 1:4

Thus, in the immersion direction (advancing), contact angles varied from 45.35 ± 1.38 (angle specific for the HA 0.3% solution) to $49.46 \pm 3.11^\circ$ (for the Carbomer 940 0.3% solution). On the emersion (receding) direction, the angles diminished, from 43.97 ± 0.94 (HA solution 0.3%) to $15.04 \pm 0.19^\circ$ (Carbomer 940 0.3% solution). The experimental results from Table 2 present in a comparative manner the contact angles obtained using both methods. The angles measured at the plate immersion surface were similar to those obtained on the goniometric pathway. On the emersion direction, it can be noticed a reduction in the contact angles more obvious for the series HAC 1:1 - HAC 1:4, and Carbomer 940 0.3% hydrocolloid solutions. The difference calculated between advancing and receding angles was defined as the hysteresis angle, with values calculated for each formulation and expressed as mean values and standard deviation (Mohos *et al.*, 2014). The resulting hysteresis angles varied between 1.37 and 34.59° , according to the presented data from Table 2. Elevated values were specific for solutions with a good capacity of spreading and variation of the CA in time.

Table 2. Comparative results obtained through goniometric and tensiometric analysis of the hydrocolloid systems

Analysis model	Contact angle model			Wilhelmy plate model		
Code	CA (L) ($^\circ$)	CA (R) ($^\circ$)	CA (M) ($^\circ$)	CA Adv ($^\circ$)	CA Rec ($^\circ$)	Hysteresis ($^\circ$)
HA 0.3%	38.00 ± 1.30	36.67 ± 1.79	37.33 ± 1.40	45.35 ± 1.38	43.97 ± 0.94	1.374 ± 0.44
HAC 1:1	46.01 ± 0.42	42.94 ± 3.29	44.47 ± 1.70	42.12 ± 0.78	33.56 ± 0.39	8.56 ± 0.73
HAC 1:2	49.88 ± 0.89	50.32 ± 1.02	50.10 ± 0.90	45.48 ± 0.56	40.96 ± 0.13	4.52 ± 0.55
HAC 1:3	53.93 ± 1.35	54.68 ± 3.39	54.30 ± 2.37	51.60 ± 0.21	41.58 ± 0.26	10.01 ± 0.05
HAC 1:4	58.85 ± 1.16	57.10 ± 2.74	57.97 ± 1.87	49.52 ± 1.62	32.20 ± 1.14	17.32 ± 0.52
Carbomer 0.3%	62.32 ± 0.96	59.78 ± 1.34	61.05 ± 0.85	49.64 ± 3.11	15.04 ± 0.19	34.60 ± 2.92

The method was used as well to study the wettability phenomena in contact with hydrophilic and lipophilic surfaces of bicontinuous microemulsions prepared with tetradecane or methyl oleate as lipophilic phases stabilized with sucrose esters and pentanol (Vargas-Ruiz *et al.*, 2017). The increase of the hysteresis emphasized the affinity of the microemulsion for the analyzed substrate, explaining substance adsorption at the solid/liquid interface.

Wilhelmy plate method was considered a useful approach for measuring dynamic contact angles of the studied hydrocolloids, completing the information obtained in the goniometric study.

CONCLUSIONS

Based on the obtained results and observations, this study emphasized a complementary characterization method in the analysis area of superficial phenomena, applied in the formulation of liquid and semi-solid pharmaceutical systems with bioactive ingredients. The designed hydrocolloids aimed to offer an improvement in the management of oral pathologies and were characterized by adequate superficial characteristics. The surface tension values were specific for the oral cavity environment. Moreover, the comparative goniometric and tensiometric studies offered a comprehensive perspective on the contribution of the static and dynamic contact angle for the assessment of the hydrocolloids' wettability. In both analysis models, Carbomer 940 influenced the variation in contact angle, emphasizing its role in increasing the samples consistency and mucoadhesion effect. Wilhelmy plate model supported the results through the calculation of dynamic contact angle and hysteresis, being a mark for the estimation of the hydrocolloid spreadability on a surface and their practical application for buccal administration in oral candidiasis.

Acknowledgment

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FABRIC DATABASE FOR E-LEARNING PLATFORM IN THE FIELD OF VIRTUAL CLOTHING PROTOTYPING

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Virtual clothing prototyping generates numerous benefits within the professional clothing design process. In order to become a professional, students have to train and they need adequate learning instruments. There is a lack of open-source web applications for training of students in this domain. This paper presents an e-learning platform in the field of virtual clothing prototyping and a related learning material of a fabric database, required by the e-learning platform's algorithms. This e-learning platform for training purposes simulates the fit of a garment on a 3D human avatar in four steps: selection of the avatar, selection of the fabric, selection of the garment and style. All these elements are included in four databases, namely the fabric database, the garment database, the fashion database and the 3D human model database. In order to support training of higher education students with the e-learning platform, a curriculum of learning materials was prepared and structured in relation to these four databases. The learning material on the fabric database includes valuable aspects on digitalization of a real fabric, some basic properties as well as the selection mechanism of the e-learning platform. These learning materials were conceived by an European partnership in frame of the Erasmus+ project DigitalFashion (<https://digitalfashionproject.eu/>).

Keywords: training, students, professionals

INTRODUCTION

Digital fashion is a field of fashion that uses 3D technologies to simulate virtual garments or to reproduce digital models of physical products, thus becoming a major contributor to a global sustainable fashion industry, by promoting innovation, reducing waste and encouraging conscious consumption. Virtual prototyping is considered as one of the main solutions to address sustainability in the early stages of the product design process, thus being part of the widely *no-waste philosophy*. Designers, pattern makers and producers can all benefit from 3D virtual prototyping to reduce the amount of generated waste (Morandi and Tonelli, 2023; Zero Waste Europe, 2023).

Virtual prototyping has the origin in early 60', when Ivan Sutherland, a computer scientist from Massachusetts Institute of Technology (MIT), pioneered the first CAD system, named Sketchpad "to make possible for a man and a computer to converse rapidly through the medium of line drawings". Sketchpad allowed users with minimal computer knowledge to create and manipulate digital drawings using a light pen and a display screen. The first digital library was obtained, under tape form, with collected pictures for possible future use (Sutherland, 1963).



Figure 1. Sutherland using Sketchpad system (University of Minho)

Up to the present days, more and more sophisticated software solutions for 3D virtual prototyping of garments were developed. The three most powerful CAD/CAM software provider companies are: Prisma Tech, with its leading fashion software CLO, Lectra, with 50 years of industry experience and Adobe, the provider of Adobe Substance 3D toolset (Prisma Tech, Lectra, Adobe).

In a digital world, virtual prototyping represents a highly desirable work modality in the field of clothing design and is relevant for a large category of beneficiaries. Among the key beneficiaries are the higher education students, who need a series of simple and accessible digital learning instruments. Therefore, in the last few years, a series of e-learning instruments were developed in the frame of Erasmus+ projects, for correlated textile domains such as:

- Smart textiles prototypes and basic disciplines (Skills4Smartex project: <http://www.skills4smartex.eu/>)
- Software for design and modelling of fabric structures (OptimTex project: <http://www.optimtex.eu/>)
- Innovation in textile industry (Destex project: <http://www.destexproject.eu/>)

In order to meet the training challenges of students learning virtual prototyping of clothing, six European research and educational providers have joined expertise, for designing and implementing an e-learning platform in this domain. The e-learning platform is first of all a web application with free access on the Internet – it requires single registration (<https://digitalfashiondieu.com/login>). Other software solutions for virtual clothing prototyping, such as CLO 3D, Lectra Modaris or Adobe Substance 3D, are proprietary desktop applications and are quite cost-intensive. The e-learning platform of virtual prototyping developed in the frame of the Erasmus+ DigitalFashion project (www.digitalfashionproject.eu) is thus meant to meet the training needs of students in the clothing design domain.

Aside from students, the clothing industry is another major beneficiary of virtual prototyping. This advanced technology enables the development of digital garment models before physical production and fast customization according to customer preferences, thus minimizing material waste and enabling rapid design adjustments. Furthermore, factories can simulate production processes, enhancing workflow and machinery use, resulting in significant cost savings and enhanced productivity. Overall, the integration of virtual prototyping in textile manufacturing not only improves efficiency but also aligns with sustainability goals by minimizing resource consumption and waste. This is why the e-learning platform developed within the DigitalFashion project is also useful for introducing basic concepts of training for industry professionals.

The most important gain of e-learning is the technological advance reached through an interactive collaboration between higher education institutions, fashion industry specialists, software solutions suppliers and final clients which should become as “digitally compatible” as possible. To tackle this challenge, the higher education institutions have to permanently

collaborate with partners from industry and continuously incorporate into the curriculum, the digital tools used in the industry. This permanent interaction not only captivates students' curiosity and interest, but also facilitates better comprehension and knowledge retention, which will generate better employability competencies (Zhang, 2021; Conlon and Gallery, 2024; Niyogushimwa, 2023; Grosu *et al.*, 2022).

THE PROJECT AND THE E-LEARNING PLATFORM

The Erasmus+ DigitalFashion project (Collaborative Online International Learning in Digital Fashion) is a Higher Education strategic partnership project for the period 2022-2025. INCOTP – Bucharest coordinates a prestigious European partnership of research and educational providers in the field of clothing design: ENSAIT – France, HOGENT – Belgium, University Maribor – Slovenia, CITEVE – Portugal and TUIASI – Romania. The project accomplished four project results (PR), which are already available on the project's website www.digitalfashionproject.eu:

PR1 – a new methodology for the clothing design training on European level, which underlined the need of virtual prototyping and 3D design, based on a survey with 35 participating clothing companies.

PR2 – four databases required for the algorithm structure of the e-learning platform: fabric database, garment database, fashion database and 3D human model database.

PR3 – the e-learning platform as web application, available at the URL: <https://digitalfashiondleu.com/login>

PR4 – the new curricula explaining the process of virtual prototyping based on the programmed e-learning platform – also available on the project website and on the Moodle platform www.advan2tex.eu/portal/.

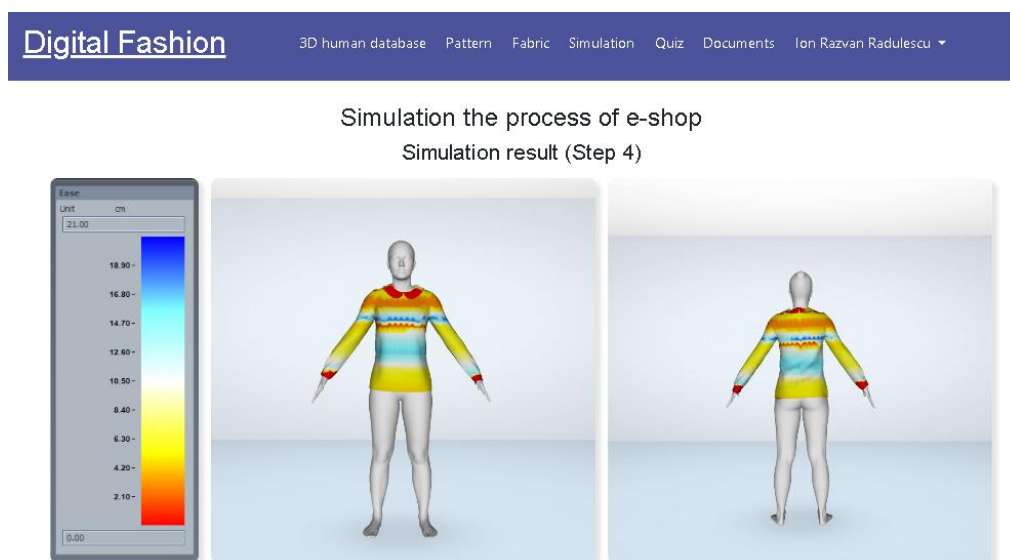


Figure 2. Print screen of the e-learning platform with simulation of the garment fit on 3D human model

The e-learning platform is programmed with algorithms that access four databases: a fabric database, a garment database, a fashion database and a 3D human model database. Figure 2 presents the fit of a blouse garment of a certain fabric on the 3D human avatar. Based on these databases a curriculum of learning materials presenting and explaining the process of virtual clothing prototyping was conceived.

FABRIC DIGITALIZATION PROCESS

The curriculum regarding the fabric database includes explanations related to fabric properties, real and digital (virtual) fabrics and transition from real to digital fabrics. The fabric database of this project consists in a structured and organized collection of types of fabrics, widely used in clothing industry and suitable for the garment models selected in the project. In the frame of the Digital Fashion Project, new representative physical samples of fabrics and garments have been collected from the project partners in order to construct a digital fabric database and then demonstrate the complete digitalization process to fashion designers. Creating a 3D digital garment requires inputs of the corresponding digital fabric properties. Digitalizing fabric technique is based on image processing and machine learning algorithm. In this project the draping image was taken using a Cusick Drape Tester, i.e. orthogonal projections of the drapes of textiles were taken using a digital camera. In addition, the drape coefficients (DC) and the number of nodes were calculated using Drape Analyser software.

From the point of user view, the prediction process for fabric technical parameters is realized by inserting the drape image of real fabric and exploiting a comprehensive digital fabric database implemented in the Lectra Modaris 3D Fit Software. The Lectra database is a large dataset of fabric properties, including the contour of fabric drape and associated drape features. The Lectra database includes 111 pictures of drape and for each digital fabric 23 properties (23 columns) are provided, including: Drape shape, Number of fabric, Average amplitude of drape, Average distance of drape, Maxim peak dimension, Minim valley dimension, Number of peaks, Weight, Commercial name or colour, Composition, Thickness, Armor, Warp/Weft Contexture, Weft Bending, Warp Bending, Drape coefficient, Nb folds, CisT, CisC, FlexT, FlexC, Color, and Patterns. One example is given in Figure 3.

Digitalize Fabric Process

Upload a drape image

No file chosen

Upload file name: MSF1.jpg

Estimated Drape Parameters:

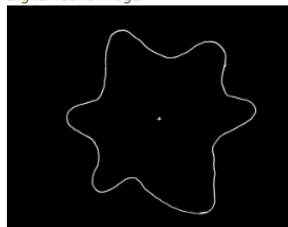
AA: 25.0, AD: 314.38, MP: 365.09, MV: 250.5, NoP: 6

Drape Image:



The Closest Fabric is 51

Digital Fabric Image:



Digital Fabric Properties: [0 Drape 0 N 51 AA 36.3 AD 188.41 MP 236.05 MV 136.22 NoP 6 Weight 323 Nom commercial ou coloris Beige Composition laine Epaisseur en mm 1.27 Armure sergé croisé 2 lie 2 Contexture Chaîne / Trame 14,4/17,8 Bending Chaîne 3.546875 Bending Trame 3.091099 Drape Coefficient 0.350291 Nb plis 6.0 CisT 0.327 CisC 0.339 FlexT 0.39 FlexC 0.31 Coloris 0 Motifs 0]

Figure 3. Digitalized fabric result

THE FABRIC PROPERTIES

It is important to explore and understand the visual and mechanical properties of textile materials for an informed selection of fabric to achieve a specific look, texture, lines, and drape of the final product. By comprehending the properties of textile materials, designers can create aesthetically pleasing and functional products that meet consumers' needs and expectations. The fabrics in the database were characterized according to the following properties:

- material/ fabric composition, by their exact fibrous composition;
- material /fabric weight, in grams per square meter;
- drapability, the property of the fabric to form mobile folds under the action of its weight;
- fabric image, which shows colour, pattern, texture and finishing;
- fabric colour (according to Pantone Code/RGB code);
- construction – knitted or woven – and types of waves/knits;
- elasticity, on warp/wales - ability to stretch and then return to its original shape and size;
- thickness- the distance, in mm, between the two faces of the fabric, measured under a certain pressure;
- see through (yes/no) the propriety of the fabric to allow the passage of a ray of light;
- feel/touch (slippery, stiff, smooth, soft, little rough) a sensation when the material touches the skin.

The fabric database developed in the Digital Fashion project framework comprises a total of 49 real fabric samples (labelled F1-F49), categorized based on their intended garment use, namely Men's shirts, Men's trousers, Women's blouses, and Women's skirts. Their important fabric parameters related to the garment feel, comfort and fitting are described and the criteria for selecting a digital twin fabric (from the Lectra database) of the real fabric are outlined. Fabric database contains representative weaves and knits (Figures 4 and 5):







Code	Image	Composition (%)	Weight (gm ⁻²)	Thickness (mm)
F1		100% cotton	200	0,38
F2		97% cotton, 3% elastane	115	0,22
F6		55% cotton + 45% cellofibre	103,3	0,228
F7		100% cotton	114,52	0,462
F8		100% cotton	138,54	0,292
F9		68% polyester, 29% viscose, 3% elastane	345	0,65

Figure 4.

Example of woven fabrics in the database






Code	Image	Composition (%)	Weight (gm ⁻²)	Thickness (mm)
F3		100% cotton	145,8	0,48
F4		100 % cotton	210,2	0,467
F5		100 % cotton	163,86	0,674
F15		45% polyacrylonitrile, 55% ?Poly?	315,8	1,86
F45		78% polyamide, 22 elastane	238	0,536

Figure 5.

Examples of knitted fabrics in the database

THE FABRIC SELECTION ON THE E-LEARNING PLATFORM

By using the e-learning platform, users can select the real fabrics from the fabric database according to their demands. Appearance images, draping images, draping properties and mechanical properties of each fabric are provided to the user to facilitate selection of the appropriate fabric (Figure 4).

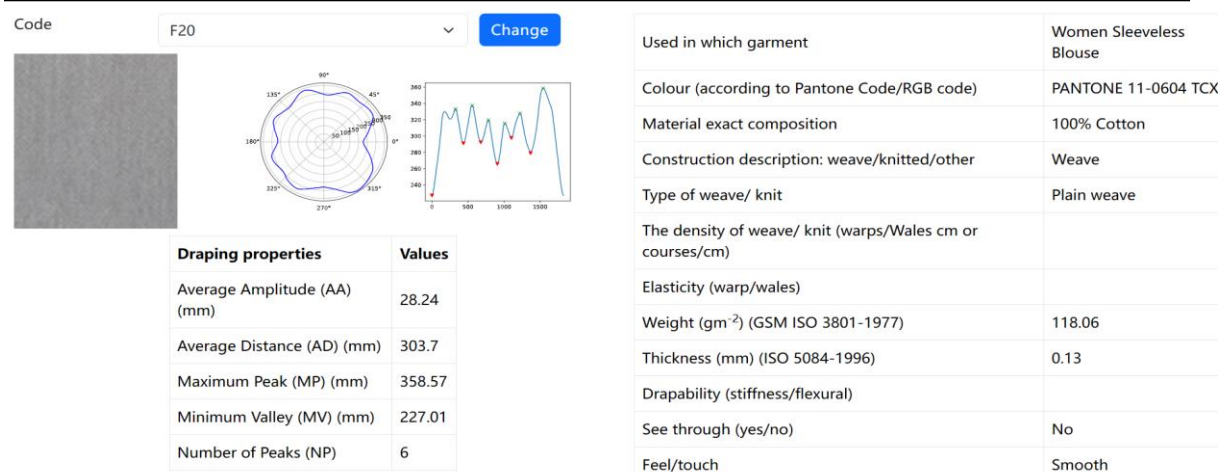


Figure 4. Fabric selection on the e-learning platform

The database includes fabric details such as raw material composition, fabric's specifications (e.g. weight, fabric identity, source and Lectra pairing number, fabric image, colour code), construction description (type of weave/knit, the density of weave/ knit, thickness, elasticity, bending and stiffness properties, visual references as transparency, drapability, feel and touch). After the user selects a real fabric, the system will recommend digital fabrics with similar performance to the user. This recommendation is based on intelligent algorithms. The idea is to cluster the existing digital fabrics (in Lectra database) into different clusters based on the similarity of their draping parameters to identify the group that is the closest to the real fabric (closest group).

CONCLUSIONS

This paper presents modern educational materials in the field of virtual clothing prototyping, meant to be used by students and professionals. The benefits of virtual garment prototyping are undeniable, while our training solution is a free web application. The educational materials developed within the Erasmus+ project DigitalFashion are going to be presented to professionals within multiplier events and to high school students within workshops. The module "Fabric database" aims to develop specific competencies related to fabric knowledge, fabric construction, and the properties of both real and digital fabrics. The entire learning material is available at URL: https://digitalfashionproject.eu/?page_id=2489.

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HISTOGRAMMICALLY COMPARING MATHEMATICAL SIMULATION RESULTS IN MODELING A STOCHASTIC PROCESS EXHIBITING BOTH CONTINUOUS AND DISCRETE VARIATIONS

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The current paper aims to present several mathematical simulation results which have been obtained by modeling a Lévy stochastic process, exhibiting two continuous variations followed by a discrete one. In modeling such a process, we surely had to decompose it into three sub-processes: the first one is a deterministic linear drift (a time-depending simple linear function); the second one is a standard (one-dimensional) Wiener process (a stochastic process respecting three essential properties, namely: independence, stationarity and continuity); the third one is a CFD Poisson stochastic process, which is discrete (as formerly stated). The comparison was made by analyzing different sets of histograms. These sets have been plotted by histogrammically comparing two jump distributions, namely Pareto distribution vs. normal distribution. The analyzed Lévy stochastic process, exhibiting both continuous and discrete variations, has been computed upon its discrete part, by using an appropriate skeleton structure, the main advantage of such a computation being the efficiency of it, although it is known that a weakness in using “discrete skeletons” is that the location of large jumps in the simulation model cannot be precisely identified.

Keywords: Lévy stochastic process, Wiener process, histogram.

INTRODUCTION

Lately, “Lévy processes” turn out into an interesting topic in several areas, among which: finances, economics, statistics, mathematics, physics or engineering.

Indeed, Lévy processes are often used by financial traders so as to conclusively describe the volatility of the market in the real-world, but also in order to develop risk-free theoretical scenarios. They are used in economics, for developing continuous time-series models. Also, they can be used in life-table statistics and actuarial mathematics for computing various risk parameters in insurance/reinsurance, as well as by physicists and engineers, *e.g.*, in assessing parameters to characterize different kinds of turbulence.

Lévy processes’ importance was also recently demonstrated in some new fields, such as mathematical finance – as by Eberlein (2020) – that they are able to properly approximate various observations in financial market, regarding jumps or spikes of the prices, their accuracy exceeding the one of the pure Wiener processes, as shown by Barndorff-Nielsen *et al.* (2001).

In the textile and leather industry, understanding stochastic processes with both continuous and discrete variations is crucial for optimizing production processes, quality control, and predictive maintenance.

For example, in dyeing and finishing, where chemical reactions and color variations are influenced by time-dependent factors, a Lévy process model can help predict and manage these variabilities, reducing waste and improving consistency.

Additionally, in leather treatment and textile wear testing, stochastic modeling can simulate and anticipate wear patterns or defects, enhancing the ability to preemptively address quality issues and extend the lifespan of materials.

Through simulations based on accurate stochastic processes, companies can make more informed decisions and improve resource efficiency.

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CHOSING THE LÉVY PROCESS FOR THE STUDY

As it is well known, the term of “Lévy process” generically refers to a motion of a point that has random successive independent displacements remaining identical across the time.

The first part of the Lévy process that we have chosen for this work is the simplest deterministic process, named “linear drift”, which will be denoted in our paper as “ μt ”.

The second part of our Lévy process is the Wiener process, which will be denoted as “ σB_t ”, where B stands for “Brownian”, because the Wiener process is often called “Brownian motion”.

The third (and final) part of the chosen Lévy process is a Poisson process compound upon N_t random variables: $k = X_1, X_2, \dots, X_{N_t}$.

As always, a sum of Lévy processes is also a Lévy process, we obtain the following compound Lévy process (consisting in two continuous variations followed by a discrete one):

$$Y_t = \mu t + \sigma B_t + \sum_{k=1}^{N_t} X_k \quad (1)$$

Discrete simulation is important in studying the properties of our compound Lévy processes, as the Wiener process continuously generates random movements occurring between its jumps.

Consequently, we intend to simulate the discrete parts of the compound process by using a “discrete skeleton”, $\{(P_k, A_k, M_k)\}$, which corresponds to variables called: “prior”, “after” and “maximum”, these variables being related to valuable information about the process (before and after a random jump, as well as for the maximum value of Y_t by the time of its k^{th} jump).

INTRODUCING THE SIMULATION MODEL

As previously discussed, the simulation model will be not continuous, but discrete, as the values of the Lévy process are collected in a “discrete skeleton”, $\{(P_k, A_k, M_k)\}$.

Considering the expression (1) and assuming discrete time – following Applebaum (2004):

$$T \sim \exp(\lambda) \quad (2)$$

one can further define:

$$V = \max_{0 \leq t \leq T} \mu t + \sigma B_t \quad (3)$$

and

$$W = \left(\max_{0 \leq t \leq T} \mu t + \sigma B_t \right) - (\mu T + \sigma B_T) \quad (4)$$

The discrete simulation can be performed according to an appropriate algorithm, that will be described in what follows.

ALGORITHM PROPOSED TO SIMULATE THE CHOSEN LÉVY PROCESS

The algorithm will be proposed according to the previously presented purpose.

Algorithm: Simulation of the Lévy process, assuming discrete time

Set $P_0, A_0, M_0 = 0, 0, 0$

for iteration, $k = 1, 2, \dots, n$ do

Simulate V_k, W_k, X_k

Set:

$$P_k = A_{k-1} + (V_k - W_k)$$

$$A_k = A_{k-1} + (V_k - W_k) + X_k$$

$$M_k = \max\{M_{k-1}, A_{k-1} + V_k, A_{k-1} + (V_k - W_k) + X_k\}$$

end for

The code implementation in the algorithm above is written using the R programming language.

In the settings of this simulation, X_k are independent and identically distributed (i.i.d). random variables having the distribution F , which are defined as the size of the k^{th} jump, V and W are two normally distributed independent random variables, whose intensities are:

$$\phi_1 = \sqrt{\frac{\mu^2}{\sigma^4} + \frac{2\lambda}{\sigma^2}} - \frac{\mu}{\sigma^2} \quad (5)$$

and

$$\phi_2 = \sqrt{\frac{\mu^2}{\sigma^4} + \frac{2\lambda}{\sigma^2}} + \frac{\mu}{\sigma^2} \quad (5')$$

respectively.

The function to be implemented has the following arguments: n (total number of iterations), μ, σ, λ (parameters from which it becomes possible to compute the intensities given above (ϕ_1 and ϕ_2)), a (threshold to be discussed in estimating first-passage probabilities), X (argument that selects which is the type of distribution for the jump components), and “jump_size” (mean of the distribution).

There are three types of return values for this function: “final” – for the values of the “skeleton” at the iteration n , so $\{(P_n, A_n, M_n)\}$; “path” – for the values of the “skeleton” at each iteration, k and “first passage” – for a true/false value which depends on exceeding or not the value of constant a (introduced above).

As far as simulation of jumps while keeping fixed parameters, a set of 800 simulation runs will be performed, so as to later prove the variability of a simulation upon twenty simulation runs (keeping the same parameters).

First simulation is run for fixed parameters μ, σ, λ and assuming all the values for jump_size to be equal to the unity and F is chosen as a normal distribution.

LÉVY PROCESS' EVOLUTION

The values of A and M are approximately identical; the support of the normal distribution belongs to the interval $[0, \infty)$, so values of jump_size are either null or strictly positive, whereas $A_k \geq P_k$.

There cannot be identified any predominant components in terms of absolute values, so

the studied process should mostly be characterized by the positive linear drift and the values of `jump_size`. From the set of 800 simulation runs, the difference between the simulations runs seems to be more noticeable within the interval $[480, 500]$.

LÉVY PROCESS' DECOMPOSITION AND COMBINING THE DISCRETE PARTS

As stated before, the composed Lévy process can be decomposed into three sub-processes, the first one being a deterministic linear drift (μt) – which is continuous, the second one being a stochastic Wiener process (σB_t) – which is also continuous and the third one being a stochastic Poisson process ($\sum_{k=1}^{N_t} X_k$), – which is discrete, as presented by Costa (2023).

As far as the first part is concerned, as the name itself suggests, the linear drift is a simple linear function of the time, with coefficient $\mu \in \mathbb{R}$.

As far as the second part is concerned, it is well known that a stochastic process $W(t)$ is called Wiener process if it respects the following three properties: independence, *i.e.*, $[W(t + \Delta t) - W(t)]$ does not depend on $W(\tau)$ for any $\tau \leq t$; stationarity, *i.e.*, the distribution of $[W(t + \Delta t) - W(t)]$ does not depend on t and continuity, *i.e.*:

$$\lim_{\Delta t \rightarrow 0} \frac{P(|W(t+\Delta t) - W(t)| \geq \delta)}{\Delta t} = 0 \quad (6)$$

for $\delta > 0$, as proved by Kim and Park (2024).

As far as the third part is concerned, the Poisson process N_t – with its intensity $\lambda \geq 0$ – can be used to define processes having non-continuous increments, in terms of the number of events in non-overlapping intervals:

$$P(N(t) = n) = \frac{(\lambda t)^n}{n!} e^{-\lambda t} \quad (7)$$

this process occurring at arrival times S_1, S_2, \dots, S_n and interarrival times T_1, T_2, \dots, T_n , where the connection between arrival and interarrival times is that S is the sum of T , *i.e.*:

$$S_n = \sum_{k=1}^n T_k \quad (8)$$

In order to avoid an inefficient simulation of the Wiener process, the composed Lévy process would be better described with the two discrete parts (linear drift and Wiener process) combined together.

They could be looked at as one discrete function, which would be defined as $[V_k - W_k]$, describing the movement of Y_t between jumps X_k and X_{k-1} – *i.e.*, between T_k and T_{k-1} .

At one side, as between consecutive jumps the combined component $\{\mu t + \sigma B_t\}_{t \geq 0}$ follows a volatile pathway as a consequence of the stochasticity of B_t component, the maximum value of the combined component within the interval $[T_k - T_{k-1}]$ will be denoted as V_k :

$$V_k = \max(\mu t + \sigma B_t), \quad t \geq 0 \quad (9)$$

V_k being the value of the combined component $\{\mu t + \sigma B_t\}_{t \geq 0}$ exactly at the moment t_{\max} (with $0 = T_{k-1} \leq t_{\max} \leq T_k$) in which V_k reached its maximum value within the interval – *n.b.* that, for every interval, t is set to be null.

On the other side, W_k is defined as:

$$W_k = \max(\mu t + \sigma B_t) - (\mu T + \sigma B_T), \quad t \geq 0 \quad (10)$$

Consequently, the change of Y_t within the time interval $[T_k - T_{k-1}]$, which might be attributed to the continuous part of the compound process, is equal to $V_k - W_k$, which means:

$$\mu T + \sigma B_T = V_k - W_k \quad (11)$$

INTERPRETING THE “DISCRETE SKELETON”

Within the “discrete skeleton”, $\{(P_k, A_k, M_k)\}$, the value P_k (prior to the next jump X_k) is obtained by adding to the value A_{k-1} (after the last jump, X_{k-1}) the change caused by the continuous process between jump X_k and X_{k-1} (represented as the difference between the values V_k and W_k):

$$P_k = \underbrace{A_{k-1}}_{Y_t \text{ after } X_{k-1}} + \underbrace{(V_k - W_k)}_{\text{linear+Wiener}} = A_{k-1} + V_k - W_k \quad (12)$$

The value A_k , obtained after X_k , is equal to the sum between the value P_k , registered before the jump, and the size of the jump, X_k :

$$A_k = P_k + X_k = A_{k-1} + (V_k - W_k) + X_k = A_{k-1} + V_k - W_k + X_k \quad (13)$$

The value M_k is the maximum value taken by Y_t during the k^{th} jump, meaning that it is equal to the maximum value of all points in the interval $[0, S_k]$ registered within the respective time period.

$$M_k = \max(M_{k-1}, A_{k-1} + V_k, A_{k-1} + V_k - W_k + X_k) \quad (14)$$

TESTING PARETO DISTRIBUTION VS. NORMAL DISTRIBUTION

First-passage probability is defined for a fixed value, $a \geq 0$; in this case, it is the probability that the compound Lévy process ever exceeds a ; as to estimate this probability, we shall compare the value a to the maximum value of this process – *i.e.*, to the M coordinate of the “discrete skeleton” $\{(P_k, A_k, M_k)\}_{k \in \mathbb{N}}$, so if M_k does not reach level a (whatever value it can take), we can conclude that the path cannot ever go beyond a – as remarked by Kindap and Godsill (2024).

The compound Lévy process was tested for two distributions of the values of $\text{jump_size } Y_k$, in each case the mean jump_size being equal to the unit. Six histograms were drawn.

Namely, we histogrammically have compared – by recording the values found in each small interval – the first-passage probabilities for a Pareto distribution (with a shape parameter equal to 1.04) to a normal distribution (with a standard deviation equal to 10) – *n.b.*, the normal distribution is the case in which the Wiener process is neither negatively-oriented nor dominant.

We used these two distributions to generate different values of jump_size of the Poisson process, in order for them to serve as input data in the simulation of the Lévy process.

We shall show here, in Figures 1-3, the resulting first-passage probability for each member of the “discrete skeleton” $\{(P_k, A_k, M_k)\}_{k \in \mathbb{N}}$, by means of three sets of histograms, recorded from a hundred values of $\{(P_{800}, A_{800}, M_{800})\}$, corresponding to a Pareto and, respectively, to a normal distribution, in each figure being involved just one member of the “discrete skeleton”.

For simplicity, in both cases, we have kept the same value for the parameters $\{\mu, \sigma, \lambda\}$, all of them having the same value, $\mu = \sigma = \lambda = 1$.

It was easily proved that the Pareto distribution exhibits a smaller variance, whereas only the normal distribution can take negative values, meaning that in this case the Lévy process can have both positive or negative jumps, *i.e.*, just for the normal distribution, we could sometimes find cases in which $A_k < A_{k-1}$. Moreover, the values of A can be significantly different from the ones of M only in the normal distribution (and this happens, again, because this is the only one in which the jumps can be negative).

One may also observe that, whereas all the three Pareto distribution histograms are quite asymmetric (appearing in the range [1100, 1540] and having the majority of the values in the range [1210, 1430] and a strong peak between 1210 and 1320, the results are more evenly distributed for the normal distribution histograms: they appear in the range [1200, 2860] for P and A and [1310, 2860] for M, being mainly represented in the range [1450, 2440].

The histograms previously presented are considered to be representative, because no other component of the Lévy process is dominant, so they have proved that variance differences are remarkable from the histograms themselves, consequently limiting all the discussion only to a single run of simulations.

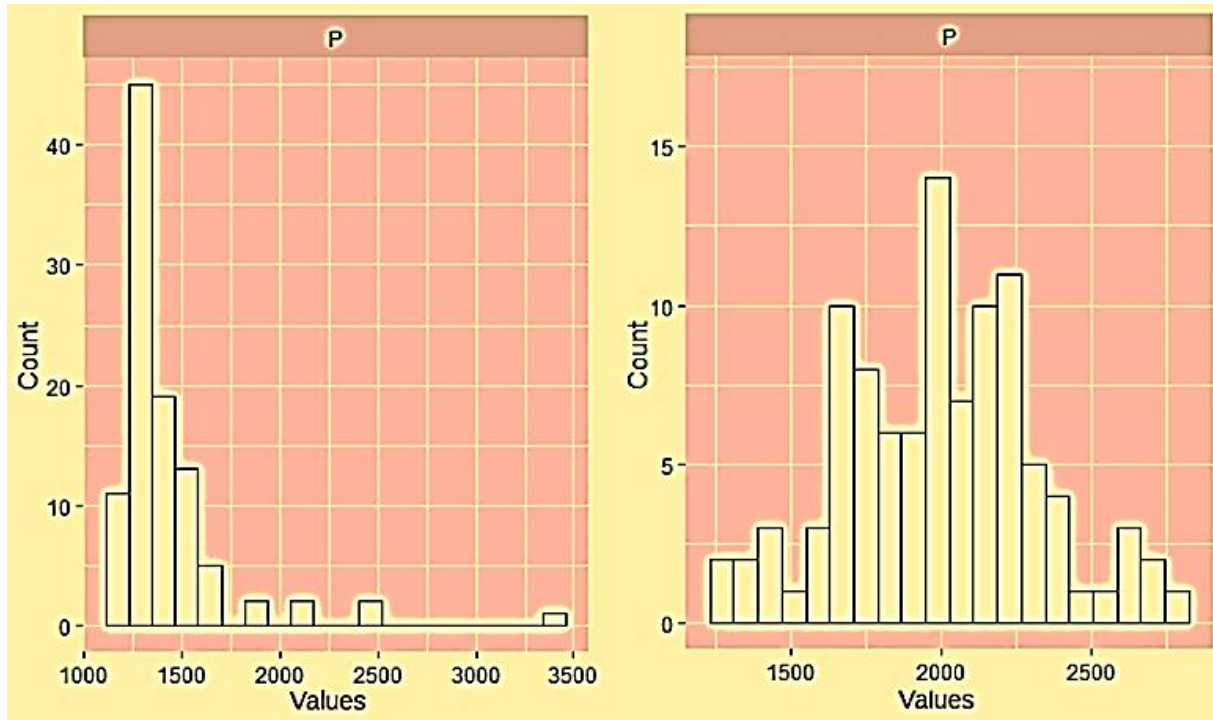


Figure 1. Histograms recorded for P from a hundred values of $\{(P_{800}, A_{800}, M_{800})\}$: Pareto distribution (left) vs. normal distribution (right), with all parameters presented above

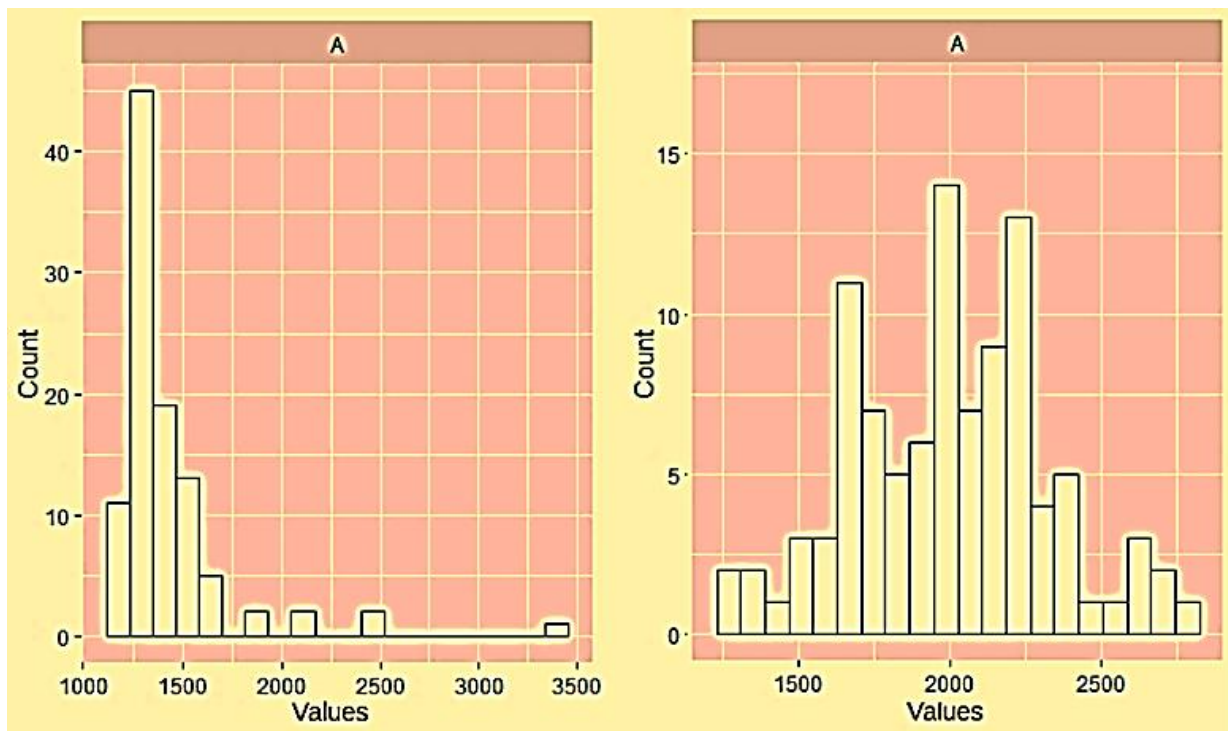


Figure 2. Histograms recorded for A from a hundred values of $\{(P_{800}, A_{800}, M_{800})\}$: Pareto distribution (left) vs. normal distribution (right), with all parameters presented above

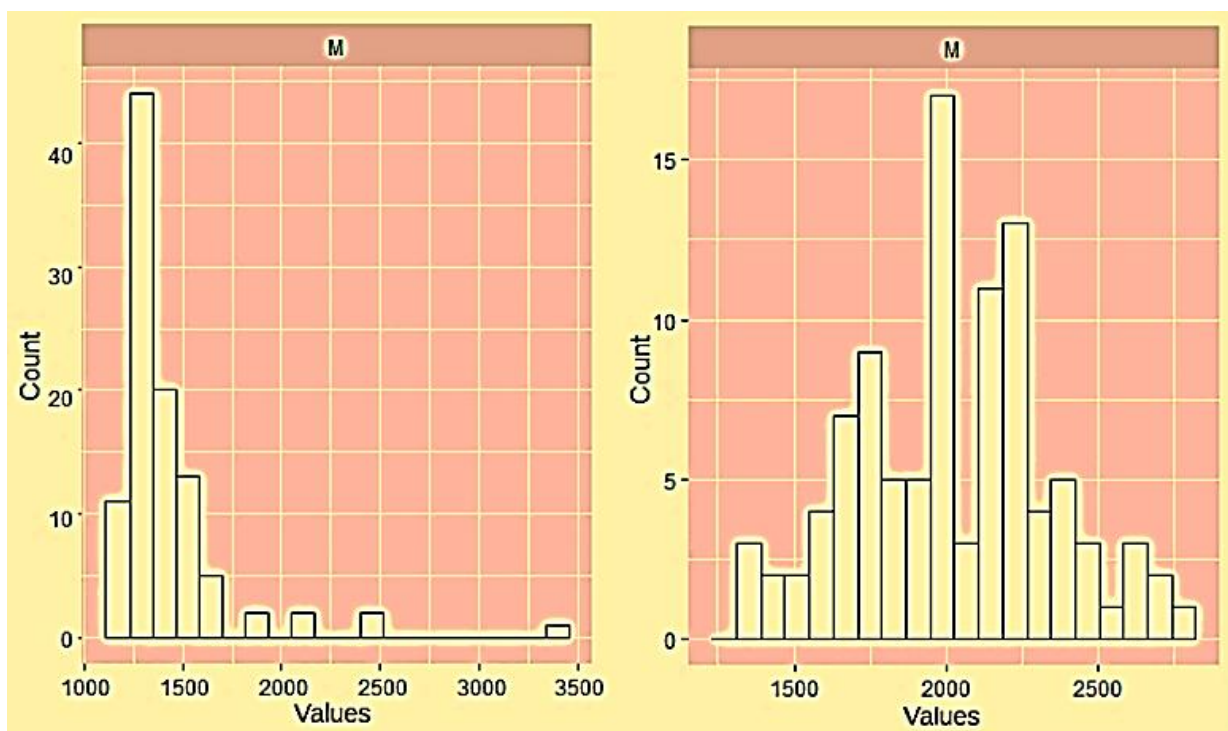


Figure 3. Histograms recorded for M from a hundred values of $\{(P_{800}, A_{800}, M_{800})\}$: Pareto distribution (left) vs. normal distribution (right), with all parameters presented above

CONCLUSION

Within this work, taking into account how difficult it can sometimes be to simulate the

continuous Wiener process, a compound Lévy process was computed upon its discrete parts, by using an efficient “discrete skeleton” structure.

The main benefit of this procedure is the computation efficiency, although a drawback of using “discrete skeletons” consists in the fact that the location of large jumps cannot be identified with precision within this mathematical simulation.

We have demonstrated that the Wiener process component inside the composed Lévy process is the one responsible for determining the variance of Y_t .

The values taken by the first-passage probability during the Lévy process were collected in a “discrete skeleton”, $\{(P_k, A_k, M_k)\}$.

These correspond to three variables meaning – as previously mentioned: “prior”, “after” and “maximum” (all of them giving useful information regarding the process at different times, either before or after a random jump of the process).

When experimenting with two different distributions of the i.i.d. variables X_k ($1 \leq X_k \leq N_t$) which can characterize the stochastic Lévy process, namely Pareto vs. normal distribution, we have observed some interesting aspects about the values that can be taken by the jumps in each case.

Finally, by analyzing any potential differences that could occur in variance given by these two statistical distributions, we have found that the presented histograms are representative for any of the three members of the “discrete skeleton”, $\{(P_k, A_k, M_k)\}$, as no other component of the process is dominant, leading to the conclusion that variance differences are noticeable from the graphs themselves, so the discussion can be successfully restricted to a single simulation run.

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IMPLEMENTING JACOBI ALGORITHM *VERSUS* GAUSS-SEIDEL ALGORITHM IN SOLVING A DISCRETIZED PROBLEM

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As it is well known that performance of algorithms generally relies both on the hardware that they are run on and on the way that they are implemented and executed the present paper aims to show that the executions' wall-clock time might be affected by parallelizing the code, although this could sometimes become a hard task. Within this work, based on Poisson partial differential equation, we shall present different aspects of a comparison between implementing Jacobi algorithm in solving a discretized problem and implementing Gauss-Seidel algorithm in solving the same. On one hand, the results obtained by implementing the Jacobi algorithm benefited very much from the parallelization, so the first case has shown both good performance and scalability when introducing supplementary threads; as its structure always needed to exhibit a complete data copy computed in its former iterations, it became easier for us to have the code parallelly executed, because there was an insignificant risk of data races' hitting. On the other hand, the results obtained by implementing the Gauss-Seidel algorithm managed to solve the discretized problem, although its performance was not as good as the one of the Jacobi algorithm, so the second case has shown a good execution performance in varying sizes of sequentially executed data, taking into account that, as far as parallelizing was concerned, the performance was unreliable and random.

Keywords: Jacobi algorithm, Gauss-Seidel algorithm, Poisson partial differential equation.

INTRODUCTION

As the Poisson equation lately becomes an interesting topic in describing the heat distribution within a uniform environment, we shall analyze through this paper the model of a cubic room, for which we shall complementarily implement the Jacobi algorithm and the Gauss-Seidel algorithm, in a sequential way, in order to solve a certain discretized problem.

By the end of the paper, we shall be able to express which one of these two algorithms is most appropriate for the chosen model, *i.e.*, which one of them is most suitable for this purpose.

PRELIMINARY STATEMENTS

For calculus' simplicity, the cubic room will be described in the 3D cartesian coordinate system as presented in Figure 1, *i.e.*, centered in the center of it – denoted by \mathcal{O} (0, 0, 0) – and having [-1, 1] as definition domain for all the coordinate axes. The vertices of the cube will be:

- \mathcal{A} (1, 1, 1);
- \mathcal{B} (-1, 1, 1);
- \mathcal{C} (-1, -1, 1);
- \mathcal{D} (1, -1, 1);
- \mathcal{E} (1, 1, -1);
- \mathcal{F} (-1, 1, -1);
- \mathcal{G} (-1, -1, -1);
- \mathcal{H} (1, -1, -1).

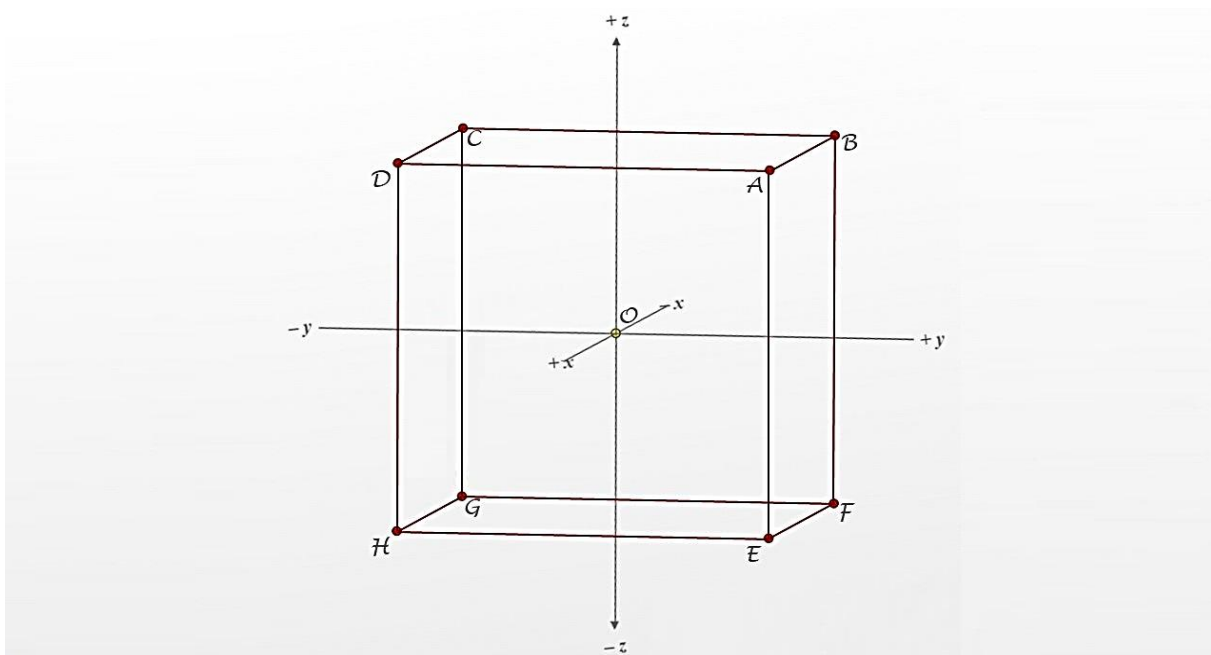


Figure 1. Presenting the position of the cubic room within the 3D cartesian coordinate system

Among the six walls, only one will be set to be cold – having a temperature of 0°C , whereas the rest of them will be set to have room temperature – more exactly, 20°C .

A radiator which is capable to emit a $200^{\circ}\text{C}/\text{m}^2$ radiation shall be placed near to the cold wall, namely at the location specified by the following function:

$$f = \begin{cases} 200, & x_1 \leq x \leq x_2; y_1 \leq y \leq y_2; z_1 \leq z \leq z_2 \\ 0, & \text{otherwise} \end{cases} \quad (1)$$

in which: $x_1 = -1, x_2 = -3/8; y_1 = -1, y_2 = -1/2; z_1 = -2/3, z_2 = 0$, as shown below, in Figure 2.

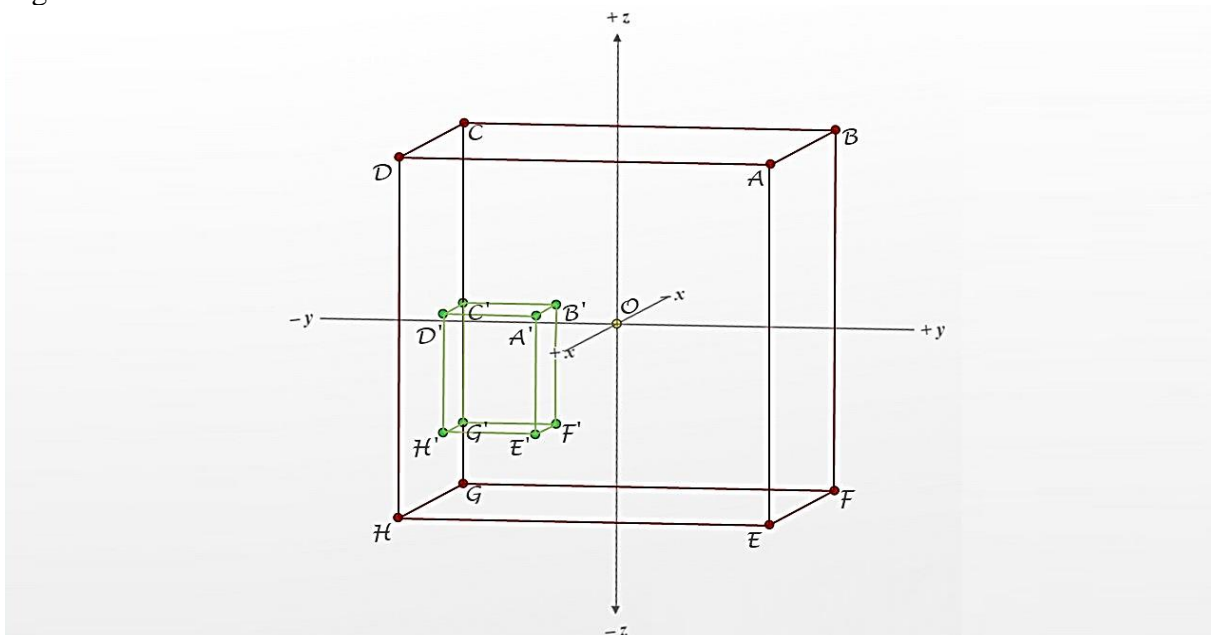


Figure 2. Presenting the position of the radiator inside the cubic room within the 3D cartesian coordinate system

The vertices of the smaller cube (representing the radiator placed inside the cubic room) will be, therefore:

$$A' (-3/8, -1/2, 0);$$

$$B' (-1, -1/2, 0);$$

$$C' (-1, -1, 0);$$

$$D' (-3/8, -1, 0);$$

$$E' (-3/8, -1/2, -2/3);$$

$$F' (-1, -1/2, -2/3);$$

$$G' (-1, -1, -2/3);$$

$$H' (-3/8, -1, -2/3).$$

The dissipation of the heat shall be described by selecting grid points inside the cubic room and estimating the heat values, by using appropriate differential equations.

The cube will fit into three different L-type caches, which will be denoted as L, L' and L''.

SPECIFICATIONS OF THE COMPUTING CLUSTER MACHINE USED FOR STUDY

As to execute the tests for this study, a computing cluster machine shall be used, whose hardware and software specifications are gathered in Table 1:

Table 1. Hardware and software specifications of the computing cluster machine used for study

Hardware specifications	Sockets	2
	CPUs/socket	12
	No. of CPUs	24
	CPUs Model	Intel Xeon Processor E5-2650
	Architecture	64-bit
	Cache L	32 kB
	Cache L'	256 kB
	Cache L''	61440 kB
Software specifications	Cubic matrix implementation	C-native way, indexed with triple pointers
	Optimization flags	-O3 and -unroll-loops
	C compiler	GCC v9.2.0

For both algorithms proposed, it would be interesting to express the maximum size of the variable N, denoting the number of grid points chosen inside the cubic room (on each axis),

In the first case (Jacobi algorithm), we will find, for each L-type cache:

$$N = \sqrt[3]{L/24} - 2 \quad (2)$$

whereas in the second case (Gauss-Seidel algorithm), we will similarly find, in each case:

$$N = \sqrt[3]{L/16} - 2 \quad (3)$$

Using these formulas, we shall calculate and report below – in Table 2 – the maximum size of N for each L-type cache.

Table 2. Maximum size of N for each L-type cache for both Jacobi and Gauss-Seidel algorithms

	Cache	Jacobi algorithm	Gauss-Seidel algorithm
Maximum size of N	L	9	10
	L'	20	23
	L''	134	154

The limits imposed for the dimensions of the grid placed inside the cubic room shown in Figure 1 are established in order to fit this entire room into each of the three caches presented before.

POISSON PARTIAL DIFFERENTIAL EQUATION

Having the limits calculated and presented before, it will become possible to establish the values of N that shall be used for our tests. Namely, the values chosen for N will be equal-to, just-above and just-below these limits, noting that all the rest of the values that N will take through the test will be in-between the limits, as to permit us to determine the behavior of both algorithms, as long as the size of the data will be kept within the above specified L-type cache sizes – as stated by Borawake and Hiwarekar (2024), as well as by Goona *et al.* (2021).

In computational fluid dynamics, Poisson partial differential equation is appropriate to describe the distribution of temperature within a particular space, such as our cubic room.

So, the main problem will be to estimate steady-state distribution of temperatures within the cubic room presented in Figure 1, taking into account the influence of the radiator placed inside it, as shown in Figure 2.

As it is well known, the equation governing this kind of diffusion process is the Poisson partial differential equation, whose general form is:

$$\frac{\partial^2 \Phi}{\partial x^2} + \frac{\partial^2 \Phi}{\partial y^2} + \frac{\partial^2 \Phi}{\partial z^2} = -f(x, y, z), \quad x_1 \leq x \leq x_2; \quad y_1 \leq y \leq y_2; \quad z_1 \leq z \leq z_2 \quad (4)$$

in which $\Phi = \Phi(x, y, z)$ represents the temperature that diffuses in space and $f(x, y, z)$ is the function already specified in Equation 1 (having there given also the limits of the intervals for x, y, z) – as shown by Amjad and Abdullah (2021), Mohanty and Niranjana (2024), Yu *et al.* (2024).

BEHAVIOR OF JACOBI ALGORITHM

Presenting Jacobi Algorithm

The Jacobi algorithm uses the grid structure of the cub so as the values of f and Φ will be stored in 3D arrays, and the values corresponding to the inner grid of Φ will be initialized to arbitrary values, as presented by Khrapov and Volkov (2024).

Afterwards, a duplicate of Φ shall be introduced, denoted as Φ_{old} , which will be initialized as identical with Φ . Having both versions of Φ , the Jacobi algorithm will alternately compute the values within the grid until reaching the convergence. The number of cubic data arrays which represent (in the present case) $\Phi = \Phi(x, y, z)$ is two. Consequently, the Jacobi algorithm will iterate over one set of computations, alternating between the two versions of Φ , allowing the algorithm to permanently keep track of latest data, which will be furthermore computed upon, but also stored in a second array, so latest data can never be overwritten until completely used.

For the Jacobi algorithm, there will be triple nested loop iterations, *i.e.*, loop iterations through all dimensions corresponding to the cubic arrays (excluding the cube boundary). During each iteration, the new values of Φ will be computed using the equation bellow:

$$\Phi_{i,j,k}^{n+1} = \frac{1}{6} \left(\Phi_{old,i-1,j,k}^{(n)} + \Phi_{old,i+1,j,k}^{(n)} + \Phi_{old,i,j-1,k}^{(n)} + \Phi_{old,i,j+1,k}^{(n)} + \Phi_{old,i,j,k-1}^{(n)} + \Phi_{old,i,j,k+1}^{(n)} + \Delta^2 f_{i,j,k} \right) \quad (5)$$

where the Δ symbol appearing in the equation above stands for the distance between the grid points placed in the same dimension.

Indexing will be made in the order: $i \rightarrow j \rightarrow k$, *i.e.*, the outer-most loop will be run through depth, the middle one – through rows and the inner-most one – through columns.

The algorithm will be ended when the difference between the values obtained for two Φ grids during two consecutive iterations will become so small as to conclude convergence. In other words, the difference between Φ and Φ_{old} (when compared to a specified threshold) should become insignificant for the algorithm to end.

Implementing Jacobi Algorithm

Implementing Jacobi algorithm will be carried out using a single thread only (procedure usually called “sequential way of implementation”), following the theoretical algorithm.

The first three arguments will be the cubic arrays of f , Φ and Φ_{old} . The constant N will represent the total number of grid points inside the cube – in all dimension – for each of the cubic data arrays, whereas the final argument will be the tolerance against which the difference between iteration will be taken into comparison. We shall also take into account the maximum number of iterations performed before ending the algorithm (*i.e.*, until reaching the convergence).

BEHAVIOR OF GAUSS-SEIDEL ALGORITHM

Presenting Gauss-Seidel Algorithm

The Gauss-Seidel algorithm is able to approximate the solution of the Poisson differential equation by using the same 3D grid as Jacobi algorithm, as shown by Khrapov and Volkov (2024).

Generally, the difference between these algorithms consists in the number of cubic data arrays which represent (in the present case) $\Phi = \Phi(x, y, z)$ – temperature that diffuses in space.

As far as the Gauss-Seidel algorithm is concerned, the number of cubic data arrays which represent $\Phi = \Phi(x, y, z)$ is reduced to just one (comparatively to two, as already stated for the Jacobi algorithm).

The values for the grid corresponding to the observation of Φ are again initialized by making an arbitrary guess. Consequently, during each iteration, the new values of Φ will be computed using the equation bellow:

$$\Phi_{i,j,k}^{n+1} = \frac{1}{6} \left(\Phi_{i-1,j,k}^{(n)} + \Phi_{i+1,j,k}^{(n)} + \Phi_{i,j-1,k}^{(n)} + \Phi_{i,j+1,k}^{(n)} + \Phi_{i,j,k-1}^{(n)} + \Phi_{i,j,k+1}^{(n)} + \Delta^2 f_{i,j,k} \right) \quad (6)$$

where the Δ symbol appearing in the equation above stands again for the distance between the grid points placed in the same dimension.

Equation (6) shows that this method of computing new values of Φ implies that, for a certain iteration, the computed values will depend on some other values, previously calculated during the same iteration.

Implementing Gauss-Seidel Algorithm

The core of the Gauss-Seidel algorithm is mostly equivalent to the one of the Jacobi algorithm, the main difference being the absence of the duplicate of Φ , which used to be called Φ_{old} , so the update calculations shall be directly performed upon the cubic data array Φ .

Compared to the other algorithm, this one does not show any reference to the previous values, at different grid points, so a temporary variable must be used in order to store any new

value. Afterwards, a computation of the squared difference between the two values shall be performed, so that eventually the old one can be replaced by the new one in the cubic data array.

After each iteration, the updated value of Φ must be compared to the grid matrix previously obtained, permanently checking if convergence is achieved, in order to stop the algorithm.

The implementation of Gauss-Seidel algorithm within our study will also be a sequential one, as in the former case.

JACOBI ALGORITHM VERSUS GAUSS-SEIDEL ALGORITHM

The performance of an algorithm is relied both on the hardware it runs on, as well as the way it is implemented and executed with respect to said hardware.

In order to ultimately compare the performances of the two algorithms submitted to our study, we shall express a formula giving the lattice updates per second, which can be denoted as $L(N)$.

$$L(N) = \frac{I \cdot N^3}{t} \quad (7)$$

where I stands for the number of iterations performed until achieving convergence, N is – as usually – the number of points inside the grid (considered in all dimensions), whereas t is the total wall-clock time of the algorithm.

We shall also calculate – in both cases – the parameter named floating-point operations per second (denoted as flop/s), which represents a measure of computer performance while developing each of the two algorithms. In order to find the expression of flop/s, the number of lattice updates per second – $L(N)$ – needs to be multiplied with F , that represents the floating-point operations number in the loop that runs through columns in each algorithm.

$$\text{flop/s} = L(N) \cdot F \quad (8)$$

Hence, combining the last two equations, we get the expression for flop/s, which will be considered as the main performance indicator for both algorithms:

$$\text{flop/s} = \frac{I \cdot N^3 \cdot F}{t} \quad (9)$$

What we can ascertain is that there is a significant performance difference between the two algorithms, in the sense that Jacobi algorithm performance varies proportional to the value of N , whereas Gauss-Seidel algorithm maintains constant performance, not being able to increase more than around one hundred updates of the lattice per second.

CONCLUSION

By making a comparison between Jacobi algorithm and Gauss-Seidel algorithm (using their sequential implementations), we can observe that, focusing on the lattice updates realized per second for different values of the number of points inside the grid, for the same size of the problem, Jacobi algorithm requires a greater number of iterations for the convergence to be achieved than Gauss-Seidel algorithm, but this observation should not automatically lead to the conclusion that the second algorithm performs stronger than the first.

Indeed, assuming that both algorithms submitted to comparison are to be executed for an entire set of values given to the number of points inside the cubic grid, maintaining this as the only changeable parameter (the maximum allowed number of iterations, as well as the tolerance

threshold remaining static during the test), the performance will be mainly expressed by the number of updates of the lattice per second.

In these terms, as previously stated, Jacobi algorithm prevails over Gauss-Seidel algorithm.

It is worth noticing that, in the textile and leather industry, efficient algorithmic performance is critical for processing large datasets involved in quality assessment, pattern recognition, and defect detection. Parallelizing algorithms like Jacobi and Gauss-Seidel in solving Poisson partial differential equations can optimize these processes by reducing computational time for simulations and data analysis. For instance, Jacobi's algorithm, with its favorable scalability in parallelization, can be applied in modeling heat distribution in fabric treatments or predicting wear in leather surfaces. Meanwhile, even though the Gauss-Seidel algorithm may not parallelize as efficiently, its sequential strength makes it valuable for smaller, detailed simulations, such as stitching pattern assessments or localized stress testing in textiles. By improving algorithm performance in these areas, this research supports faster and more reliable quality control in textile and leather production, ultimately enhancing product durability and efficiency of production.

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DESIGN OF NEW STRUCTURED BIOEMULSIONS, BASED ON VEGETABLE EXTRACTS AND SURFACTANTS, USING INNOVATIVE BIOTECHNOLOGIES

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New bioemulsions were created using innovative biotechnologies based on: sage, geranium and lemongrass extracts and 3 surfactants mixture, Lauryl glucoside/Tween 20/Tween 80, for different concentrations of extract plants, to improve surface properties. A comparison was made for the way to solubilize and encapsulate the three vegetable extracts in emulsions. The order of introduction the components into the developed biotechnologies, the working conditions and especially the choice of the concentration of surfactants >CMC, is essential in the solubilization of vegetable extracts and obtaining the desired bioemulsions. The bioemulsions were characterized by optical microscopy with or without three types of vegetable extracts at 23-50°C. The changes in the aggregation process were observed for each type of emulsion, the influence of temperature and the solubilization of vegetable extracts. By dynamic light scattering (DLS) the stability, concentration, particle size, polydispersity, and zeta potential of bioemulsions were observed. FTIR measurements highlighted the interaction mechanism of surfactants with vegetable extracts from the created bioemulsions. A mechanism of solubilization of vegetable extracts in surfactant micelles was proposed in this research. Vegetable extracts are hydrophilic and attach to the hydrophilic groups of the chains. For Tween 80, the amount of solubilized vegetable extract is higher than in the case of Tween 20, because it has more hydrophilic groups. Van der Waals interaction forces are responsible. The modeling of release speed in water for each vegetable extract solubilized and encapsulated in bioemulsions can be done based on the first-order profile.

Keywords: structured bioemulsions, innovative biotechnologies, vegetable extracts

INTRODUCTION

Three oil vegetable extracts from geranium, lemongrass, and sage were selected to be solubilized and encapsulated in bioemulsions based on mixture of Lauryl glucoside/Tween 20/Tween 80. We compared the way to solubilize and encapsulate the three vegetable extracts in emulsions. Lauryl glucoside is a surfactant used in cosmetics and laundry detergents. It is a glycoside produced from glucose and lauryl alcohol. Lauryl glucoside is a non-ionic surfactant and member of the alkyl glucoside family which are substances formed by mixing alcohols and sugar and/or glucose. This ingredient is usually sustainably sourced from palm kernel oil, corn sugar or coconut. It improves the cleansing process without stripping necessary moisture (Das *et al.*, 2022).

Tween 80 is a polyethylene sorbitol ester, also known as Polysorbate 80, PEG (80) sorbitan monooleate, polyoxyethylenesorbitan monooleate. Tween 80 is widely used in biochemical applications including: solubilizing proteins, emulsifying and dispersing substances in medicinal and food products (Hughes *et al.*, 2021; Nuraje *et al.*, 2013). Tween 80 is an antibacterial agent, schematic representation in Fig. 1.

Tween 20 is a polyoxyethylene sorbitol esteris, a frequently used member of the polysorbate family. These have been used as emulsifying agents for the preparation of stable oil-in-water emulsions. Tween 20 has been used in pre-extraction of membranes to remove

peripheral proteins (used at 2% for extraction of membrane-bound proteins). It has been used as a blocking agent for membrane-based immunoassays at a typical concentration of 0.05% (Vărășteanu, 2014). A schematic representation of Tween 20 is shown in Fig. 2.

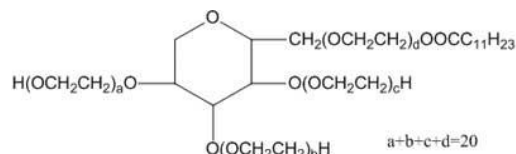
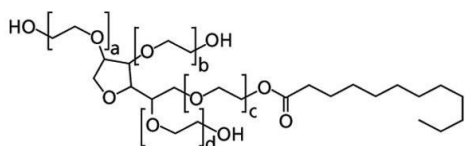


Figure 1. Schematic representation of Tween 80 Figure 2. Schematic representation of Tween 20

Tween is a group of non-volatile surfactant derivatives derived from glycerol esters (Baker and Sanders, 1986). Tween 20 and Tween 80 vary in chemical and physical properties and are usually solubilized or suspended in water. Tween 20 is mainly used as an effective binding agent in the production of foam and other polymers by means of its high solubility and low boiling point. Tween also has other important uses as a thermosetting agent in the process of manufacturing thermosetting plastics and as an adhesive for repairing paper materials. The most important usage of Tween is its application as an oil absorber and emulsifier. It is also used in the manufacturing of water-based and oil-based goods like shampoos, facial masks, hair gels, ointments, soaps, and cleansers. These goods are usually produced using emulsifying waxes. These three surfactants selected have been used as emulsifying agents for the preparation of stable water/in water emulsions.

In this research new structured bioemulsions based on oil vegetable extracts and surfactants were created and analyzed by: optical microscopy, FTIR-ATR spectroscopy and dynamic light scattering (DLS) and microbiological tests.

MATERIALS AND METHODS

In order to obtain new structured bioemulsions, the following materials have been used: Lauryl glucoside, Tween 20 and Tween 80, ethanol as co-solvent from Sigma-Aldrich; oil vegetable extracts of: geranium, lemongrass, sage from “VIORICA” company. The experimental techniques used in this paper consist in: optical microscopy with an ELTA 90 Medical Research S.R.L. equipment; DLS with a Zetasizer-nano “MALVERN” equipment, with measuring range between 0.3 nm- 60.0 μ m and zeta potential determination with an accuracy of $\pm 2\%$; FTIR-ATR spectroscopy with a JASCO spectrophotometer. The resistance to the action of bacteria of the 1-4 samples of bioemulsions was determined according to SR EN ISO 20645/2005.

The bioemulsions obtained in this research are presented in Fig. 3: **sample 1** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside; **sample 2** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and geranium extract; **sample 3** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and lemongrass extract; **sample 4** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and sage extract.

RESULTS AND DISCUSSIONS

Obtaining Bioemulsions with Solubilized and Encapsulated Vegetable Extracts

The encapsulation of vegetable extracts in bioemulsions is a two-step emulsification process. The result are multiple emulsions with vegetable extracts encapsulated due to the properties of the 3 surfactants (Tween 20, Tween 80, Lauryl Glucoside) used, to orient and form emulsions at the nano and micro levels.

Multiple bioemulsions are complex systems, also called ‘emulsions of emulsions’, in which the dispersed phase droplets contain a continuous phase with other dispersed droplets. In the first step (I) – introduction in water/ethanol at 1:1 ratio of the surfactants: Lauryl Glucoside (c=5%), Tween 20 (c=5%), and Tween 80 (c=5%), with HLB from 7 to 10, by homogenizing and stirring at room temperature for 1 hour; speed at 1000 rpm; in the second step (II) – vegetable extract (c=6%), (from geranium, sage or lemongrass) is added or not (for multiple emulsion without extracts) at pH=6 adjusting with a phosphate buffer solution (PBS), and homogenized by stirring at 50°C, for 30 minutes, speed at 1000 rpm and returned to room temperature, stirring for another hour. Multiple structured bioemulsions are obtained with solubilized vegetable extracts, Fig. 4.

Multiple bioemulsions are fragile systems, so the choice of emulsification methods is of particular importance in the success of obtaining vegetable extracts solubilized and encapsulated in emulsions with the desired properties.



Figure 3. a) Photographic image of **sample 1** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside; b) Photographic images of: **sample 2** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and geranium extract; **sample 3** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and lemongrass extract; **sample 4** – multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and sage extract

Characteristics of Bioemulsions with Solubilized and Encapsulated Vegetable Extracts

Dynamic Light Scattering (DLS)

The four samples obtained and presented in the Fig. 3 were characterized by dynamic light scattering. Dynamic light scattering tests showed that all 4 bioemulsions with or without vegetable extracts are nano and microstructured. The size, percentage of the particles and Zeta potential were determined (indicating their stability), Table 1.

The experimental results of dynamic light scattering showed that the size of the particles and their distribution differ depending on the way of encapsulation of the plant extract in bioemulsions and the type of plant extract used. Zeta potential shows a tendency towards agglomeration. The influence of the three surfactants selected (Lauryl glucoside/Tween 20/Tween 80) was to obtain stable bioemulsions with encapsulated vegetable extract. For the bioemulsions with sage extract, the particles with the smallest dimensions of 55.7 nm were obtained.

Table 1. Experimental results of dynamic light scattering for the four bioemulsions obtained

No. sample	Sample at room temperature	Average diameter (nm)	% Intensity	Zeta Potential (mV)
1	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside	1209.6	76.3	-33.5
		32.33	19.1	
		13.20	4.6	
2	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and geranium extract	412.1	91.7	-52.3
		58.13	7.39	
		1000	1.0	

No. sample	Sample at room temperature	Average diameter (nm)	% Intensity	Zeta Potential (mV)
3	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and lemongrass extract	163.7	100	-38.2
4	Multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside and sage extract	341.3	93.6	-47.2
		55.7	6.4	

Optical Microscopy Tests

The optical microscopy images from Fig. 4 showed the four bioemulsions with or without vegetable extract encapsulated at room temperature and 50°C. All four bioemulsions presented in Fig. 4 had a good encapsulation process of vegetable extract. Fig. 4 shows oriented and agglomerated structures. The results are in agreement with literature data (Das *et al.*, 2022; Hughes *et al.*, 2021; Nuraje *et al.*, 2013; Vărășteanu, 2014) related to the formation of structures in multiple bioemulsions.

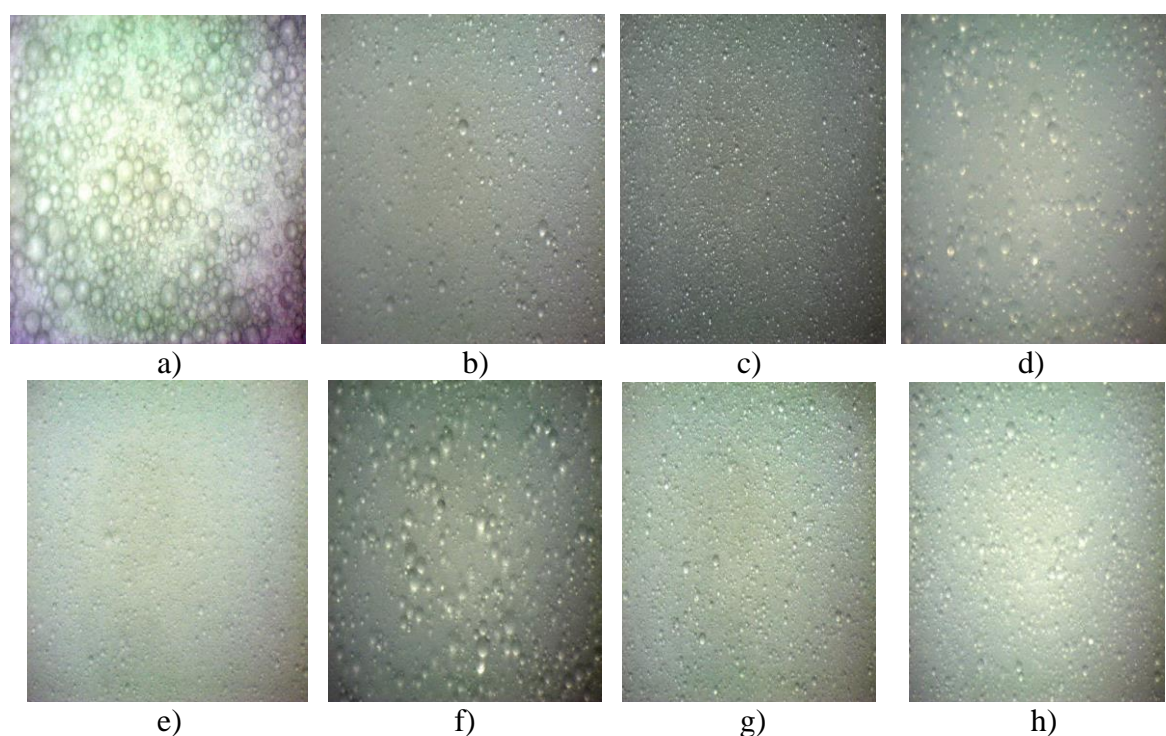


Figure 4. Optical microscopy images (1000x) for samples 1-4 at room temperature and 50°C: a) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside at room temperature; b) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside at 50°C; c) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with geranium extract at room temperature; d) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with geranium extract at 50°C; e) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with lemongrass extract at room temperature; f) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with lemongrass extract at 50°C; g) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with sage extract at room temperature; h) multiple emulsion based on Tween 20/Tween 80/Lauryl Glucoside with sage extract at 50°C

It is observed that the introduction of a vegetable extract into the bioemulsion leads to a decrease in the size of the structures formed both at room temperature and at 50°C (Fig 4.a reported to c, e, g).

FTIR-ATR Spectroscopy Tests

An FTIR-ATR spectrophotometer was used to analyze bioemulsion samples **1-4** (Fig. 3) and each extract selected or surfactants Tween 20, Tween 80 (Fig. 5).

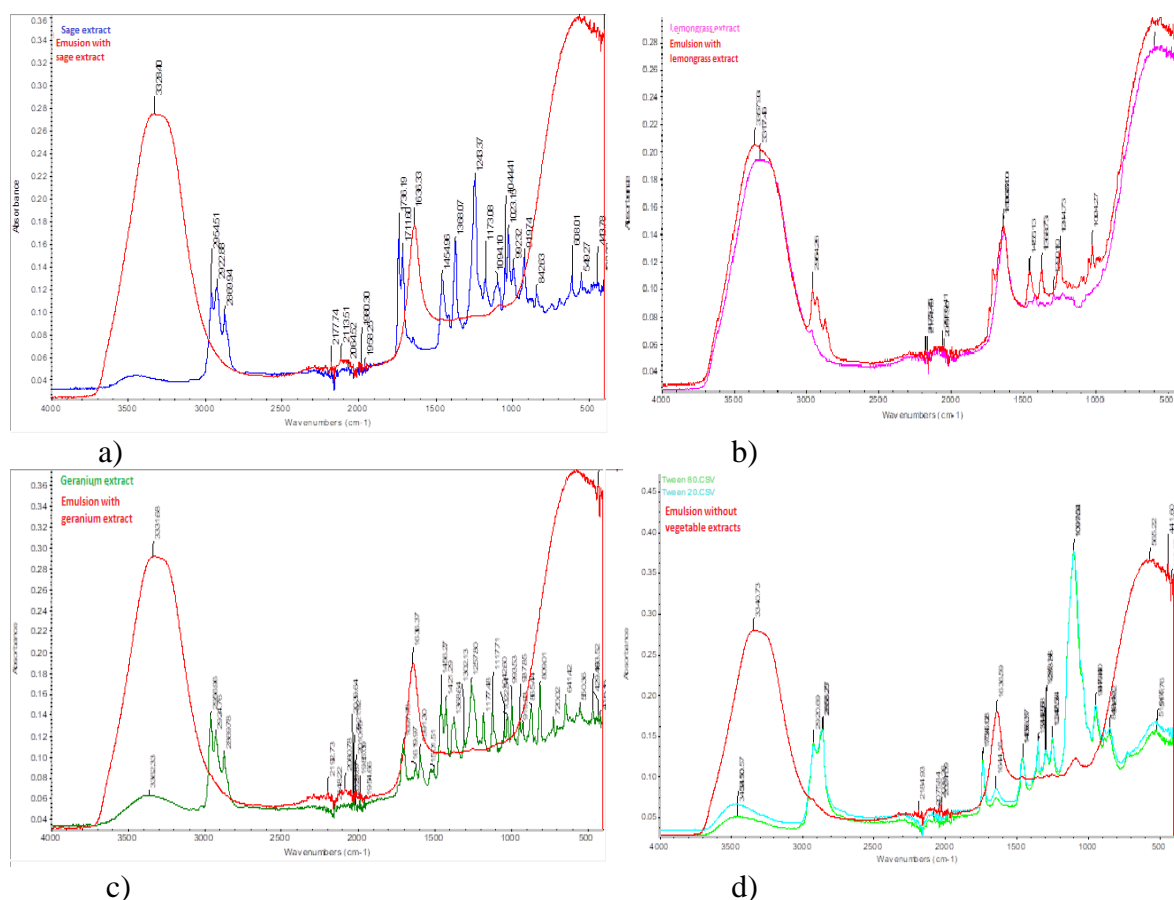


Figure 5. Overlapping FTIR-ATR fingerprint spectra of: a) sage extract -- and bioemulsion with sage extract--; b) lemongrass extract -- and bioemulsion with lemongrass extract--; c) geranium extract -- and bioemulsion with geranium extract--; d) emulsion without vegetable extracts -- and Tween 80—Tween 20--

All analyzed bioemulsions (samples **1-4**) have a maximum absorption at the wave number 3343 cm^{-1} due to the presence of Tween 20 and Tween 80 surfactants in emulsions (Fig. 5-d).

Microbiological Tests

The four samples were also microbiologically analysed, to determine behaviour to bacterial attack of *Staphylococcus aureus* and *Escherichia coli*, carrying out analysis three days from inoculations. The best results in order, were obtained both for *Staphylococcus aureus* and *Escherichia coli* for samples **4>2>3>1**.

Specimens of the material to be tested are placed on two-layer agar plates. The lower layer consists of a culture medium without bacteria while the upper layer is seeded with the selected bacteria. The level of antibacterial activity is assessed by examining the area of bacterial growth in the area of contact between the agar and the test tube, and if applicable the area of the zone of inhibition around the test tube.

Method used:

- replication of the bacteria used in the test: *Escherichia coli* ATCC 11229 (gram-negative), *Staphylococcus aureus* ATCC 6538 (gram-positive). We work with a pure, freshly propagated culture;

- dry sterilization of laboratory glassware in an oven at 180°C;
- preparation of the culture medium, characteristic of the test bacteria used, namely: -Nutrient Agar for the genus *Escherichia coli* and Mannitol Salt Agar for the genus *Staphylococcus aureus*;
- wet sterilization in the autoclave and Erlenmeyer vessels with culture media;
- the samples must be circular, with a diameter of 25±5 mm.

The agar volume is prepared for the bottom layer without bacteria. In each sterilized Petri dish (10 ± 0.1) ml are placed and the agar is allowed to solidify. The amount of agar for the top layer is prepared and cooled to 45°C on a water bath. 150 ml of agar are inoculated with 1 ml of bacterial working solution (1-5 x 10⁸ cfu/ml). The container is shaken vigorously to distribute the bacteria evenly. To each Petri dish (5±0.1) ml are added and the agar is allowed to solidify. The samples are placed on the surface of the nutrient medium and then incubated at 37°C.

The Mechanism Proposed for Vegetable Extracts Solubilization (Encapsulation) in Surfactant Micelles

The mechanism proposed for encapsulation of vegetable extracts (sage, geranium, lemongrass) in three surfactant micelles (Tween 20/Tween 80/Lauryl Glucoside) and obtained structured bioemulsions were investigated using FTIR-ATR spectroscopy, DLS and optical microscopy, Fig. 6.

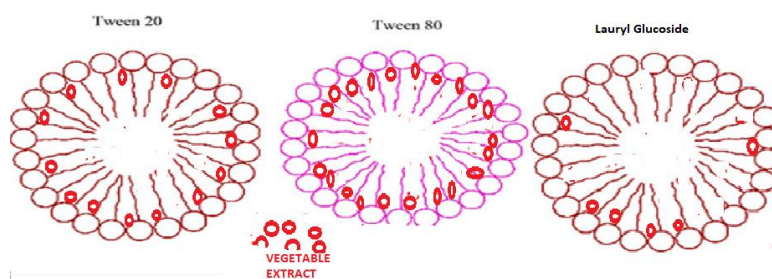


Figure 6. Mechanism proposed for vegetable extracts selected, solubilization (encapsulation) in surfactant micelles of: Tween 20, Tween 80, Lauryl Glucoside

The results have outlined three distinct processes depending on the surfactant concentration. In the pre-micellar range, the variation in absorbance and peak was attributed to the attraction of the initially head group to the β-diketone group of each selected vegetable extract. At surfactant concentration in intermediate/micellar region including CMC, a second type of interaction is observed, corresponding to the attachment of alkyl chains of surfactant to aryl groups of each selected vegetable extract and displacement of head group from β-diketone group of the vegetable extract. Finally at surfactant concentration higher than the CMC, in the postmicellar region, a type of interaction is observed, which corresponds to the encapsulation/solubilization of each selected vegetable extract into micelles, predominantly in monomeric form (Fig. 7).

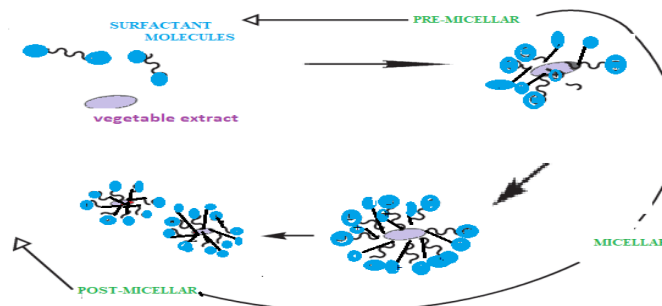


Figure 7. Schematic presentation of a proposed mechanism of interaction between selected vegetable extracts and surfactant molecules of Tween 20, Tween 80, Lauryl Glucoside

Modeling the Encapsulation Process of Selected Vegetable Extracts in the Obtained Structured Bioemulsions

Modeling encapsulation process of vegetable extracts selected in structured bioemulsions obtained was carried out taking into account all the parameters: the concentration and type of surfactants, the ratio between vegetable extracts selected and surfactants, micellar critical concentration of surfactants, speed and time of stirring, temperature, pH, average diameter of particles, zeta potential.

In this research is modeling the speed yield of each vegetable extract in water (which is encapsulated in bioemulsions) for the best results of samples: **4** and **2**. The dependence of absorbance to the encapsulated each vegetable extract from samples: **4** and **2**, yielded in water, on the time, was also analysed. A modeling program is created that can calculate the speed of yielding in water, of each vegetable extract encapsulated in emulsions for samples: **4** and **2** (for which the best results were recorded). The calculation modeling program created is in VBA with the Excel Worksheet interface. The modeling program was applied for the two selected samples: **4** and **2**. According to the modeling program, for sample **4**, the speed yield in water of *vegetable extract-sage* encapsulated in emulsion is: speed of yielding in water, of vegetable extract encapsulated in sample 1 is $v = 0.00000422$ unit. of Abs/min. For sample **2**, the speed yield in water of *vegetable extract-geranium* encapsulated in emulsions is, according to the modeling program: speed of yielding in water, of vegetable extract encapsulated in sample **2** is $v = 0.0076597$ unit. of Abs/min. It is observed that speed yield of vegetable extract in water (which is encapsulated in emulsions) is different depending on the type of surfactant used and its interaction with each vegetable extract selected (geranium or sage). The modeling of release speed in water, of each vegetable extract encapsulated in bioemulsions can be done based on the study of Baker and Sanders (1986). In Table 2 the theoretical results of three models on a data set (for sample **4**) are presented comparatively, and in Fig. 8, the comparative graphic representation.

Table 2. Comparison of failure profiles (Baker and Sanders, 1986)

	$S \cdot 10^5$	a	σa	b	σb	c	σc
Zero order	4.4	0.496645	5.39E-05	-0.00015	1.97E-05		
Order one	2.41	0.49589	0.000156	0.000956	8.46E-05	0.407194	0.173009
Radical order	2.32	0.496968	4.93E-05	-0.00045	3.12E-05		

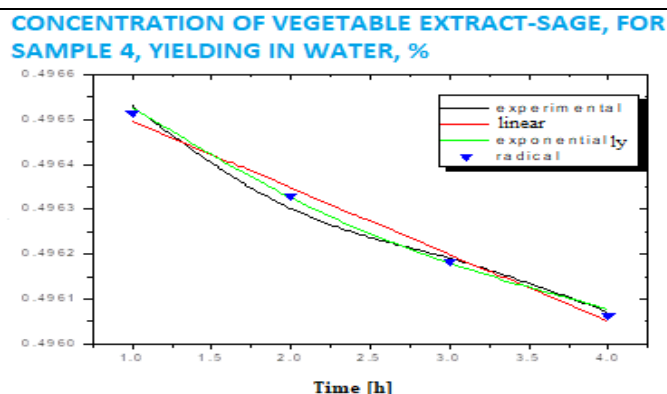


Figure 8. Comparison of yield profiles for sample **4**

The **first order** failure profile was chosen because there are two values of the absorbance (initial and final), and the failure of speeds yield of vegetable extract-sage (sample

4) in water are very low. The modeling of release speed in water, of vegetable extract-sage encapsulated in bioemulsion (sample 4), can be done based on the first-order profile.

CONCLUSIONS

Tween 20, Tween 80, Lauryl glucoside are surfactants that play a critical role in bioemulsion preparations, especially in forming stable bioemulsions and solubilizing vegetable extracts in aqueous solutions.

The paper proposed a mechanism of solubilization (encapsulation) of vegetable extracts (sage, geranium, lemongrass) in surfactant micelles of: Tween20, Tween 80, Lauryl Glucoside. For Tween 80, the amount of solubilized vegetable extract is higher than in the case of Tween 20, because it has a larger hydrophobic chain. Van der Waals interaction forces are responsible for this.

The changes in the aggregation process were observed for each type of bioemulsion, the influence of temperature, the solubilization (encapsulation) of each vegetable extracts by dynamic light scattering and optical microscopy.

The mechanism of interaction for surfactant micelles and each vegetable extract selected was created. Also, the encapsulation of each vegetable extract selected in bioemulsions was modeled taking into account all the parameters.

The versatility, compatibility and stability-enhancing properties of bioemulsions created, make them valuable ingredients in a variety of industries including the leather or textile industries.

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STUDY ON THE COMPOSITION OF VULCANIZED RUBBER MIXTURES FROM WASTE TIRE

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As a result of the development of the automobile industry, the number of used tires has significantly increased. Since they are non-biodegradable and represent a danger to the environment, methods have been developed to capitalize on them, namely: by reusing, by recycling or recovering energy. The following materials are generally found in the tire composition: 14-30% natural rubber, 11-30% synthetic rubber, 20-30% carbon black, 12-27% steel, 0-10% textiles, 7-14% other components. From the mixture of vulcanized rubber existing in used tires, following a grinding and sieving process, rubber granules and rubber powder can be obtained. They can be used to make polymer composites for different fields. The properties of these types of materials are greatly influenced by the composition of the rubber powder, especially the filler content and the type of natural or synthetic rubber that the initial products had. The purpose of this study is to analyze the composition of the vulcanized rubber mixture from four types of used tires. The samples were analyzed by: determining the ash content and acetone extract, Burchfield test and FTIR spectroscopy. The identification of the type of rubber by the Burchfield test and FTIR spectroscopy showed that the samples contain natural rubber, or a mixture of natural rubber with butadiene-styrene rubber. The ash test and the acetone extract, together with their FTIR analysis, indicated that the crosslinking of the rubber mixtures was obtained with sulfur in the presence of vulcanization accelerators and zinc oxide. The amounts of resins, antioxidants, mineral oils, waxes or fatty acids is below 10%. The development of methods for determining the composition of rubber powder can contribute to obtaining new types of polymer composites with improved properties.

Keywords: waste tire rubber, ash, acetone extract, FTIR.

INTRODUCTION

The dynamic increase in the production of rubber products, especially those used in the automotive industry, is responsible for the large amount of vulcanized rubber waste, especially in the form of used tires. More than 17 million tons are produced globally each year. China, the European Union (EU), the US, Japan and India generate the largest amounts of used tire waste – almost 88% of the total number of used tires worldwide (Sienkiewicz *et al.*, 2012; www.jatma.or.jp). For example, in the EU, the number of used tires increased from 2.1 million tons in 1994 to 3.4 million tons in 2010, and 3.55 million tons in 2020, respectively (Table 1) (Sienkiewicz *et al.*, 2012; Sienkiewicz *et al.*, 2017; www.etrma.org).

The diversification of chemical compositions and three-dimensional structures existing in vulcanized rubber from tires (see Table 2) is the main reason why they are highly resistant to biodegradation, photochemical decomposition, chemical reagents and high temperatures. Given the increased number of used tires, they represent a serious threat both to the natural environment and to human health due to fire risks and their use as a suitable habitat for rodents, snakes and mosquitoes (Sienkiewicz *et al.*, 2012; www.jatma.or.jp; Jahirul *et al.*, 2021).

The progress made in recent years in the management of polymer waste has led to the perception of used tires as a potential source of energy or new raw materials. The development

of studies on their more efficient recovery and recycling and the restrictive legal regulations of the European Union regarding the management of used tires, have led to solutions that favor the transformation of this substantial flow of rubber waste into new polymeric materials or alternative fossil fuels (Sienkiewicz *et al.*, 2012).

Table 1. Recovery of used tires in 2010 and 2020 (www.etrma.org, Sienkiewicz *et al.*, 2012)

Recovery Method	2010		2020	
	Romania	UE	Romania	UE
Amount of used tires collected (kT)	33	3420	54	3555
Amount of scrap tires recovered (kT)	33	2600	54	3265
• Recycled materials (kT)	1	1200	0	1920
• Civil engineering and public works (kT)	0	240	0	112
• Energy recovery (kT)	32	1160	54	1249
The quantity of unrecycled tires (kT)	0	170	0	290
Percentage of recycled tires (%)	100	95	100	92

Table 2. Component materials of tires (%) (Sienkiewicz *et al.*, 2012; Ramarad *et al.*, 2015)

Ingredient	Amount (%)	Function
Natural rubber	14-30	Structural element
Synthetic rubber	11-30	Properties improvement
Carbon black	20-30	Rubber reinforcement
Steel	12-27	Casing reinforcement
Textile	0-10	Structural element
Additives (antioxidants, curing system, etc.)	7-14	Rubber performance improvement

The legal ban on landfilling tires has been driven by increased levels of recovery and recycling. In this sense, the policies of most countries regarding the disposal of used tires are based on their selective collection and management through the following methods (www.jatma.or.jp; www.etrma.org; www.rma.org; www.wbcsd.org): reprocessing for reuse (extending the life of tires), energy recovery, pyrolysis, product recycling, material recycling (grinding and devulcanization), etc.

Currently, end-of-life tires have become the source of materials for obtaining “environmentally friendly” polymer composites with useful properties. Thus, rubber granules and powder can be obtained from used tires following a grinding and sieving process; they can be reused to obtain different types of composites with elastomeric or thermoplastic polymer matrix. The method of manufacturing polymer composites using rubber powder from recycled tires is very simple and can be done using already known technologies, machines and equipment used in the polymer industry. The implementation of these composites in industrial production does not require large investments. These composites are used to manufacture items such as: floor materials, windshield wipers, washers, strips, molds, cable housings, shoe soles, etc. (Sienkiewicz *et al.*, 2017). The properties of these types of materials are influenced by the method of obtaining the rubber powder (cryogenic or ambient temperature grinding), the size of the rubber granules, the crosslinking method of the rubber mixture, the filler content and the type of natural or synthetic rubber that the initial products had. At the same time, their properties depend on the type of polymer matrix used and, therefore, on the nature of the interactions between the matrix, the type and size of the rubber granules and the amount of rubber powder in the composite (Ramarad *et al.*, 2015; Formela, 2022; Kiss *et al.*, 2022).

The purpose of this paper is to determine the composition of vulcanized rubber mixtures derived from four types of used tires and to compare the results obtained with the existing data in the scientific literature.

EXPERIMENTAL

Materials

During the experiments, four types of vulcanized rubber mixtures were analyzed, which were obtained from four types of used tires, according to Table 3.

Table 3. Types of used tires for which the composition of the rubber mixture is determined

Sample code	Type of used tire	Producer	Characteristics	Photo
1	Nankang Cross Season 185 -60 - 15	Nankang Rubber Tire Corporation Ltd., Taiwan	All season tires The speed index: H Load index 88	
2	Laufenn 225 – 65 – 16 C	Hankook Tire Co. Ltd., Hanover, Germany	Winter tires Speed index: R Load index: 112/110	
3	Nokian Hakka Green 195 – 65 - 15	Nokian, Finland	Summer tires Speed index: H Load index: 95	
4	Radbeug 185 – 65 – 15 Power	Radburg Center SRL, Romania	Summer tires Speed index: T Load index: 88	

Laboratory Analyses

The vulcanized rubber samples obtained from used tire waste were tested to determine the composition by: Burchfield test, determining the ash, determining the acetone extract and FTIR spectroscopy.

Burchfield test – the identification of the type of rubber present in the post-consumer tire rubber waste by a Burchfield test was carried out using Burchfield reagent 1, which consists of: 1 g of para-dimethylaminobezaldehyde, 0.01 g of hydroquinone and 100 ml of absolute methyl alcohol, to which are added 5 ml of concentrated hydrochloric acid. The color change that occurs during the test indicates the presence of a specific elastomer. The method was the following: about 1 gram of the ground rubber sample was placed in a glass test tube with fusible glass, and in another test tube, 2 ml of reactive solution type 1 was poured. The tube filled with rubber sample was heated with a flame, until the vapors that released reached the second tube with type 1 reactive solution. The color of the solution after cooling and mixing was noted. Then the solution was diluted with 5 ml of methanol and the specimen was introduced in a boiling water bath (at 100° C) for 3 minutes. The color obtained was noted, because the color change provides information about the type of elastomer (Braun, 1986; Verleye *et al.*, 2001; Burchfield, 1946).

The determination of the ash content (mineral substance content) – in accordance with ISO 247, Rubber, Determination of ash — Part 1: Combustion method. For the determination, an amount of 5-6 g of waste rubber sample was cut into parallelepipedal pieces with sides of about 2 mm and then placed in a porcelain crucible previously brought to constant weight. The crucible with material was burned with the help of a gas bulb, in the niche, until the organic material was exhausted, after which it was placed in a calcination furnace at high temperature (800°C) until reaching constant mass. Ash content was calculated using relation (1) (Test Methods of Rubber Materials and Products, Matador Rubber S.R.O., 2007):

$$\text{ash content}(\%) = \frac{m_i}{m_f} \times 100 \quad (1)$$

in which: m_i is the mass of the material weighed initially and m_f is the mass of the residue after calcination, in g.

The determination of the acetone extract – according to ISO 1407, Rubber, Determination of solvent extract, Method B. The method is based on the property that resins have to dissolve in certain organic solvents. An amount of 5-6 g of sample cut into small pieces (about 2 mm) was weighed and a medium porosity filter paper cartridge was formed. The sample cartridge was inserted into the Soxhlet apparatus (mounted on a water bath) and attached to the extraction flask previously brought to constant weight (by heating to 105°C). The required amount of acetone was added and extracted until the liquid was observed in the level tube (18 hours). After extraction, the cartridge was removed, the acetone was removed by distillation, and the flask was dried and brought to a constant mass (in an oven at 105°C for 3 hours). The acetone extract content was calculated from the relation (2):

$$\text{acetonic extract}(\%) = \frac{m_1 - m_2}{m} \times 100 \quad (2)$$

in which: m_1 is the mass of the flask with residue, m_2 is the mass of the empty flask, and m is the mass of the rubber waste test sample, in g.

Fourier Transform Infrared Spectroscopy (FTIR) spectra of samples was obtained using Nicolet iS50 FT-IR spectrophotometer in the wave number ranging from 400 cm^{-1} to 4000 cm^{-1} . To analyze the type of rubber in the samples, the samples were tested as such (coded 1T-4T) as well as by analyzing the uncured elastomer that dissolved after immersing the samples in toluene for 72 hours. Before testing, the toluene was evaporated by keeping the samples at room temperature for 3 days, then placing them in an oven at 80°C for 8 hours. These samples were coded 1N-4N.

RESULTS AND DISCUSSION

Results of the Analyses Carried out in Order to Identify the Types of Elastomers

According to the scientific literature, car tires generally contain natural rubber, or a mixture of natural rubber with other types of elastomers (Ramarad *et al.*, 2015; Formela, 2022). The identification of the types of elastomers present in the analyzed tire waste was achieved both by a Burchfield color test and by FTIR spectroscopy.

The results obtained from the Burchfield test (Fig. 1) indicate that all types of analyzed waste contain natural rubber, because the color initially obtained was brown (Fig. 1b). After adding 5 ml of methanol and keeping the samples in a boiling water bath for 3 minutes (Fig. 1c), samples 1-2 had a dark blue violet color, the other samples had a dark green color. According to scientific literature (Verleye *et al.*, 2001; Burchfield, 1946) samples 3 and 4 contain a mixture of natural rubber (NR) and styrene-butadiene rubber (SBR), and samples 1 and 2 contain natural rubber.

In order to verify the results obtained with the elastomer identification by the Burchfield test, FTIR analyses of the samples were performed (Fig. 2), as well as the analysis of the unvulcanized elastomers, which were extracted in toluene (Fig. 3). Analyzing the obtained FTIR spectra, in all the samples the existence of the bands corresponding to natural rubber is observed, namely: the specific bands from 810-814 cm^{-1} corresponding to the C-H deformation vibration from the chemical group cis -C=C-, those from 1370-1376 cm^{-1} and 1427-1498 cm^{-1} corresponding to the deformation of the C-H bond from -CH₃ and -CH₂, or those from 1600-1665 cm^{-1} due to the stretching of the C-C bond from -C=C-, as well as the bands from 2910-2921 cm^{-1} and 2847-2852 cm^{-1} specific C-H symmetrical or asymmetrical from -CH₂- or -CH₃. For samples 3 and 4 the band at 1012 cm^{-1} specific to C-H (trans) from the -(CH=CH)- groups of the butadiene chains of SBR can be observed in Fig 2., and in Fig. 3 the band at 699-700 cm^{-1} can be seen, specific to C-H deformation in monosubstituted benzene, specific to polystyrene chains in SBR. Other absorption bands that can be observed are those from 450-550 cm^{-1} and the bands

from 600-700 cm^{-1} specific to C-S from sulfur vulcanization. The bands from 952 cm^{-1} appear due to the deformation of the C-H bond from trans -C=C- , while the bands from 720-722 cm^{-1} and 740-746 cm^{-1} may be due to the C-H bond from -CH_2 . For samples 3-4 bands of low intensity can be observed in the range 2000 -1660 cm^{-1} specific to the C-H deformation corresponding to SBR polystyrene chains (Roy & De, 1992; Manaila *et al.*, 2022).

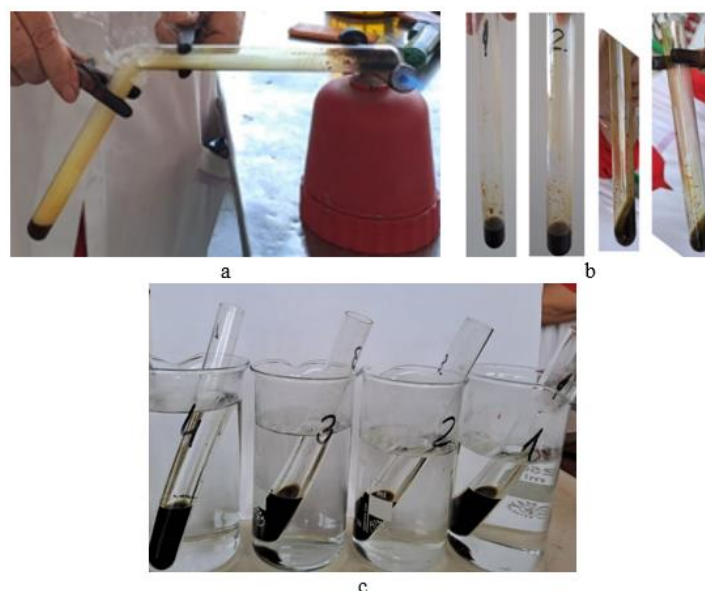


Figure 1. Images during the identification of the type of rubber from waste tires by the Burchfield test: (a) the two test tubes, with the rubber sample and the respective reagent type 1, (b) the colors obtained initially, (c) the colors obtained after the addition of methanol and immersion in a hot water bath

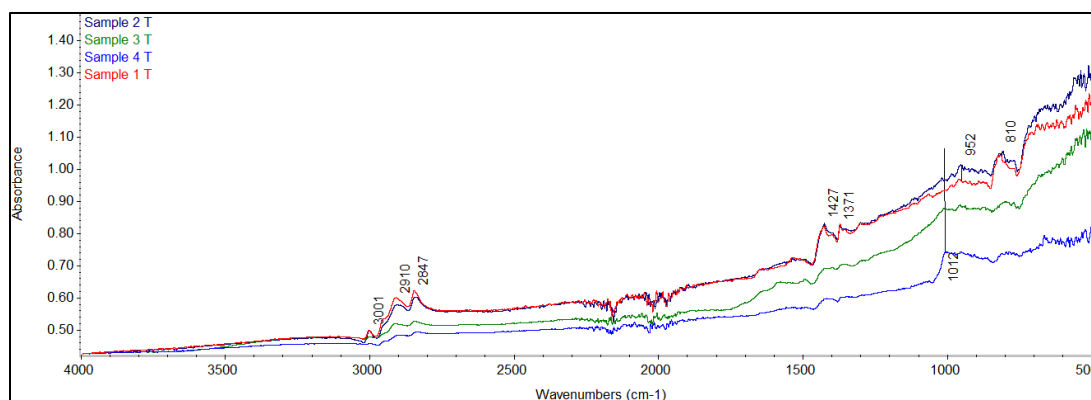


Figure 2. FTIR spectra of vulcanized rubber samples from used tires (coded 1T-4T)

Ash Analysis

The amount of ash obtained from tire waste provides information regarding the amounts of inorganic compounds in the powder such as metal oxides, inorganic dyes or inorganic fillers. According to the results presented in Table 4, it can be observed that for all four samples small amounts of ash were obtained, of 2.26-3.74% (Fig. 4a). FTIR spectroscopy was performed to identify the ingredients present in the ash (Fig. 5).

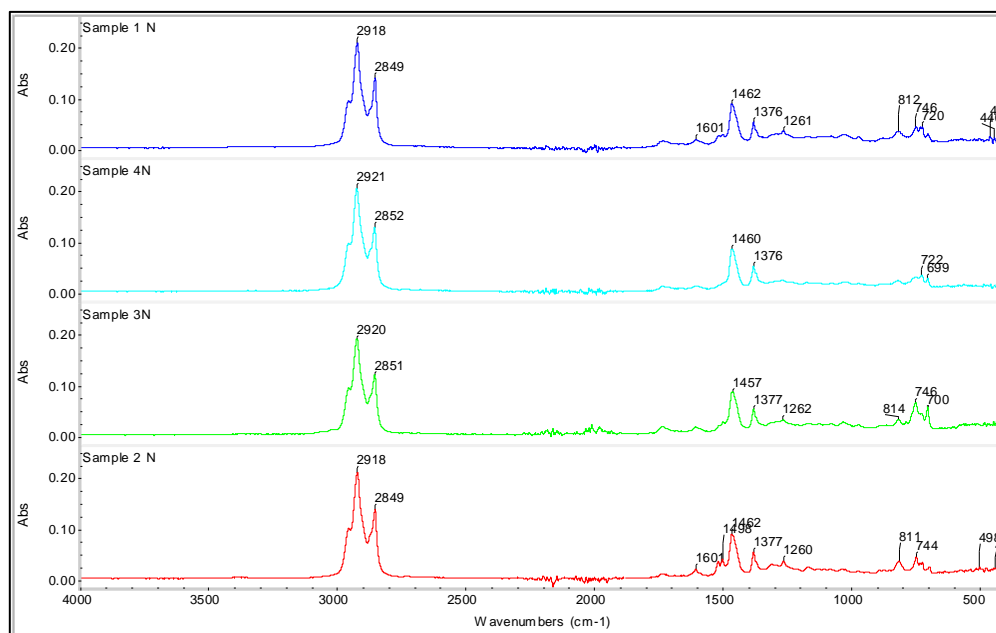


Figure 3. FTIR spectroscopy of the unvulcanized elastomer extracted in toluene (immersion 72 hours) – coded 1N-4N

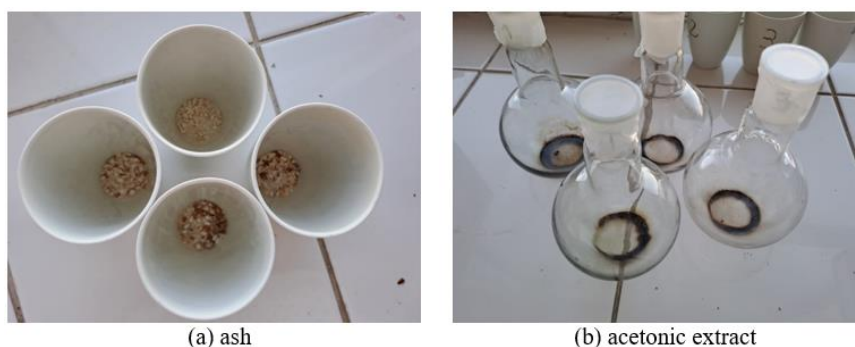


Figure 4. Images of the ash samples (a) and the respective acetone extract (b) after analyses

Table 4. Results of ash and the acetone extract determination from the analyzed samples

Sample code	Type of used tire	Ash, %	Acetone extract, %
1	Nankang Cross Season 185 -60 -15	3.74	5.73
2	Laufenn 225 – 65 – 6 C	2.88	8.27
3	Nokian Nakka Green 195 – 65 -15	2.26	5.02
4	Radbeug 185 – 65 – 15 Power	3.64	9.88

The FTIR spectrum of the ash obtained from the analyzed samples (Fig. 5) can indicate the presence of precipitated silica through the band at 1101 cm^{-1} specific to Si-O, the bands from $869\text{--}978\text{ cm}^{-1}$ specific to Si-OH, or the bands from $475\text{--}673\text{ cm}^{-1}$ due to Si-O-Si bonds, but it can also indicate the existence of ZnO and sulfur bridges (bands from $550\text{--}450\text{ cm}^{-1}$) (Eissa *et al.*, 2022; Katumba *et al.*, 2008).

Acetone Extract

The amounts of resins, free sulfur, antioxidants, mineral oils, waxes, organic accelerators, fatty acids can be identified through the acetone extract. The amount of acetone extract in the samples was 5.02-9.88% (Table 4). From the FTIR spectra of the acetone extracts shown in

Fig. 6, it can be concluded that the samples may contain several types of ingredients (such as organic acids, aldehydes, ketones, esters, mineral oils and waxes, etc.), and the composition of the acetone extracts obtained is similar for the analyzed samples (Coates, 2000).

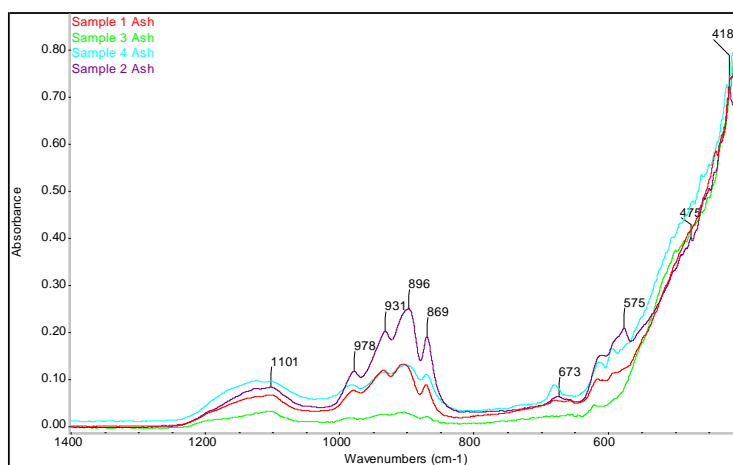


Figure 5. FTIR spectroscopy of ash, coded 1-4 Ash

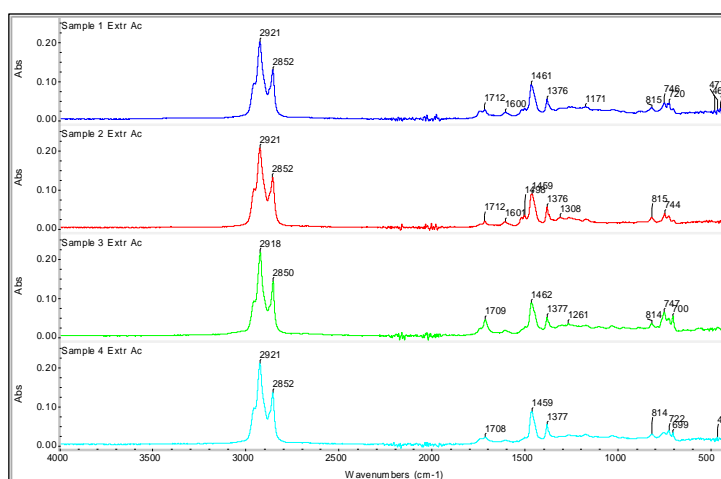


Figure 6. FTIR analyses of acetone extracts for the analyzed samples, coded 1-4 Extr Ac

CONCLUSIONS

Four vulcanized rubber samples from four different types of used tires were analyzed. From the Burchfield test and FTIR Spectroscopy, it was concluded that samples 1 and 2 contain natural rubber, and samples 3 and 4 contain a mixture of natural rubber and butadiene-styrene rubber. The amount of ash obtained after the analyses is in the range of 2.26-3.74% and according to the FTIR spectra, it can be composed of zinc oxide, sulfur and precipitated silica. These compounds can come from vulcanization with sulfur and accelerators in the presence of activators. According to the results obtained with the acetone extract, it was observed to be 5.02-9.88%, being composed of resins, antioxidants, waxes, organic accelerators or fatty acids. The experimental data obtained are in accordance with those existing in the specialized literature (Ramarad *et al.*, 2015; Formela, 2022).

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DEVELOPMENT AND CHARACTERIZATION OF SALICYLIC ACID-BASED MICROEMULSIONS FOR TOPICAL APPLICATION

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The goal of the study was to design topical microemulsions using salicylic acid as a poorly soluble model drug. The microemulsions were selected by building a pseudoternary phase diagram, starting from four main components namely oleic acid, Tween 80, propylene glycol, and ultrapure water. The systems were obtained by solubilizing salicylic acid into the oil and the stabilizer mixture (surfactant/co-surfactant – S/CoS), using water titration. Then, the following parameters were studied: pH, conductivity, refractive index, mean droplet size, rheological descriptors, and surface tension. The organoleptic properties were specific for the selected ingredients, and the pH values were considered appropriate for applying the microemulsion to the skin. The conductivity confirmed the microemulsion O/W type, while the refractive index values were specific for isotropic systems. The mean droplet size varied between 117-272 nm, under the influence of stabilizers, using two methods: the Cumulant model and the Sparse Bayesian Learning algorithm. The rheological analysis offered information related to the internal structure of microemulsions, characterized by Newtonian or non-Newtonian flow. The non-Newtonian behaviour was evaluated through Ostwald-de-Waele, and Herschel-Bulkley models respectively. The surface tension was specific for dispersions stabilized by adequate quantities of S/CoS and varied between 30.82-34.71 mN/m. The developed salicylic acid-based microemulsions presented adequate physico-chemical characteristics, making them promising solutions for treating certain dermatological disorders such as acne, psoriasis, or cutaneous keratosis.

Keywords: salicylic acid, topical microemulsions, physicochemical characteristics.

INTRODUCTION

In the actual context, an integrated approach to managing dermatologic conditions involves a rapid diagnosis and a targeted treatment to rebalance normal cutaneous metabolism (Zhang *et al.*, 2023). From the most encountered diseases diagnosed in different age groups, acne, psoriasis, cutaneous keratosis, and specific forms of dermatitis lead to imbalances in normal cellular division, and present common manifestations like inflammatory events, squamous lesions, skin discomfort, and pronounced damage at sun exposure, impacting the psycho-social life quality of the individuals (Richard *et al.*, 2022; Dreno *et al.*, 2021).

Salicylic acid (SA), a classic β -hydroxy acid with evidenced therapeutic applications, contributes to the healing of mild cutaneous lesions as a function of concentration, being a constituent of a broad range of dermatocosmetic products due to anti-inflammatory, antimicrobial, exfoliating, and anti-seborrheic properties. Additional benefits of using SA are UV protection and reduction of erythematous reaction through protein transcription inhibition (Kornhauser *et al.*, 2010). Recent evidence conceived in the demonstration of melanogenesis inhibition of SA from ginseng root, and anticancer activity in skin melanoma (Liu *et al.*, 2021; Ausina *et al.*, 2020). Several attempts were conducted to study SA solubilization, as

key compound in the formulation of nano-sized medication for treating acne, psoriasis, warts or combined with a second drug to improve anti-inflammatory, keratolytic, or antibacterial activity (Anicescu *et al.*, 2022, Sevinç Özakar *et al.*, 2022).

There is a high interest in studying microemulsions (MES) for topical application of dermatologic drugs. Known as thermodynamically stable and isotropic nanodispersions, oil in water (O/W) MES possess an enhanced capacity of solubilizing and entrapping lipophilic drugs into an oil core stabilized in an aqueous media with the aid of a surfactant and a cosurfactant (Talianu *et al.*, 2019).

The objective of this study followed the development and characterization of topical microemulsions with SA solubilized into an oil phase, intended to be applied in the treatment of dermatologic conditions known to create imbalances in normal cellular metabolism.

MATERIALS AND METHODS

Materials

Salicylic acid was acquired from Chemical Company, oleic acid vegetable from Merck, Tween 80 from Carl Roth, propylene glycol (PG) from Sigma Aldrich, Ultrapure Milli-Q water was generated from a Milly-Q® Direct 8 Water Purification System (Merck Millipore), being the aqueous phase.

Methods

Design of the Pseudoternary Phase Diagrams

To prepare stable O/W microemulsions entrapping salicylic acid it was mandatory to study the stability areas of the O/W and W/O microemulsions, of some lamellar phases and unstable regions. For a chosen ratio of Tween 80/PG of 2:1, nine different oil/stabilizer mixture dilution lines of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 were considered to explore the formation of microemulsions using water titration method. The diagram was plotted using Triplot 4.1.2. (Todd Thompson Software, LA, USA). All dispersions were inspected and six stable points of microemulsions were chosen as it shows in Figure 1.

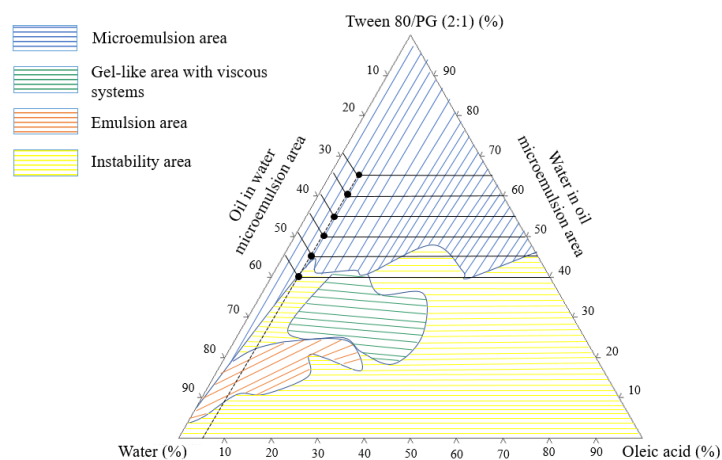


Figure 1. Pseudoternary phase diagram built to explore the formation of MEs

Preparation of the O/W Microemulsions

Following the data presented in Table 1, the calculated quantity of salicylic acid was accurately weighed at a Sartorius MC1 Laboratory LC 620 P analytical balance (Sartorius, Gottingen, Germany), and solubilized in oleic acid under magnetic stirring using DLAB MS-H380Pro thermostated stirrer (DLAB Scientific, Beijing, China). Afterward, the mixture of Tween 80 and PG (in a ratio of 2:1) was slowly added to the oily dispersion. The homogenization was continued, and the preparation process was succeeded by adding distilled water through meticulous titration until 20 g of microemulsion was obtained. The resulting dispersions were left to equilibrate for 24 hours and further characterized.

Table 1. Composition of the O/W microemulsions

Ingredients (w/w, %)	MES 1	MES 2	MES 3	MES 4	MES 5	MES 6
Oleic acid	5	5	5	5	5	5
Tween 80/PG (2:1)	40	45	50	55	60	65
Salicylic acid	1	1	1	1	1	1
Distilled water	54	49	44	39	34	29

pH Determination

The pH of the microemulsions was evaluated using a Mettler Toledo pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland).

Conductivity Determination

The conductivity of the microemulsions was assessed to confirm their phase behavior. Corning 441 conductivity meter (Cole Parmer, Vernon Hills, USA) was used, and the measurements were recorded in triplicate, at 24 ± 0.5 °C.

Refractive Index Determination

The refractive index of the microemulsions was obtained using a Krüss DR 201-95 digital refractometer (Krüss Optronic, Hamburg, Germany). Distilled water with a refractive index of 1.3330 was used to calibrate the apparatus. The measurements were recorded in triplicate at 24 ± 0.5 °C.

Dynamic Light Scattering Determination (DLS)

The mean droplet size and the polydispersity index (PDI) were assessed using DLS technique on diluted samples (Talianu *et al.*, 2024). VascoKin particle analyzer (Cordouan Technologies, Pessac, France), equipped with a 638-nanometer laser functioning in a backscattering mode was used over the analysis process to explain droplet behavior using the Cumulant and Sparse Bayesian Learning (SBL) algorithm.

Rheological Evaluation

The flow behavior of the microemulsions was studied using a rotational viscometer, Multi-Visc Rheometer (Fungilab, Barcelona, Spain) (Dănilă *et al.*, 2024), equipped with an LCP standard spindle at 24 ± 0.5 °C. The operational conditions were previously reported (Anicescu *et al.*, 2022).

Superficial Analysis

The surface tension of the microemulsions was tested with a Sigma 700 force tensiometer in quinduplicate mode (Biolin Scientific, Finland) (Hamed *et al.*, 2022). Under the guidance of Du Noüy ring method, the platinum ring interacted with the microemulsion sample measuring the maximum force of detachment at the liquid surface.

RESULTS AND DISCUSSION

The organoleptic characteristics of the microemulsions translated as aspect, color and odor were associated with the composition. From Figure 2 it can be seen that the microemulsions presented different aspects from opalescent to transparent appearance, being observed how the stabilizer mixture affects their internal structure.



Figure 2. Microemulsions observed at 24 h after preparation

The pH values varied in the range 2.90 ± 0.01 - 3.51 ± 0.01 , being strongly influenced by the presence of acidic species of salicylic acid and oleic acid, and gradually increased to the maximum value with the increase in the stabilizer content.

Conductivity evaluation confirmed the O/W type of the microemulsions. The experimental values ($\mu\text{S}/\text{cm}$) varied between 48.30 ± 0.10 - 104.27 ± 0.15 $\mu\text{S}/\text{cm}$ as a function of the aqueous phase content. Similarly, the refractive index increased with the S/CoS concentration from 1.3750 ± 0.0001 to 1.4175 ± 0.0001 , giving information about the isotropic character. Preliminary analysis results performed in triplicate can be followed herein in Table 2.

Table 2. Experimental data of pH, conductivity and refractive index obtained in the preliminary analysis of microemulsions

Code	pH	Conductivity	Refractive index
MES 1	2.90 ± 0.01	104.27 ± 0.15	1.3750 ± 0.0001
MES 2	3.02 ± 0.01	102.53 ± 0.12	1.3848 ± 0.0001
MES 3	3.04 ± 0.00	99.17 ± 0.51	1.3856 ± 0.0000
MES 4	3.26 ± 0.01	83.90 ± 0.56	1.4050 ± 0.0001
MES 5	3.35 ± 0.00	66.17 ± 0.31	1.4096 ± 0.0001
MES 6	3.51 ± 0.00	48.30 ± 0.10	1.4175 ± 0.0001

During dynamic light scattering analysis, the mean droplet size and droplet size distribution were assessed following two methods as presented in Table 3. According to the Rayleigh theory of light scattering, the logarithmic autocorrelation function of intensity in time (μs) was obtained to calculate the diffusion coefficient and the mean droplet size by applying the Stokes-Einstein equation.

Table 3. Cumulative results obtained over the DLS analysis, presenting the mean droplet size values processed by the Cumulant and the SBL models

Code	Z_{average} (nm)	PDI	Cumulant Model			Sparse Bayesian Learning model		
			D _{10%} (nm)	D _{50%} (nm)	D _{90%} (nm)	D _{10%} (nm)	D _{50%} (nm)	D _{90%} (nm)
MES 1	272.73	0.390	215.44	451.46	946.03	102.81	472.82	716.84
MES 2	218.82	0.322	170.97	326.63	653.52	107.68	297.78	623.99
MES 3	208.59	0.325	163.25	311.87	623.99	889.50	297.78	750.60
MES 4	160.71	0.295	135.68	259.21	495.20	74.39	179.07	716.84
MES 5	130.85	0.272	98.17	187.57	358.28	64.75	163.25	375.23
MES 6	117.50	0.290	98.17	187.54	358.28	53.82	163.25	225.64

With the aid of the Cumulant model, Z_{average} values of the mean droplet size were obtained together with the PDI. As it was valued in Figure 3 presenting the dynamics of droplet size distribution - case (a), the droplet diameter was influenced by composition. The increase in S/CoS (%) determined a decrease in droplet size from 272.73 nm to 117.5 nm. Moreover, the size distribution was well represented by D10%-D90% domains as presented in Table 3, where the best results were attributed to the MES 5 and MES 6 systems. At concentrations of 60-65% Tween 80/PG (%), the corresponding peaks were shifted towards 100 nm.

Because it is hard to find samples with a single monodispersed domain of droplets, a variation in droplet size distribution can be emphasized using a continuous multimodal analysis algorithm. Considering the variation of PDI between 0.272 and 0.390, it was worthwhile to make a SBL analysis. Thus, the droplet size distribution was quantified as well through D10%-D90% domains and graphically represented in Figure 3 - case (b).

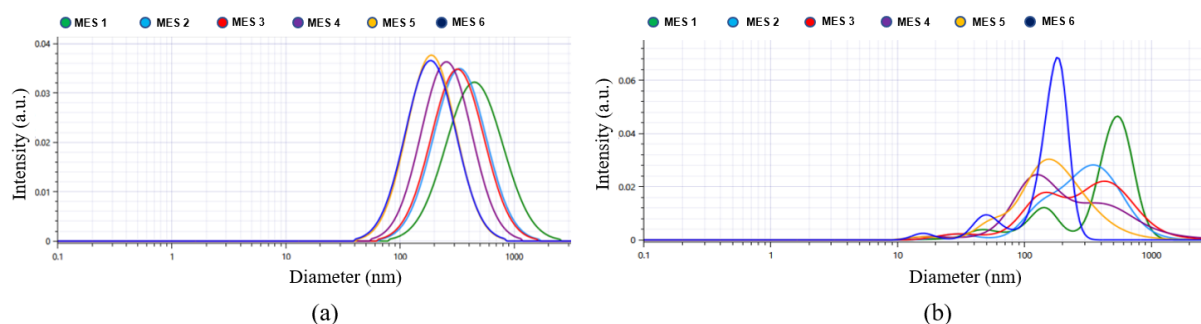


Figure 3. Cumulative profiles of intensity (a.u.) as a function of diameter (nm) analyzed based on Cumulant model - case (a), and profiles of intensity (a.u.) as a function of diameter (nm) analyzed based on SBL model – case (b).

It can be noticed a good result for MES 6, where the high-intensity peak was placed between 100-200 nm, followed by low-intensity peaks under 100 nm, reflecting the existence of small droplets with trimodal distribution. The droplet size diameter represents in this way an important parameter to predict the behavior of the microemulsions in topical application and consequently, the drug passage through skin pathways.

Recent studies centered on cosmetic emulsions development found that the rheological profile is influenced by the stabilizer activity. It was stated that the stabilizer (S/CoS) is a determinant component for stability and spreadability, and together with the bioactive ingredients, contributes to an adequate application at the skin level (Dănilă *et al.*, 2019).

Over the rheological evaluation, non-Newtonian and Newtonian flows were assessed, being strongly connected with the variation in droplet size distribution. Rheological profiles of the shear stress (Pa) as a function of shear rate (s^{-1}) were presented in Figure 4, while the rheological descriptors can be seen in Table 4. MES 1 - MES 3 followed a pseudoplastic flow, described by the Ostwald-de Waele model for MES 1 with consistency index (K) values of $0.937 \text{ Pa}\cdot\text{s}^n$, while in the case of MES 2 and MES 3, the Herschel-Bulkley model was quantified by elevated K values with τ_0 of 1.343 Pa and 2.782 Pa (Figure 4 - case (a)).

Corroborating the viscosity with the droplet size variation, the three nanodispersions behaved as fluid emulsion-like systems with larger droplets over 500 nm and pseudoplastic behavior.

At a constant concentration of the oil phase, the increase in stabilizers S/CoS (Tween 80/PG) determined a reduction in droplet size, and a different rheological behavior resulting in clear microemulsions with a laminar flow.

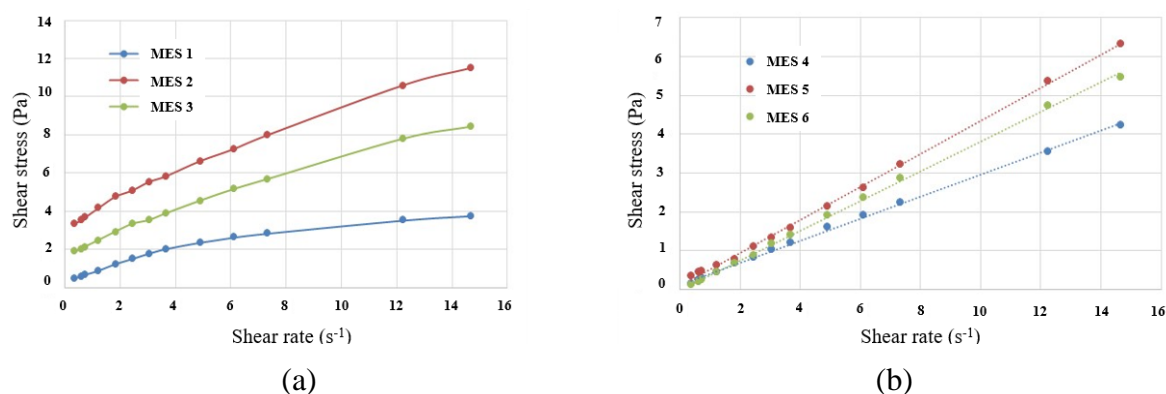


Figure 4. Rheological profiles of shear stress (Pa) as a function of shear rate (s^{-1}) for the tested MES 1 – MES 6 at $24 \pm 0.5^\circ\text{C}$ representing the non-Newtonian flow (a) and Newtonian flow behavior (b)

Consequently, the Newtonian profiles of the MES 4 - MES 6 nanodispersions presented in Figure 4 – case (b) were described by viscosity values between 0.283 and $0.424 \text{ Pa}\cdot\text{s}$, unaffected by the variation of shear stress and shear rate. The model was well fitted by regression coefficients placed between 0.9991 and 0.9995, as mentioned in Table 4. The values obtained in this case are closely related to the results reported in the literature, as recently emphasized for an optimized methotrexate topical microemulsion with a viscosity of $0.103 \text{ Pa}\cdot\text{s}$ and Newtonian flow (Mishra *et al.*, 2024).

The most elevated viscosity values were recorded for MES 2 and MES 3 and can be justified by the flow behavior transitions observed on the phase diagram.

Table 4. Rheological descriptors for the non-Newtonian and Newtonian flow behavior of the tested microemulsions

Code	Model	R	τ_0 (Pa)	K, ($\text{Pa}\cdot\text{s}^n$)/ viscosity ($\text{Pa}\cdot\text{s}$)	n
MES 1	Ostwald-de Waele	0.9929	0	0.937	0.534
MES 2	Herschel-Bulkley	0.9995	2.782	1.178	0.747
MES 3	Herschel-Bulkley	0.9994	1.343	0.993	0.738
MES 4	Newton	0.9991	0	0.2836	1
MES 5	Newton	0.9993	0	0.4241	1
MES 6	Newton	0.9995	0	0.3815	1

The surface tension of the microemulsions placed around 30.825 ± 0.008 mN/m and 34.710 ± 0.503 mN/m indicated the formation of stable systems, with minimal values obtained for MES 4 – MES 6 prepared with the highest S/CoS amount. The results are graphically presented in Figure 5. The reduction in surface tension promoted by the S/CoS mixture allows appropriate spreading onto a surface, facilitating the microemulsion application on the skin area and the enhanced formulation activity at the cellular level.

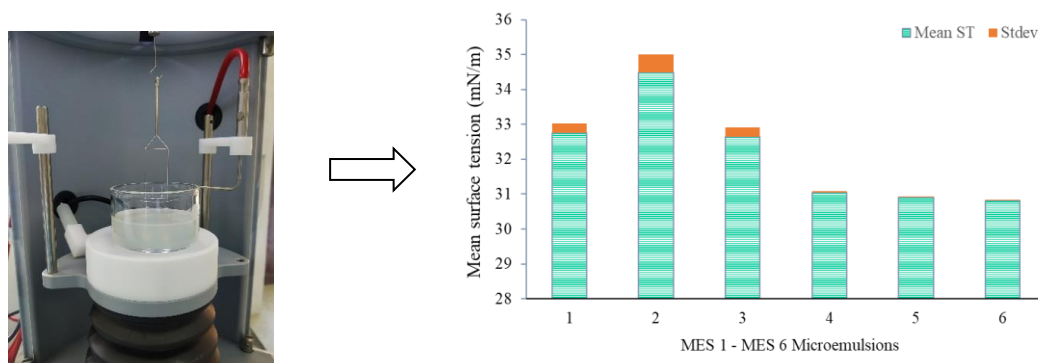


Figure 5. Variation of surface tension for the tested MES 1 - MES 6 at $24 \pm 0.5^\circ\text{C}$, analyzed with Du Noüy ring method

It can be appreciated that the obtained data offer important information concerning the stability of the interfacial film at the oil/water interface, as previously explained in the case of surfactant migration phenomena from the interface (Koli *et al.*, 2021), on the rule the smaller the surface tension variation, the higher the stability will be.

CONCLUSIONS

The obtained microemulsions were stable and the composition appropriately influenced their physicochemical parameters. The designed systems can be used as solubilizing vehicles for salicylic acid by selecting suitable oil phases and stabilizers in calculated amounts, using pseudoternary phase diagram plotting. The physical parameters analyzed over the experimental process, namely conductivity, refractive index, droplet size, rheological behavior, and surface tension lead to the depiction of two model microemulsions. Thus, the MES 5 and MES 6 had reduced droplet sizes of 130.85 nm and 117.50 nm, respectively, according to the Cumulant model, with the lowest PDI values. The SBL algorithm contributed to the droplet dynamics understanding and identification of different droplet populations leveraging the system stability. As fluid systems, described by Newtonian flow and low surface tension, a targeted effect and adequate skin contact allow the drug passage. To conclude, the designed microemulsions could be emphasized as potential systems of interest in the current management of dermatologic conditions.

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FUNCTIONAL CLOTHING DESIGN FOR THE ELDERLY

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Functional clothing represents the evolutionary segment in the technical textiles market. The domain of functional clothing is large and varied, each functionality having its own set of specifications, material needs, and corresponding technologies and methods. Garments for persons with special needs are part of the functional clothing category and are meant to improve the quality of life of those whose body shape, mobility or dexterity differs from the norms. This paper presents the advantages of virtual fashion design, that for persons with special needs (the elderly) lead to more comfortable, fit, and easy to wear garments, from the perspective of clothing design. The steps involved in developing the virtual prototypes were: i) 2D design of the basic patterns in accordance with the anthropometric data from the size table using the Pattern Design Software (PDS); ii) Simulation of functional clothing on a virtual mannequin using the Optitex 3D Suite software. The 2D patterns made with the PDS software were placed on the parameterized model and the types of seams were defined for the virtual assembly by simulating their sewing. The landmarks were deformed according to the shape of the human body; iii) Evaluation of garment fit on the body: after the completion of the 3D simulation process of the product, the appearance of the product and the way it fits on the body were analyzed.

Keywords: functional clothing, elderly, virtual prototyping

INTRODUCTION

The domain of functional clothing is large and varied, each functionality having its own set of specifications, material needs, and corresponding technologies and methods. It is well known that all types of clothing perform multiple functions from aesthetic to basic protection. Functional clothing is defined by that garment specifically designed or engineered to meet the settled performance for the user, under extreme conditions (Gupta, 2011a & 2011b).

The process of design and engineering of functional clothing is based on the outcomes of an objective assessment of multiple user requirements thus tending to become a very complex and iterative project. Clothing with functional abilities cannot simply be mass produced like everyday apparel. The adaptability of all the stages of the manufacturing process is tested to produce functional clothing that ensures workability, desired functional performance and comfort. Special synergies between apparel and technologies are required for embedding the functionality within the garment and development of textile and apparel with built-in technologies that can enhance the end product with extended functions and better comfort (Neves *et al.*, 2015a; Joshi, 2024).

The aging process causes a deficiency of cardiovascular functions, muscle resistance, joint flexibility, sensory and brain functions (Neves *et al.*, 2015b).

Regarding the inevitable and irreversible changes in the elderly body, the following occurrences and risks can be highlighted: 6% of average height loss; decrease in muscle mass, which leads to other disorders, such as decrease in bone density, and joint flexibility, growth of adipose tissue, changes in the abdomen, bending and deposition of fat in certain areas of the body. Age-related medical conditions, such as arthritis or osteoporosis, can lead to a change in body shape and functional limitations, such as dexterity and mobility (Chung *et al.*, 2024).

Today, advanced computer simulation techniques and garments virtual prototyping are indispensable for the development of garments and their fitting on the 3D body models within a virtual environment, as well as real-time virtual clothes try-on (Jevsnik *et al.*, 2012 & 2017). Considering the direct contact between skin and clothing, information about anthropometry, biomechanics and ergonomics is of extreme relevance for the development of suitable models for different user segments, with various needs.

Modelling is a process that starts from the observation of the body and its mapping, and it ends with the dressing test of a body, in real conditions. Therefore, to the development of an adequate modelling is necessary to know anatomy as well as the functioning of the body (Neves *et al.*, 2015a). Garments for persons with special needs are part of the functional clothing category and are meant to improve the quality of life those whose body shape, mobility or dexterity differs from the norms.

The Tex4Age project aims to develop functional textile products (articles of clothing and textile materials for environment of the elderly) intended to improve the quality of the elderly life by approaching innovative technologies that integrate the Safe-by-Design concept. This paper presents the virtual prototyping of functional clothing for elderly that is more comfortable, fit, and easy to wear from the perspective of clothing design.

EXPERIMENTAL

Clothing products for people with special needs (the elderly) are generally limited in regards to the fit on the body and ease of dressing/undressing, which is problematic. These people want to be treated like any other group, they have a strong desire to have elegant and functional clothing, at the same time.

Designing clothing for people with special needs (the elderly) is an extremely complex activity. The complexity and the dual, apparently contradictory, nature of the requirements are imposed by: functionality in relation to the type of activity, comfort, aesthetics, reliability and maintenance needs of the wearers; the impact on human health and well-being define the design of clothing/textile products for the environment of people with special needs as a multi-criteria design problem.

The needs analysis revealed that, for the users of clothing items/textile products, designed for the environment of people with special needs, the products must fulfill the following key needs, in order of priorities: User comfort; Protection; Functionality; Durability under conditions of frequent wear; Aesthetics; Applicability for several categories of people with special needs; Acceptability by users; Reasonable cost. Starting from the identified key needs, the performance parameters that clothing items/textile products must fulfill for the environment of people with special needs (the elderly) were established (Zhang *et al.*, 2015).

When manufacturing clothing, for these people, the anthropometric standards elaborated for the population with proportional body dimensions cannot be used, nor the classic design rules. In order to design and manufacturing functional clothing for the elderly, the anthropometric database of the Romanian population between the ages of 6 and 60+ was refined, according to certain criteria, data obtained in the anthropometric surveys carried out by The National Research & Development Institute for Textiles and Leather (INCDTP), during the period 2008-2014, in the historical regions of Romania, in Bucharest, at the “Prof. Dr. N. Paulescu” National Institute of Diabetes, Nutrition and Metabolic Diseases and in public order structures, through a three-dimensional scanning process. The scan was performed with the Anthroscan 3D Mobile Scanning System from Human Solutions-GmbH, provided by INCDTP, which contains a family of ScanWorX-Anthroscan software modules for 3D body visualization and automatic processing and evaluation of anthropometric data (Bruniaux *et al.*, 2016; Nakić and Bogović, 2019).

The Specific Anthropometric Database

The primary 3D anthropometric database of INCDTP contains 6150 scanned bodies, of which: 2850 children, girls and boys, 1800 adult subjects, women and men, 1500 obese and elderly subjects and 150 subjects from public order structures. Primary anthropometric database was filtered, according to certain criteria (e.g. sex, age, and Body Mass Index, etc.) to constitute the databases specific to people with special needs (the elderly), namely Anthropometric database for women aged 60+, and for men aged 60+.

Each anthropometric dimension of the specific databases was subjected to a one-dimensional statistical processing by calculating the statistical parameters for the main dimensions of the body (bust and hip circumference, for women and chest and waist circumference, for men). Dimensional typology selection (establishing body shape variants to be included in the anthropometric dimensions table) took into account the frequency of the investigated sample, different combination of possible value of main dimensions. Thus, frequency values bigger than 5% were selected. Determination of body types, for these group of people with special needs, was realized by analyzing the frequency of the difference between Ps (hip circumference) and Pb (bust circumference), on women and Pt (waist circumference) and Pb (chest circumference), on men. The types of the bodies with the highest frequency were selected, and for these the sizes of the garments and the body dimensions that will be used in the design of the patterns were established.

Virtual Garment Design for the Elderly

The development of functional clothing for people with special needs is complex, costly, and time consuming. The use of computational modelling and simulation can reduce development time and production costs of functional clothing for elderly (Avadanei *et al.*, 2022). Therefore, in a first step, we made the virtual prototypes of functional clothing for elderly using the OptiTex software suite.


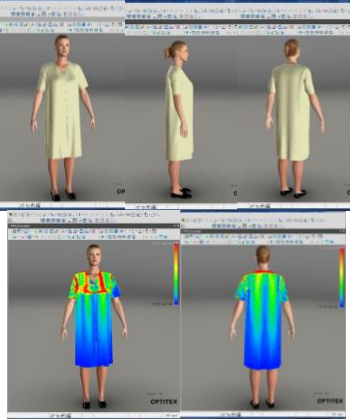
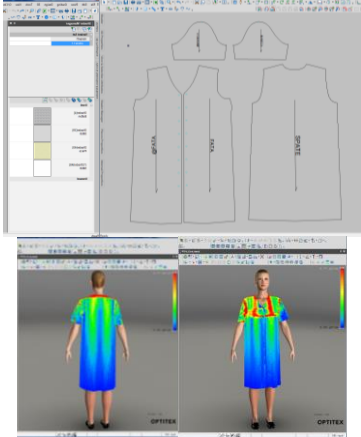
The stages completed were the following: i) 2D design of the basic patterns in accordance with the anthropometric data from the size table using the Pattern Design Software (PDS); ii) Simulation of functional clothing on a virtual mannequin using the Optitex 3D Suite software. The 2D patterns made with the PDS software were placed on the parameterized model to the dimensions in the anthropometric data table and the types of seams were defined for the virtual assembly by simulating their sewing. The landmarks were deformed according to the shape of the human body; iii) Evaluation of the fit of the product on the body: after the completion of the 3D simulation process of the product, the appearance of the product and the way it fits on the body were analyzed (Nakić *et al.*, 2019).

In order to create the virtual prototype, functional clothing products were selected from the most common range used by the elderly.

RESULTS AND DISCUSSIONS


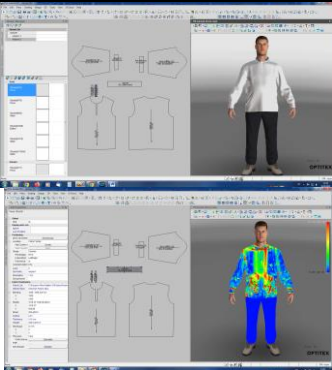

Based on the mentioned algorithm, the virtual prototypes of functional clothing for the elderly were obtained. In the following tables are exemplified two of them.

Table 1. Dress for the elderly female

<i>Conceptual model</i>	<i>Parameterized mannequin and basic patterns: vB body type, size 48</i>	<i>Model patterns, virtual prototype on the body and checking the body-product fitting by viewing the tension map.</i>
		

The model is designed for people of different sizes and body type vB. The virtual prototype made with model patterns when analyzing the degree of body-product fit, on the mannequin parameterized according to body type and size, using the tension map, shows a high pressure in the shoulder area (normal for a product with shoulder support) and breasts. After correcting the model patterns in the breast area, when resuming the product simulation, a correct positioning of the product on the body and a reduction of the tension exerted in the breast area were highlighted.

Table 2. Blouse for the elderly men

<i>Conceptual model</i>	<i>Parameterized mannequin and basic patterns: vD body type, size 56</i>	<i>Model patterns, virtual prototype on the body and checking the body-product fitting by viewing the tension map.</i>
		

The basic patterns were designed for Body type D, size 56. Visualization of the product made with the basic patterns, on the parameterized mannequin, indicates a proper placement and correct patterns. The analysis of the tension map of the product made with the model patterns, shows a good fit, without pressure on the body and sleeves well placed on the hands, without creases.

CONCLUSIONS

The quality of life for the elderly can be substantially improved through a clothing design adapted to conformation, needs, special functional requirements, but also with functionality and aesthetics improved.

Design and engineering of functional clothing for the elderly is a complex and challenging process. Starting from the needs analysis, the key needs of functional clothing for

the elderly were identified, which were the basis for establishing the key performance parameters. The established performance parameters were translated into design requirements, based on which the raw materials, the realization technologies, the conception (design) of functional clothing were identified.

The solutions and advantages offered by the OptiTex software suite were used for the virtual prototyping of the functional clothing for the elderly prior to the physical realization and verification of its compliance through 3D simulation on an avatar.

The use of 3D virtual prototyping offers many advantages as: the possibility to check the aspect of the product and how it fits on the 3D model of the human body, without requiring physical development of a prototype; the reduction of the time to produce the first prototype; a significant reduction of the manufacturing costs; the reduction of waste; the possibility of diversifying the model – by combining or altering the textures of materials.

The results obtained in the design and creation of the virtual prototype, which included the function of modeling the product according to the technical characteristics of the materials, are used to define the technological parameters of making the functional clothing products for elderly.

Acknowledgements

This work was carried out through the Core Programme within the National Research Development and Innovation Plan 2022-2027, carried out with the support of MCID, project no. 6N/2023, PN 23 26 0103, project title “Advanced functional textile materials for protection and improving the quality of life – Tex4Age”.

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ANTIBACTERIAL-TREATED TEXTILES WITH NATURAL ACTIVE COMPOUNDS

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In recent years, antimicrobial textiles have garnered significant attention for their potential to reduce infection transmission in medical and healthcare settings. This paper is focused on the assessment of antimicrobial activity of textile structures treated with essential oils (pine and eucalyptus essential oils) used for the undergarments of military personnel. For this purpose, three types of textile materials (polyester, cotton-polyester and cotton-elastane) were treated with a mixture of gum Arabic and pine essential oil and a mixture of gum Arabic and eucalyptus essential oil by exhaustion method using a Ugolini device. The antibacterial activity was assessed based on the standard SR EN ISO 20645/2005 Textile fabrics – Agar diffusion plate test, which revealed that the samples have antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*. Cytotoxicity was evaluated by using cell cultures as models with perspectives of primary exposure to the action of the agents incorporated in the test preparations. Human cell lines that can be present in the subdermal space (wounds) were chosen, namely, fibroblasts (dermal fibroblast line ATCC PCS-201-041), human monocytes-macrophages (ATCC CRL 9855) and Jurkat T lymphocytes (ATCC TIB-152). Also, cellular viability, proliferation and cytotoxicity were evaluated by MTS and flow cytometry. It has been revealed that cell viability was not significantly affected and the sample shows no cytotoxicity.

Keywords: antimicrobial, military, essential oil, cytotoxicity, cellular viability

INTRODUCTION

The interest in antimicrobial textiles has constantly increased in the last decades, especially due to their everyday use in various fields: medicine and healthcare, clothing, sportswear and footwear, upholstery and furniture, food packaging, air and water purification systems, etc. Their ability to destroy or stop the multiplication of the microbial population defines their efficacy against bacteria, viruses, and fungi (Tanasa *et al.*, 2023). Antimicrobial textiles play an important role in military clothing as many infections occur in hot and humid climates, while other conditions such as eczema are frequent in dry climates. There is a long history of skin-related medical conditions affecting the military population. It appears that approximately 27% of dermatological diagnoses registered in theatres of military operations are related to bacterial or fungal infection (Arcidiacono and Spitz, 2016). Various plant oils and extracts, such as pine, eucalyptus, rosemary and others, can naturally inhibit bacterial growth. Various plant extracts and essential oils can inhibit unpleasant sweat odours, reduce bacterial growth, possess antioxidant properties, and even serve as skin-lightening agents (Mikucioniene *et al.*, 2024). Pine (*Pinaceae*) is one of the world's most significant sources of essential oils, containing over 50 constituents, with around ten being particularly important.

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The primary components of pine essential oil include pinene, camphene, carene, sabinene, terpinolene, myrcene, α -terpineol, caryophyllene, limonene, bornyl acetate, p-cymene, phellandrene, γ -terpinene, germacrene D, and spathulenol. According to Bhalla *et al.* (2013), pine essential oil enhances the activity of white blood cells, which are crucial for eliminating microbes from the body (Bhalla *et al.*, 2013). Terpenoids, which are the primary components of pine essential oil, possess antimicrobial, antiallergic, antifungal, antiviral, antispasmodic, and anti-inflammatory properties. These properties are beneficial in the prevention and treatment of numerous diseases, including cancer (Gheorghita *et al.*, 2022). Eucalyptus (*Eucalyptus* spp.), a plant native to Australia, is primarily cultivated for its fast-growing wood and essential oil, which is used for various purposes. The essential oil, extracted from buds, leaves, bark, and fruits, possesses antiseptic, antibacterial, anti-inflammatory, antioxidant, and anticancer properties. Consequently, it is recommended for treating respiratory diseases such as flu, colds, and sinus congestion (Vecchio *et al.*, 2016). The composition of eucalyptus oil varies with geographical location and seasons, which in turn affects its biological activity. The primary compounds of the essential oil, depending on the species, include eucalyptol, p-cymene, neo-isoeugenol, limonene, and spathulenol (Gheorghita *et al.*, 2022).

MATERIALS AND METHODS

Three textile structures (P1-PES, P2-BBC/PES and P3-BBC/Elastane) were treated with 2 types of essential oils: pine essential oil (PG) and eucalyptus essential oil (EG) of 0.002% concentration and 1% gum Arabic by exhaustion method using an Ugolini device (drum 8L) with hydro module 1:10 (500 mL float). The treatment started from a temperature of 20°C, which was increased to 40°C with a gradient of 2°C/min. The samples were kept at this temperature for 30 min. The textile materials were washed for 30 min at a temperature of 30°C with Kemapon PC/LF solution. Afterwards, they were rinsed twice with hot water (30°C) and once with cold water (20°C) and dried at room temperature. Two treatment solutions with essential oils (pine essential oil and eucalyptus essential oil) of 0.002% concentration were obtained. Initially, a solution of 1% gum Arabic was made, in which the corresponding amount of essential oil dissolved in ethyl alcohol was added dropwise. The 3 samples of textile materials were treated by exhaustion with the 2 solutions obtained, for 30 min at a temperature of 40°C. After finishing the treatment, the samples were dried at room temperature for 24 hours.

The antibacterial activity was evaluated based on the standard SR EN ISO 20645/2005 Textile fabrics – Agar diffusion plate test. ISO 20645:2004 is applicable to testing finishes of hydrophilic, air-permeable materials or antibacterial products incorporated in the fibre. A minimum diffusion of the antibacterial treatment into the test agar is necessary with this procedure. In this procedure, textile samples are tested placed between two agar layers in Petri dishes. The lower layer corresponds to medium free of bacteria and the upper layer incorporates each of the two obligate bacteria a gram-positive bacterium *Staphylococcus aureus* and a gram-negative bacterium *Escherichia coli*. The nutrient media used was Nutrient Agar (NA) provided by Sanimed. After one day of incubation inhibition zone around sample is measured and according to its value sample is assessed as having good, moderate or insufficient antibacterial effect. The volume of agar was prepared for the bottom layer without bacteria, after which (10 \pm 0.1) ml was introduced into each sterilized Petri dish and the agar was allowed to solidify. The amount of gelatin for the upper layer was prepared and cooled to 45°C in a water bath. 150 ml of agar was seeded with 1 ml of bacterial working solution (1-5 x 10⁸ cfu/ml). The container was vigorously shaken to distribute the bacteria evenly. (5 \pm 0.1) ml were introduced into each petri dish and the agar was allowed to solidify. The samples

were placed on the surface of the nutrient medium and then incubated at 37°C between 18h and 24h.

The evaluation is based on the absence or presence of bacterial growth in the contact area between the agar and the sample and on the appearance of a possible inhibition zone around the samples.

The width of the inhibition zone, i.e. the zone without bacteria near the edge of the sample, is calculated according to the following formula:

$$H = (D - d) / 2 \quad (1)$$

H = inhibition zone, in millimeters

D = the total diameter of the specimen and the inhibition zone, in millimeters

d = diameter of the specimen, in millimeters.

Cell reactions were evaluated by using cell cultures as models with perspectives of primary exposure to the action of the agents incorporated in the test preparations. For this purpose, human cell lines that can be present in the subdermal space (wounds) were chosen, namely, fibroblasts (dermal fibroblast line ATCC PCS-201-041), human monocytes-macrophages (ATCC CRL 9855) and the Jurkat T lymphocytes (ATCC TIB-152). This test presents a higher degree of severity compared to the Skin Irritation test which is applied to dermal cells (healthy dermis). The use of these lines is by the regulations ISO 10993 5 2009, and with the regulations ISO 10993 (1, 6, 12,) and SR EN ISO/IEC 17025:2018.

Samples were prepared in culture medium (DMEM) not supplemented with serum, through an extraction/solubilization procedure from the collection surface, as follows:

- sample fragments (1 cm²) were weighed on an analytical balance, in a 3 mL Eppendorf tube; a negative control was also used - support material without compounds;
- 2 mL of culture medium was added over each sample;
- samples were exposed to 3 repeated cycles, consisting of: 3 cycles of ultrasonication, frequency 40 kHz, maximum intensity, duration 30 minutes, on a Sonorex Digital 10P device; 3 vortexing cycles, at maximum intensity, 3000 RPM, on a VELP ZX4 vortex homogenizer.

The operation was repeated at 24-hour intervals, during which the samples were kept at room temperature in the extraction medium. Before exposing the preparations, an additional cycle of vortexing was performed.

The extracts were administered as such, in the amount of 30 µL/well. Cell cultures were inoculated into 96-well plates at an initial density of 10,000 cells/well and initially grown for 48 hours until the culture was 80% confluent. After this level was reached, the medium was removed and replenished with medium according to the cell type. 50 µL of the sample were administered in triplicate in each well, then incubated for 48 hours (incubator with 5% CO₂, 37°C, humidity 90%). After incubation, the serum was collected and serum-free DMEM medium (180 microL) and MTS reagent (3 - (4,5 - Dimethylthiazol - 2 - yl) - 5 - (3 - carboxymethoxyphenyl) - 2 - (4-sulfophenyl) - 2H-tetrazolium) 20 µL were added. They were incubated for 3 hours as above, after which the optical density was measured in a microplate reader at a wavelength of 490 nm against a representative DMEM medium blank.

The cellular cytotoxicity stage can be approached in several ways to evaluate cellular viability, proliferation, cytotoxicity or cell lysis. To achieve this goal we used MTS assay and by flow cytometry technique, the evaluation of secondary necrosis (which appears at the end of the apoptosis phenomenon).



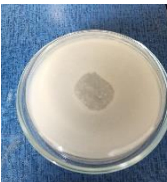
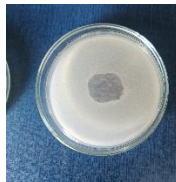


For the two assays, we used the cell line HUVEC (Primary Umbilical Vein Endothelial Cells; Normal, Human (PCS-100-010, ATCC, USA) cultivated in complete DMEM medium (10% fetal bovine serum, 1% penicillin/streptomycin solution and 1% l - glutamine) and incubated at 37°C, in an atmosphere supplemented with 5% CO₂. To carry out the tests, the

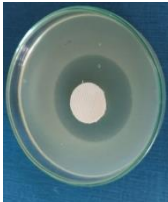
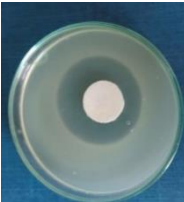


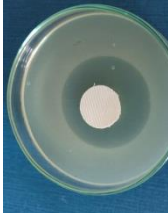
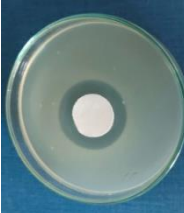
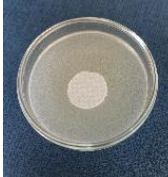

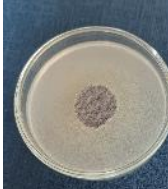

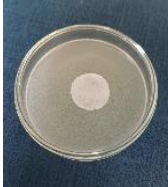

cells were multiplied and then placed in 6-well plates, at a concentration of $0.5 \times 10^6/\text{ml}$; after adhesion (24h), test agents were added. For MTS, the CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, USA) was used. The CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, USA) was used for MTS. For flow cytometry analysis, the FACScan II cytometer (Becton Dickinson, USA) was used, using the apoptosis kit (BD Pharmingen™ Apoptosis Detection Kit - Becton Dickinson, USA) and analyzing the data with DIVa 6.2 software (Becton Dickinson, USA).

RESULTS AND DISCUSSIONS

The antimicrobial activity of treated textile materials has been tested according to SR EN ISO 20645/2005 – Determination of antibacterial activity – Agar diffusion plate test (Table 1), as mentioned above on *Escherichia coli* ATCC10536 (gram-negative) and *Staphylococcus aureus* ATCC 6538 (gram-positive). According to the obtained results, treated textile structures have higher antimicrobial activity (satisfactory effect) compared to the untreated samples. Cotton-based samples present better antimicrobial activity compared to polyester samples, due to cotton's better absorption (up to 27 times its weight). Although all samples showed good results, those treated with pine essential oil present a higher inhibition zone. The absence of multiplication – even without the inhibition zone – can be considered as a positive effect because the formation of such an inhibition zone can be prevented by a small diffusion of the active substance.

Table 1. Antibacterial efficiency of the treated textile materials according to SR EN ISO 20645/2005

Sample	Inhibition zone (mm) <i>Escherichia coli</i>	Inhibition zone (mm) <i>Staphylococcus aureus</i>	Description <i>Escherichia coli</i>	Description <i>Staphylococcus aureus</i>	Evaluation	
					<i>E. coli</i>	<i>S. aureus</i>
Untreated PES			Contamination	Contamination	Insufficient effect	Insufficient effect
Untreated CO/PES			Contamination	Contamination	Insufficient effect	Insufficient effect
Untreated CO			Contamination	Contamination	Insufficient effect	Insufficient effect

Sample	Inhibition zone (mm) <i>Escherichia coli</i>	Inhibition zone (mm) <i>Staphylococcus aureus</i>	Description <i>Escherichia coli</i>	Description <i>Staphylococcus aureus</i>	Evaluation	
					<i>E. coli</i>	<i>S. aureus</i>
PGP1	H= 14 (mm) 	H= 15 (mm) 	Inhibition zone > 1 mm, no multiplication	Inhibition zone > 1 mm, no multiplication	Satisfactory effect	Satisfactory effect
PGP2	H= 17 (mm) 	H= 18 (mm) 	Inhibition zone > 1 mm, no multiplication	Inhibition zone > 1 mm, no multiplication	Satisfactory effect	Satisfactory effect
PGP3	H= 12 (mm) 	H= 10 (mm) 	Inhibition zone > 1 mm, no multiplication	Inhibition zone > 1 mm, no multiplication	Satisfactory effect	Satisfactory effect
EGP1	No zone 	No zone 	No inhibition zone, no multiplication ^c	No inhibition zone, no multiplication ^c	Satisfactory effect	Satisfactory effect
EGP2	No zone 	No zone 	No inhibition zone, no multiplication ^c	No inhibition zone, no multiplication ^c	Satisfactory effect	Satisfactory effect
EGP3	No zone 	No zone 	No inhibition zone, no multiplication	No inhibition zone, no multiplication	Satisfactory effect	Satisfactory effect

c=Absence of multiplication - even without the inhibition zone – can be considered a positive effect because the formation of such an inhibition zone can be prevented by a small diffusion of the active substance

Viability tests were carried out, by applying the samples to the mentioned cultures, performing 3 sets of determinations for each sample. The results are presented in Fig. 1.

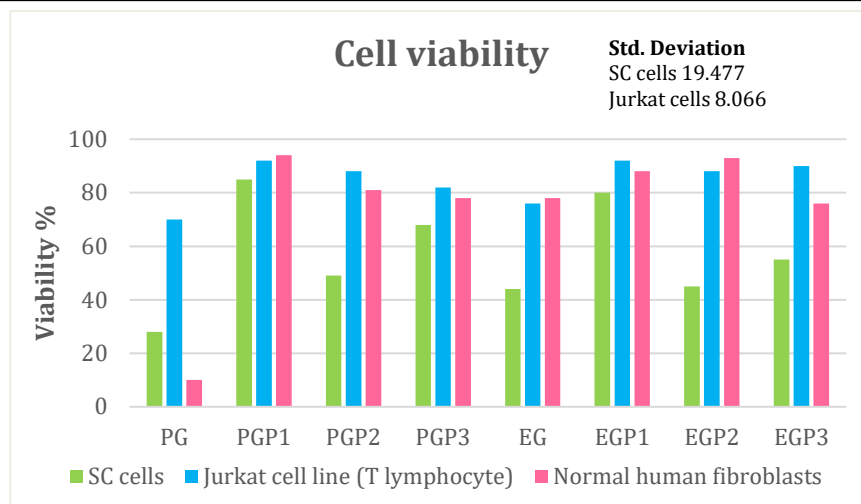


Figure 1. Cell viability – SC cells, Jurkat cell line, human fibroblasts

Cultures exposed to control sample PG showed a significant level of cytotoxicity (viability 28%), also control sample EG showed a level of viability of 43%. It should be noted that the viabilities recorded for the treated samples were systematically above the level of the controls, with the most remarkable effects for PGP1, and EGP1. In the case of the Jurkat line, a relative flattening of the results is observed, even if the control pieces showed higher cytotoxicity (viability 70, and 76% respectively), while the treated pieces resulted in viabilities between 88 and 92%, with the same maximum effects for PGP1, and EGP1.

For human fibroblast line ATCC PCS 201-041A, high cytotoxicity can be observed (only 10% viability for the PG control), and 48% viability for EG. Meanwhile, the treated samples showed low cytotoxicities, with viabilities ranging from 76-94%, again with the best results for PGP1 and EGP1, polyester samples. It should be noted that 100% polyester textile samples have the highest viability when tested on all three cell types.

For MTS and flow cytometry assays the cell line HUVEC (Primary Umbilical Vein Endothelial Cells; Normal, Human (PCS-100-010, ATCC, USA)) was used, cultivated in complete DMEM medium (10% fetal bovine serum, 1% penicillin/streptomycin solution and 1% l-glutamine) and incubated at 37°C, in an atmosphere supplemented with 5% CO₂.

In order to carry out the tests, the cells were multiplied and then arranged in 6-well plates, at a concentration of 0.5×10^6 /ml; after adhesion (24h) the test agents were added. For MTS, the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, USA) was used.

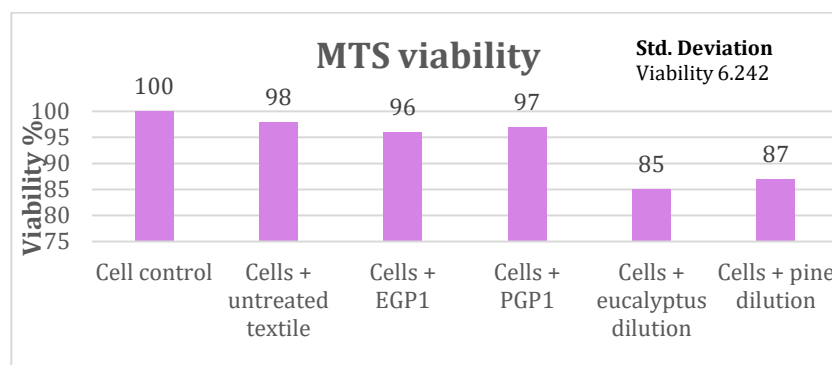


Figure 2. Determination of cell viability in the case of HUVEC cells treated with various possibly cytotoxic agents, using the MTS viability test

Fig. 2 presents the cell viability results obtained by MTS assay for the untreated textile support, the 100% polyester textile support treated with pine essential oil (PGP1) and the 100% polyester textile support treated with eucalyptus essential oil (EGP1). From the data analysis, it can be observed that cell viability was not significantly affected compared to the cell control (untreated cells).

For flow cytometry analysis, the FACScan II cytometer (Becton Dickinson, USA) was used, together with the apoptosis kit (BD Pharmingen™ Apoptosis Detection Kit – Becton Dickinson, USA) the data were analysed with DIVa 6.2 software (Becton Dickinson, USA).

Fig. 3 presents the results of cell viability and cell necrosis for the untreated textile support, the textile support of 100% polyester treated with essential pine oil (PGP1) and the textile support of 100% polyester treated with eucalyptus essential oil (EGP1).

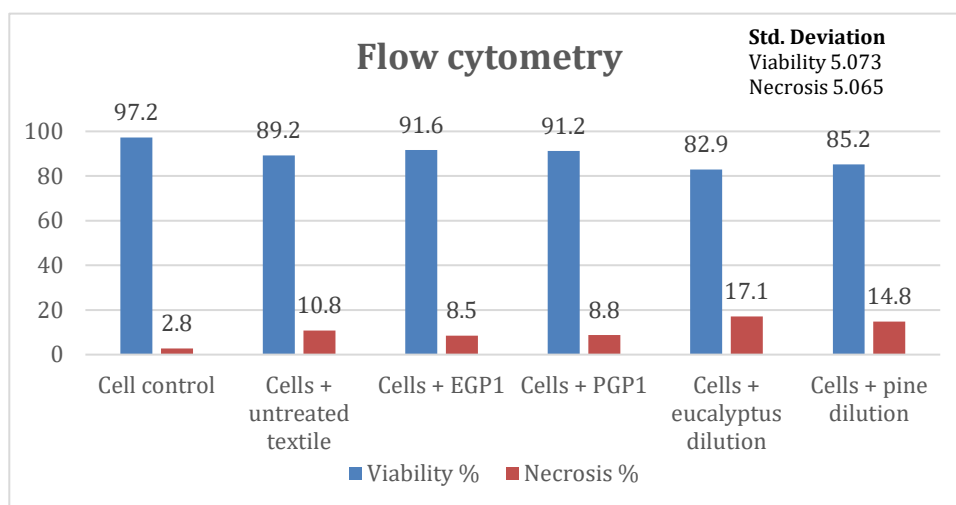


Figure 3. Determination of viability and necrosis in HUVEC cells using propidium iodide labelling of cells treated with possible toxic agents

From the data analysis, it can be observed that the level of necrotic cells does not vary significantly between the textile structure of 100% polyester treated with pine essential oil (PGP1) and the textile structure of 100% polyester treated with eucalyptus essential oil (8.8% and 8.5%) and does not show significant cytotoxicity. Necrotic cells level is very close to the control (untreated textile sample) – 10.8%.

CONCLUSIONS

The evaluation of antibacterial resistance according to SR EN ISO 20645/2005 standard demonstrated the effectiveness of treatments with essential oils. The results are considered “satisfactory” since no bacterial growth was observed beneath the samples. The *in vitro* testing of textile structures treated with pine (P) and eucalyptus (E) essential oils aimed to evaluate cell reactions using cell cultures (human cell lines that may be present in the subdermal space) as models with perspectives of primary exposure to the action of the agents incorporated in the test preparations. The use of these lines was in accordance with the regulations ISO 10993 5 2009, and with the regulations ISO 10993 (1, 6, 12,) and SR EN ISO/IEC 17025:2018. The experimental results showed that the treated textile supports present a biocompatibility between the limits of “moderately cytotoxic” to “practically non-cytotoxic”. Considering that the main application is to ensure the isolation of possible open superficial lesions from pathogenic agents (especially pathogenic bacteria), it can be considered that especially the

products of 100% PES treated with pine and eucalyptus oils can be developed and applied as topical products. Also, by analyzing the data obtained for cell viability, by performing the MTS test, it was found that it was not significantly affected by the samples treated with essential oils. From the analysis of the results obtained by the LDH evaluation, it has been concluded that the level of necrotic cells does not vary significantly between the textile structure of 100% polyester treated with pine essential oil (PGP1) and the textile structure of 100% polyester treated with eucalyptus essential oil and does not show significant cytotoxicity. Necrotic cells level is very close to the control (untreated textile sample).

Acknowledgements

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GREEN BIOSYNTHESIS OF AgNPs BY *Lactobacillus acidophilus* AND THEIR USE

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Silver nanoparticles nowadays are considered one of the most researched materials in science and technology. These nanoparticles are up to a hundred nanometers in size and differ from ordinary metals in their unique physical and chemical properties. Silver nanoparticles also have great potential in biomedicine. They can be used as antimicrobial agents since silver is highly active against bacteria and fungi. Silver nanoparticles can also treat wounds, promoting rapid healing and preventing infections. The paper presents parameters of green biosynthesis of silver nanoparticles using *Lactobacillus acidophilus* UCM B-2691. Using the methods, it was established that silver nanoparticles can be obtained only with the help of the supernatant of the culture liquid of *L. acidophilus* UCM B-2691 by selecting the optimal parameters of green biosynthesis. It was established that nanoparticles with a size of 35 nm can be obtained at a temperature of 50°C. The pH of the medium is also important. To obtain nanoparticles the pH value must be alkaline. The best results were observed when the pH was at 13. The obtained results were checked visually, spectrophotometrically and using the BeNano 90 Zeta nanoparticle size analyse by photon correlation spectroscopy (PCS).

Keywords: nanoparticles, *Lactobacillus acidophilus*, green biosynthesis.

INTRODUCTION

Many physical and chemical methods are used for nanoparticle synthesis. However, because of a growing concern over unnecessary and excessive emissions from production, there is a threat to the environment. Therefore, it is advisable to use “green” methods of metal nanoparticle production. It is also important to optimize various biological methods to achieve a high percentage of product yield and minimal costs for production (Brar *et al.*, 2022; Wang *et al.*, 2020; Voloshyna *et al.*, 2023).

The nanoparticles themselves, their properties, use and production methods play an important role in modern scientific and industrial research. In particular, biological synthesis opens up new horizons for research and use. Undoubtedly, the chemical and physical production of metal nanoparticles is also effective, yet, an energy- and financial-intensive process, without mentioning excessive environmental pollution. Biological methods, such as synthesis by microorganisms (bacteria, fungi) or biological plant extracts are becoming increasingly popular. Biological molecules such as enzymes and metabolites are used to reduce metal ions into nano-sized particles (Brar *et al.*, 2022; Voloshyna *et al.*, 2023).

The size and shape of the nanoparticles affect their properties which can only be partially explained what makes nanoparticles interesting for further research. Silver nanoparticles (AgNPs) are clusters of silver atoms 1-100 nm in diameter which can be used by scientists as antimicrobial agents (Brar *et al.*, 2022; Wang *et al.*, 2020; Voloshyna *et al.*, 2023).

AgNPs are mostly obtained by chemical, physical and biological methods. Chemical production is in use mode often, even though they are more ecologically

dangerous (Wang *et al.*, 2020; Voloshyna *et al.*, 2023). Besides, the conventional methods of nanoparticle production require toxic substances that pollute the environment. One of the safest methods of nanoparticle synthesis remains the biological method by the aid of various biological agents (Voloshyna *et al.*, 2023).

Silver nanoparticles are often used in various fields of biomedicine due to their antimicrobial activity since there is a problem of resistance of various microorganisms to antibiotics. AgNPs exert antibacterial properties against a range of gram-negative and gram-positive bacteria. Also, atomic silver is proven to have anti-fungal, anti-bacterial and anti-viral properties (Ndolomingo *et al.*, 2020; Voloshyna *et al.*, 2024).

The effectiveness of silver nanoparticles is completed by several advantages such as minimized toxicity and stability. However, the use of silver nanoparticles is not limited to their antimicrobial potential but is also used to obtain and develop new biomedical products. For instance, AgNPs can be used in the development process of medicinal substances, biomaterials, optical probes, and orthopedic materials, being a part of various pharmaceutical formulations and medical devices (Prasher *et al.*, 2020). It has been found that changing the size, surface and shape of AgNPs affects the cell membrane and cell organelles, so they can be used to reduce pathogenicity and increase drug sensitivity (Prasher *et al.*, 2020; Voloshyna *et al.*, 2023).

MATERIALS AND METHODS

Characterization of *Lactobacillus acidophilus* UCM B-2691

In this work, a lyophilized strain of *Lactobacillus acidophilus* was used, which is a homofermentative, microaerophilic, short-chain gram-positive microorganism with a rod-like morphology approximately 2-10 µm in size with class II A bacteriocins. They show important thermostability and preservation of activity in a wide pH range and a strong inhibition against food spoilage and pathogenic bacteria, which makes them an important class of biopreservatives. *Lactobacillus* bacteria exist in a variety of environments from dairy products to the human gastrointestinal tract. They are, as a rule, gram-positive, immobile, do not form spores, have a round or rod-shaped nucleus and produce lactic acid as an end product of enzymatic metabolism. *L. acidophilus* is a Gram-positive bacillus that grows optimally between 37 and 42°C and can grow at temperatures up to 45°C. It reaches its highest growth at a pH between 5.5 and 6.0, and its growth ceases at a pH of 4.0. *L. acidophilus* is an obligate homofermentative organism that ferments carbohydrates to form lactic acid and is one of the least oxygen-tolerant LABs.

Cultivation of *Lactobacillus acidophilus* UCM B-2691

The lyophilized culture of *L. acidophilus* UKM B-2691 was obtained for scientific research at the Institute of Microbiology and Virology named after D. K. Zabolotny National Academy of Sciences of Ukraine. The museum plant was transplanted into a liquid nutrient medium MRS for research, and the first and second-generation seed material was obtained (24 h, 37°C, 160 rpm). In parallel, they were sown on a dense medium of MRS, controlling the purity of the culture. After reviving the culture, it was used to accumulate biomass and metabolites.

Obtaining Working Material for Green Synthesis

The 2nd generation culture was sown on MRS liquid medium and cultivated for 48 hours at 37°C, 160 rpm. Then centrifugation was performed at 3500 rpm for 30 min. After

centrifugation, the supernatant and cells were collected separately. Sterile distilled water was added to the cells in the amount of the selected supernatant and left for 24h for the final destruction of the cells.

Conducting Biogenic Synthesis of Silver Nanoparticles

Biosynthesis of nanoparticles using *Lactobacillus acidophilus* UCM B-2691 was carried out in two ways – using cell lysate and their supernatant.

After these manipulations, both experimental solutions were poured into 30 ml sterile measuring glasses and 100 mM AgNO₃ was added. After adding salt, the pH level of the cell lysate solution and the supernatant solution was measured and adjusted to the required pH levels. 3 pH levels were chosen for the experiment: 3, 7, and 13. Proofing was done using HNO₃ and NaOH, respectively. A temperature gradient of 5°C, 25°C and 50°C was established. So, 3 different pH levels and a control solution were supplied for each selected temperature. For 24 hours, the vials with the experimental solutions of both variations of the methods were kept in thermostats according to the selected temperatures.

Research of Silver Nanoparticles

Determination of the Size of Silver Nanoparticles by Photon Correlation Spectroscopy

To determine the size of nanoparticles, the method of photon correlation spectroscopy (FCS) was used using the BeNano 90 Zeta nanoparticle size analyzer. One of the advantages of using the device when determining the size of metal nanoparticles is the non-invasiveness of the method for samples, that is, the structure of molecules is not destroyed during the study. Also, 1-2 ml of the sample is enough to prepare the experimental solution. This method allows you to obtain results with high repeatability, speed and accuracy. The measurement process is almost completely automatic, which helps to reduce errors in the work process. The chosen method makes it possible to investigate the quality of experimental samples and therefore to maximize the efficiency of further use of nanoparticles. In the protocols of the conducted experiments, you can find data on the average size of the formed nanoparticles (Z-average), the value of the distribution of nanoparticles in the solution (Area %), the standard error of measurement and the value of polydispersity (PdI). Using the polydispersity index (PdI), you can find out the degree of distribution of nanoparticles in the samples - a decrease in the value of polydispersity indicates a more homogeneous solution in terms of the measured nanoparticles size and shape.

Spectrophotometric Analysis for the Presence of Silver Nanoparticles

A ULAB 102 UV spectrophotometer was used to analyse the obtained samples for the presence of silver nanoparticles. The optical density of the samples was measured to a wavelength range of 320 nm to 500 nm in steps of 5-10 nm. Distilled water was used as a control. The wave range was chosen according to a literature review of studies of the peak of the plasmon resonance spectrum of metal nanoparticles. The obtained results were used to plot graphs of the dependence of the optical density of the samples on the wavelength.

Statistical Analysis

The results of calculating the average size of nanoparticles were calculated immediately on the BeNano 90 Zeta device. Statistical processing of the results was carried out using Microsoft Office Excel 2016 software.

RESULTS AND DISCUSSION

An experiment was conducted using the lysate of *L. acidophilus* UCM B-2691 cells and the supernatant for the synthesis of silver nanoparticles. It is known that this type of bacteria can reduce silver ions Ag^+ to elemental argentum Ag^0 thanks to enzymes, metabolites, proteins and cofactors.

First of all, it should be noted that experiments using the supernatant of *L. acidophilus* UCM B-2691 cells as a substrate for the synthesis of silver nanoparticles are not widespread, but also give excellent results, compared to experiments with cell lysate of this culture as the main component of the synthesis of silver nanoparticles. Thus, taking into account the negative results in the course of the experiment with the solution of *L. acidophilus* UCM B-2691 cell lysate, namely the formation of a silver mirror and a transparent final solution, and the positive results with the use of the supernatant solution of this culture, it can be assumed that this technique is effective for using the supernatant of *L. acidophilus* UCM B-2691 cells as a substrate for the green synthesis of silver nanoparticles.

The general scheme of the experiment is shown, where it is possible to determine the main mechanisms of experimenting, presented in the table. 1. The pH range was changed from 3 to 13 and the temperature from 5 to 50°C. The first control of the possible formation of silver nanoparticles was recorded by a change in color (Fig. 1).

Table 1. Results of qualitative analysis of the synthesis of silver nanoparticles

option	t, °C	pH	Color change
1	5	3	Light yellow
2		7	Light yellow
3		13	Dark brown
4	25	3	Deep yellow
5		7	Yellow-gray
6		13	Dark brown-red
7	50	3	Deep yellow
8		7	Deep yellow
9		13	Dark brown-red

After the synthesis of silver nanoparticles, their concentration was measured using UV-Vis spectrometry and it was determined that at a temperature of 50°C and a pH of 13, the formation of nanoparticles occurred faster and the resulting nanoparticles had an absorption peak at a wavelength of 405 nm.

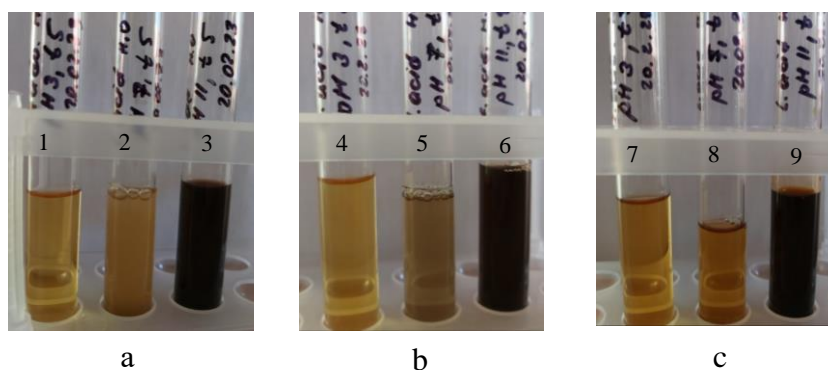


Figure 1. Visual image of synthesized silver nanoparticles at the temperature 5°C (a), 25°C (b), 50°C (c) and different values of pH 3 (1, 4, 7), pH 7 (2, 5, 8), pH 11 (3, 6, 9)

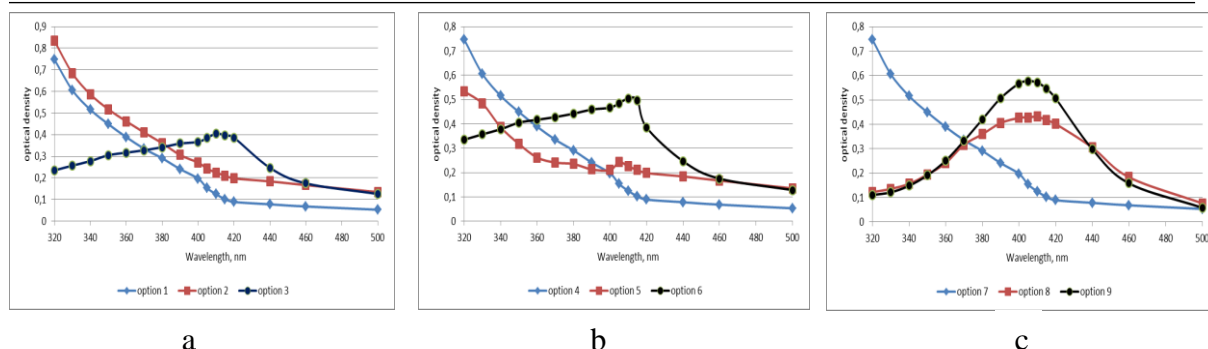


Figure 2. Spectrometric image of silver nanoparticles at temperature 5°C (a), 25°C (b), 50°C (c) and different values of pH 3 (1, 4, 7), pH 7 (2, 5, 8), pH 11 (3, 6, 9)

Thus, when comparing the sizes of nanoparticles obtained during cultivation at a temperature of 5°C (Figs. 1 a and 2 a) and a pH level of 3 (option 1), it can be seen that the nanoparticles were synthesized in the form of large clusters with an average size of 1375 nm, which were determined using BeNano 90 Zeta. Moreover, the difference in size exists due to the different pH values. Visual control was confirmed spectrophotometrically. From Fig. 2 it can be seen that there is a slight absorption peak only in variant 3, which is at 410 nm, in which we record the average particle size of 137 nm. At a neutral pH level of 7 and a temperature of 25°C (option 2), the results were almost the same when kept at a temperature of 5°C (Fig. 1 b and Fig. 2 b). After analysing the results of the study at a temperature of 25°C with different pH values and its proof, it can be seen that in the reaction mixture, where the pH was acidic (option 4), nanoparticles with an average size of 1450 nm were formed, which indicates the formation of cluster compounds and a large number of free biological molecules. However, in variant 5, with a pH level of 7, particles with a size of 965 nm were formed, which makes it clear that sufficiently large particles are formed under these conditions. After setting the pH to 11 and a constant temperature of 25°C (option 6) in the solution, the synthesis of particles with a size of 7193 nm was observed, but in the solution with the pH level adjusted to the introduction of salt, nanosilver with a size of 162 nm was formed, which gives this method an advantage in the given data synthesis characteristics. Also, under these parameters, the absorption peak of the studied variants 4, 5, and 6 was checked spectrophotometrically. The absorption peak at 415 nm was recorded only in variant 6, which indicates the formation of nanosilver particles.

But when the temperature increased to 50°C (options 8 and 9), we already recorded nanoparticles with a size of 35 nm at pH 13 and 98 at pH 7, with the values recorded spectrophotometrically, since in Fig. 2 s it can be seen that the absorption peak occurs at 410 and 405 nm in variants 8 and 9, respectively. Therefore, analysing the obtained results, we can say that pH and temperature are important in the synthesis of silver nanoparticles in the culture liquid of *L. acidophilus* UCM B-2691. The optimal temperature should be at the level of 50°C, and the pH should be alkaline.

CONCLUSIONS

The optimal conditions for the production of silver nanoparticles using *Lactobacillus acidophilus* UCM B-2691 were selected. Synthesis of nanosilver was observed by adding silver nitrate to the culture supernatant at 50°C and pH 13. A range of colors from light to dark brown was observed. Nanoparticle size analysis showed the formation of silver with z-ave: 34.66 nm, PdI: 0.242, Intercept: 0.87 and absorption peak at 405 nm.

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EVALUATION OF THE EFFICIENCY OF LIQUID LEATHER FINISHING USING POLYMERS AND MODIFIED FATS

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The efficiency of technological processes and the quality of leather products largely depend on the nature and technological capabilities of the chemical materials used. This is especially true for liquid finishing, which after tanning is responsible for the further formation of the structure and properties of the leather. Numerous publications note the advantages of using polyacrylates and modified fats in liquid finishing, however, there are references to a decrease in the strength and quality of leather dyeing when using polymers. The purpose of study was to determine the efficiency of liquid finishing of chrome leather for shoe uppers made from cattle raw materials using a new series of polymer compounds based on acrylic acid and modified fats during filling-retanning and fatliquoring. Using standard methods of studying the chemical composition, physical and mechanical characteristics and hygienic properties of the skin, as well as microscopic analysis of its structure, it was established, that the developed technology makes it possible to improve the consumer and cutting properties of leather, as indicated by an increase in many indicators: fat content, strength, elongation, yield in thickness and area, vapor permeability, resistance of the coating to wet friction, reduction of anisotropy of the main indicators of physical and mechanical properties, etc. In addition, taking into account the increasing degree of processing of solutions, the environmental load on the environment is reduced. The achieved result can be explained by the nature of the distribution and fixation of chemical materials in the structure of the dermis with the formation of strong and at the same time flexible bonds.

Keywords: liquid finishing, chemical materials, leather properties

INTRODUCTION

Active implementation of innovative developments in the production of leather, textile, non-woven materials and products allows to significantly improve the consumer properties of these materials, expand the range of their functional capabilities. The use of effective chemical materials in the form of polymer compounds, surfactants, etc. plays a significant role in this. Thanks to the physicochemical modification of structural elements and the surface, competitive products with optimized characteristics are developed. Examples include texturing, embossing, filling, which provide protection of the product from water and wind, abrasion resistance, volume, permeability of water vapor and gases, chemical resistance (Petrova *et al.*, 2023). Another example is the study of the fiber-forming properties of polymer blend melts: polypropylene-copolyamide (PP/CPA), polyoxymethylene-copolyamide (POM/CPA), POM-copolymer of ethylene and vinyl acetate (POM/EVAC), PP/EVAC, EVAC/CPA, polypropylene-polyvinyl alcohol (PP/PVA). The ability of melts of such mixtures to form fibers was assessed by the degree of longitudinal deformation of the polymer melt. The authors found that the fiber-forming properties of polymer melts can be controlled by changing the chemical nature of the mixture components, the processes of structure formation, and the introduction of various polymer additives and non-polymer additives into the binary mixture. Complex threads made from PP, POM, or EVAC microfibers have such excellent properties as high strength and initial modulus, high elasticity, exceptional softness, pleasant feel, adhesion, capacity, and wooliness without special textural procedures, high sorption of moisture, dyes, and other compounds (Tsebrenko *et al.*, 2004).

The efficiency of technological processes and the quality of leather goods also largely depend on the nature and technological capabilities of the materials used. This is especially true for liquid finishing, which after tanning is responsible for the further formation of the leather structure and properties. Traditional technologies for retanning and filling of semi-finished leather products include a wide range of plant, polymer and mineral compounds. At the same time, the limited production and high cost of plant tannins contributed to the development of synthetic compositions for retanning and filling, also with the aim of completely eliminating or partially replacing expensive plant tannins. Since there is insufficient information in the scientific literature on compositions that could fully reproduce the retanning and filling effect on the structure of semi-finished leather products, similar to the effect of plant tannins, the development of a new retanning and filling composition for leather production based on lignosulfonates and highly dispersed natural minerals has become relevant (Mokrousova, 2010).

A number of recent studies have noted the advantages of using polyacrylates (Zaiets & Andreyeva, 2023a; Canudas *et al.*, 2019; Jin *et al.*, 2019; Yi *et al.*, 2024; Dudu *et al.*, 2024) and modified fats (Zaiets & Andreyeva, 2023a; Sahu *et al.*, 2021; Nkwor & Ukoha, 2023; Niyozova, 2023; Alam *et al.*, 2024) for liquid finishing. However, there are some references to the fact that leathers retanned with acrylic polymers have lower color intensity and worse structural properties (strength) due to their high anionic activity, which changes the cationic surface of the leather, causing lower interaction of dyes and fatliquoring agents with the leather (Canudas *et al.*, 2019; Jin *et al.*, 2019; Yi *et al.*, 2024). The purpose of this study was to determine the efficiency of liquid finishing of shoe upper leather from cattle hides using a new series of polymer compounds based on acrylic acid and modified fats during retanning-filling and fatliquoring.

MATERIALS AND METHODS

To carry out the dyeing and fatliquoring processes, a number of new generation chemical materials were used in the work:

- Syntan RS-540 (Smit&Zoon, the Netherlands) – a polymer resin based on acrylic acid;
- Synthol LC (Smit&Zoon, the Netherlands) – fatliquoring mixture based on natural and synthetic oils, sulfonated triglycerides, lecithin mixture;
- Sulphirol EG-60 (Smit&Zoon, the Netherlands) – fatliquoring mixture based on sulfated natural and synthetic oils;

as well as imported materials that are widely used in practice /quebracho tannins (China) for retanning-filling; Provol BA (Zschimmer&Schwarz, Germany) – a fatliquoring agent based on lecithin; Sanodal Black (Clariant Inc, Switzerland) – anionic black dye/ and domestic materials /sodium formate, bicarbonate and carbonate for neutralization, acetic acid to enhance the spreading of the fatliquoring emulsion and improve the distribution of fatliquoring materials in the dermis; ammonium hydroxide and nonionic surfactants to increase the stability of the emulsion/.

Based on previously conducted studies of the structure, physicochemical properties and technological capabilities of a number of modern polymer compounds and modified fatliquoring materials (Zaiets & Andreyeva, 2024a; 2024b; 2024c; 2023b), a process map was created for the liquid finishing of Wet Blue – a semi-finished product of the chrome tanning method for the shoe upper leather from cattle hides, the peculiarity of which is the implementation of the retanning-filling process using Syntan RS-540 with a halved consumption of vegetable tannins (quebracho tannins), and the fatliquoring process – using modified fats Synthol LC and Sulphirol EG-60 (Zaiets & Andreyeva, 2024d):

1. Washing: water – 100-150%, surfactant – 0.3%, acetic acid – 0.5%, temperature 30 °C, duration 20 min.
2. Washing: water – 100-150%, temperature 30 °C, duration 20 min.
3. Retanning: water – 150%, chrome tanning – 4.0%; temperature 30 °C, duration 60 min; sodium bicarbonate – 0.2%, duration 60 min.
4. Washing: water – 100-150%, temperature 30 °C, duration 20 min.
5. Neutralization: water – 200-250%, sodium bicarbonate – 0.5-0.7%, sodium formate – 0.5-0.7%, temperature 30-35 °C, duration 60 min.
6. Washing: water – 100-150%, temperature 30 °C, duration 20 min.
7. Retanning-filling: water – 150%, temperature 30-35 °C, Syntan RS-540 – 3.0-6.0%; duration 40-60 min; quebracho tannins – 2.0%, 40-60 min.
8. Washing: water – 100-150%, temperature 30-50 °C, duration 20 min.
9. Fatliquoring: water – 150%, temperature 45-50 °C, duration 5 min; Synthol LC – 3.0-6.0%, Sulphirol EG-60 – 3.0-6.0%, duration 60 min; acetic acid – 1.0%, duration 60 min.
10. Washing: water – 100%, temperature 30 °C, duration 10 min.

Liquid finishing processes are carried out with constant rotation of the equipment. The consumption of materials is calculated for the technical product. A fat emulsion with a concentration of 25% is prepared by gradually adding water (temperature 45-50 °C), 2.0% surfactant and 0.5% of a 10% ammonium hydroxide solution (based on the weight of the fat) to the fat sample. Stirring is constant. After liquid finishing, the leather is processed using the traditional method of drying and tempering processes and operations and leather coating (Danilkovich & Mokrousova, 2009).

Subsequently, taking into account the created process map, rational parameters of liquid finishing were determined by mathematical modeling, providing for retanning-filling of the semi-finished product Wet blue with acrylic polymer Syntan RS-540 at a consumption of 3.0%, a temperature of 30-35 °C for 1-2 hours and fatliquoring with modified fats Sulphirol EG 60 and Synthol LC at a consumption of 3.0%, a temperature of 45-50 °C for 1.5-2 hours. The final finishing in the work was carried out by double application of a coating of the following composition, wt. parts: Compound VR (30%) – 60; acrylic AM 146 (42.5%) – 80; Rokril SW 4025 (40%) – 120; wax emulsion (20%) – 40; water – 200. Consumption – 80 g/m² (Zaiets & Andreyeva, 2024d).

The properties of the chemical and leather materials studied in the work were assessed using the analysis methods common in the leather industry (Danilkovich, 2006) in accordance with regulatory documentation, for example: DSTU EN ISO 2419: 2020 (EN ISO 2419: 2012, IDT; ISO 2419: 20 Leather. Physical and mechanical tests. Preparation and conditioning of samples; 3376: 2022 (EN ISO 3376: 2020, IDT; ISO 3376: 2020, IDT) Leather. Physical and mechanical tests. Determination of tensile strength and elongation in percent; DSTU EN ISO 17236: 2022 (EN ISO 17236: 2016, IDT; ISO 17236: 2016, IDT) Leather. Physical and mechanical tests. Determination of elongation set; DSTU ISO 3380:2022 (EN ISO 3380:2015, IDT; ISO 3380:2015, IDT) Leather. Method for determining shrinkage temperature; DSTU EN ISO 14268:2022 (EN ISO 14268:2012, IDT; ISO 14268:2012, IDT) Leather. Physical and mechanical tests. Determination of water vapour permeability; DSTU EN ISO 4048:2022 (EN ISO 4048:2018, IDT; ISO 4048:2018, IDT) Leather. Chemical tests. Method for determination of substances soluble in dichloroethane; EN ISO 5398-1:2022 (EN ISO 5398-1:2018, IDT; ISO 5398-1:2018, IDT) Leather – Chemical determination of chromium oxide content – Part 1: Quantitative determination by titration, etc.

It was experimentally established that, in comparison with the known technology, the developed technology of liquid finishing allows improving the strength, hygienic and elastic-

plastic properties of finished leather, this is indicated by the results of chemical analysis and physical and mechanical tests (Zaiets & Andreyeva, 2024d). The importance of drum dyeing for the appearance of leather and its commercial value is well known from the theory and practice of tannery production. Based on this, this study was devoted to refining the process parameters and determining the efficiency of liquid finishing using polymers and modified fats, additionally including in the proposed process flow chart after retanning-filling the process of drum dyeing under the following conditions: consumption of anionic dye Sanodal Black 4.0% of the semi-finished product weight, duration 1 hour, temperature 30-40 °C. During retanning-filling of experimental samples of Wet Blue from cow hide, 3.0% acrylic polymer Syntan RS-540 and 2.0% quebracho tannins were used; with fatliquoring with 3.0% of modified fats Synthol LC and Sulphirrol EG 60. In the control group, 4.0% of quebracho tannins were used for retanning-filling, and 6.0% of the well-known anionic fat Provol BA were used for fatliquoring. The leather was processed after liquid finishing using the well-known method for producing shoe upper leather from cattle hides (Danilkovich & Mokrousova, 2009). The leather coating was carried out in the same way as indicated above.

RESULTS

No complications were observed during the experiment. The experimental leather samples were uniformly dyed and painted black, had a clean front surface, and a pleasant handle. The color of the control samples was black with a greenish tint.

The results of chemical analysis and physical and mechanical tests presented in Table 1 indicate a positive effect of liquid finishing using 3.0% acrylic polymer, 3.0% of each of the two modified fats involved in the work, and 4.0% Sanodal Black dye on the indicators of finished leather and the choice of chemical materials from solutions, since the following occur:

Table 1. Indicators of finished leather and the degree of processing of working solutions

Indicator	Experience	Control
Leather:		
Mass part (on abs dry matter), %:	4.21	4.00
- chromium oxide		
- substances extractable by organic solvents	5.00	4.47
Tensile strength σ_1 , 10 MPa	1.89	1.78
Stress at appearance of cracks in the outer layer σ_2 , 10 MPa	1.84	1.65
$\Delta = 100 \cdot [(\sigma_1 - \sigma_2) / \sigma_1]$, %	2.7	7.3
Elongation at 10 MPa stress $L10$, %	37.9	34.0
Uniformity coefficient $K\sigma \sigma_1 / K\sigma_2 / K_{L10}$	0.89/0.86/0.79	0.78/0.74/0.62
Shrinkage temperature, °C	114.0	112.5
Vapor permeability, %	83.9	77.0
Coloring, %	70.6	59.5
Colour resistance, points:	4	3
- to dry friction		
- to wet friction	4	3
Coating resistance to wet friction, rpm	240	200
Resistance of the coating to repeated bending, points.	4	4
Output by area, %	97.6	91.8
Output by thickness, %	90.1	88.3
Spent solution:		
Degree of processing, %, after:	86.0	80.3
- retanning-filling		
- drum dyeing	74.8	65.2
- fatliquoring	83.0	76.4

- increase in the content of unbound fatty substances (substances extractable by organic solvents) in the leather by 11.8%, which improves its elastic-plastic properties (the elongation at 10 MPa stress increases by 11.5%);
- increase in overall leather strength (tensile strength) by 6.2%, and in the strength of its outer layer by 11.5%;
- decrease in the difference between overall leather strength and the strength of its outer layer by 2.7 times, which indicates a more uniform distribution of chemical materials in the dermis, which in turn improves the elastic-plastic properties and output by area;
- decrease in the anisotropy of the main indicators of physical and mechanical properties (increase in the uniformity coefficients of distribution in different directions of the leather of the tensile strength, stress at the appearance of cracks in the outer layer and elongation at 10 MPa stress by 1.1-1.2 times), which will lead to a more rational use of leather materials when cutting into foot-wear components;
- improvement in leather dyeability by 18.7% rel.;
- increase in the resistance of dyeability to dry and wet friction by 1 point;
- improvement in hygienic properties (the vapor permeability index increases by 9.0%);
- increase in output by thickness and area by 1.8% abs and 5.8% abs, respectively;
- improvement in the quality of the coating on the leather (the coating resistance to wet friction increases by 1.2 times), which will also have a positive effect on the consumer properties of leather products;
- improvement in the composition of wastewater due to an increase in the degree of solution processing after retanning-filling by 7.0% rel., after drum dyeing – by 14.7% rel., after fatliquoring – by 8.6% rel.

Improvement of the degree of dye solution processing, dyeability and coloring of leather in the experimental group showed the possibility of more economical use of dye. An additional experiment confirmed this possibility, since with a decrease in the consumption of Sanodal Black dye from 4.0 to 3.0% (i.e. by 25%), the extraction of dye from the solution, dyeability and coloring of leather really improved, the strength and vapor permeability indicators, and the resistance of dye to dry and wet friction increased (Table 2).

Table 2. Determining the possibility of reducing dye consumption

Indicator	Value for dye consumption	
	3.0%	4.0%
Degree of processing after drum dyeing, %	75.0	65.5
Coloring, %	80.1	60.2
Tensile strength σ_p , 10 MPa	2.00	1.80
Stress at appearance of cracks in the outer layer, 10 MPa	1.96	1.60
Elongation at 10 MPa stress L_{10} , %	37.0	32.5
Vapor permeability, %	85.9	76.8
Colour resistance, points:		
- to dry friction	4	3
- to wet friction	4	3

Based on the results of the conducted research, the technology of liquid finishing using polymer and modified fats can be finally presented in the form of the following simplified scheme: washing 1,2 – retanning with chromium compounds – washing – neutralization – washing – retanning-filling with organic compounds (polymer Syntan RS + tannins quebracho with a 50% reduction in vegetable tannins consumption) – drum dyeing (reduction in dye consumption by 25%) – washing – fatliquoring with modified fats Synthol LC and Sulphirrol EG 60 – washing.

Using a scanning electron microscope SEM JSM-6490-LV (GEOL, Japan), the effect of liquid finishing on the morphological structure of Crust (uncoated leather) was established, which manifests itself in a decrease in the spread of voids over the area, an increase in the density of the structure in the direction of the front surface during retanning-filling with polymer and fatliquoring with modified fats (Figure 1).

In the micrographs of the experimental samples, a more ordered structure of the dermis is observed – a more uniform separation of collagen fiber bundles and the location of interbundle spaces (Figure 1, a).

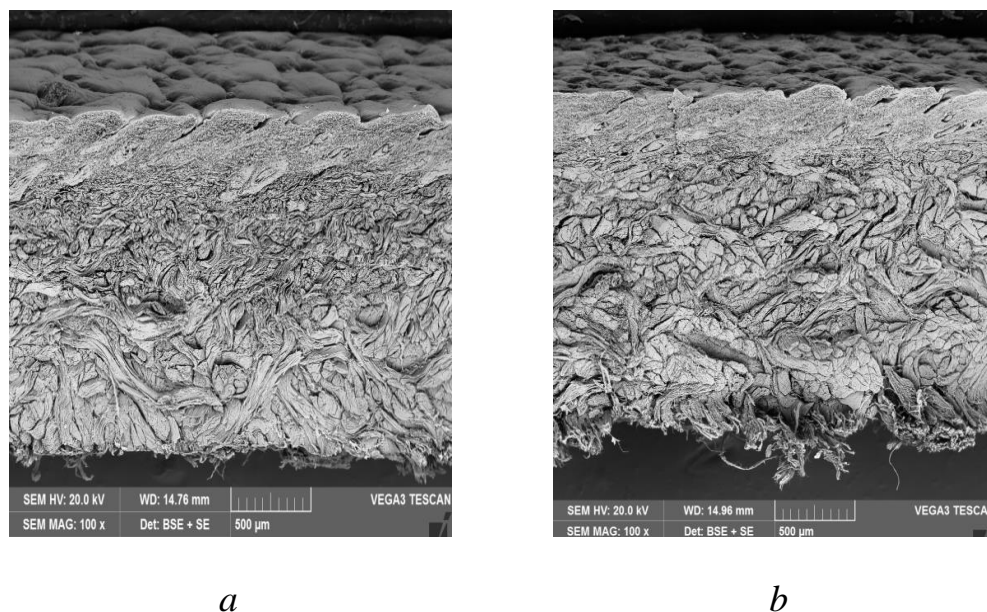


Figure 1. Electron microscopic image of cross-sections of skin samples (x100): *a* – experiment; *b* – control

Industrial testing of the developed technology confirmed the results of laboratory studies. Based on the evaluation of the properties of finished leather made using acrylic polymer and modified fats during liquid finishing, it can be concluded that such treatment improves the properties of leather, avoiding problems with the strength and coloring of traditional acrylic resins. The use of Syntan RS-540 as a retanning agent promotes the absorption of dye and fatliquoring solutions, a more uniform distribution of materials in the structure of the dermis, and a reduction in the harmful environmental impact on the environment.

CONCLUSION

Based on a comprehensive study of the structure, properties and technological capabilities of a new series of polymer compositions and modified fats, their influence on the formation of the properties of leather for shoe uppers made of cattle hides, rational parameters of the liquid finishing technology have been established, providing for retanning-filling the semi-finished product Wet blue with acrylic polymer Syntan RS-540 at a consumption of 3.0%, a temperature of 30-35 °C for 1.5-2 hours and fatliquoring with modified fats Sulphir and Synthol LC at a consumption of 3.0%, a temperature of 45-50 °C for 1.5-2 hours. The developed technology allows to improve twofold the strength, hygienic, elastic-plastic, cutting properties and some other characteristics of the finished leather while reducing the consumption of vegetable tanning agents. After including the drum dyeing process using the

anionic dye Sanodal Black in the process flow chart, the efficiency of the developed technology was assessed taking into account this process. Experiments have shown that leather is more evenly dyed black, that the dye and other reagents are more fully extracted from the working solution, and that many leather parameters are improved, including the color fastness to dry and wet friction, when using the developed technology. This result is maintained when the dye consumption is reduced from 4.0 to 3.0%, i.e. by 25%.

Industrial tests of the developed technology have confirmed the results of laboratory studies. Based on the evaluation of the properties of finished leather treated with acrylic polymer and modified fats, it can be concluded that such treatment improves the properties of leather, avoiding problems with the strength and drum dyeing of traditional acrylic resins. The use of Syntan RS-540 as a retanning agent promotes better absorption of dye and fat solutions, as well as a more uniform distribution of materials in the structure of the dermis. The obtained effect can be explained by the nature of the formation and ordering of the microstructure of the dermis under the influence of the polymer and modified fats with the formation of various, strong and at the same time flexible, mobile connections between the individual components of the “collagen-chemical material” system.

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