OBTAINING NEW BIOEMULSIONS BASED ON LAVENDER EXTRACT AND SURFACTANTS

Demetra SIMION[1*], Carmen GAIDĂU[1], Gabriela PĂUN[2], Daniela BERECHET[1], Roxana CONSTANTINESCU[1]

[1]National Research and Development Institute for Textiles and Leather (INCDTP) – Division: Leather and Footwear Research Institute (ICPI), 93 Ion Minulescu st., 031215, Bucharest, Romania

[2]National Institute for Research-Development of Biological Sciences, 060031 Bucharest, Romania

Received: 06.10.2023 Accepted: 11.12.2023 https://doi.org/10.24264/lfj.23.4.4

OBTAINING NEW BIOEMULSIONS BASED ON LAVENDER EXTRACT AND SURFACTANTS

ABSTRACT. New bioemulsions were created using biotechnologies based on lavender extract (oil) and two surfactants: Tween® 20 and Tween® 80 mixture: E1 – lavender oil/Tween® 20/water; E2 – lavender oil/ Tween® 80/water; E3 – lavender oil/ Tween® 20 and Tween® 80 (ratio 1:1)/water, for different concentrations (28.56%, 7.14%) of lavender oil, in order to improve surface properties with applications in leather industry. More concentrated emulsions with oil lavender (28.56%) were marked E1c, E2c, E3c. In the process of finishing the leathers by spraying with six types of emulsions obtained compared to an untreated leather, the aim was to improve the antifungal, antimicrobial properties as well as the softness, appearance of the leathers. The order of introducing components in the developed biotechnologies, the working conditions, and especially the choice of the concentration of surfactants >CMC, are essential in the solubilization of vegetable oils and obtaining the desired bioemulsions. Comparatively, bioemulsions were made for the version with two surfactants, but instead of lavender, immortelle was introduced, resulting in emulsions: Lemulsion, Iemulsion. The bioemulsions and leathers processed with them were analyzed by FTIR-ATR spectroscopy, DLS and microbiological tests. It can be seen that the leather with the largest amount of lavender after processing with concentrated emulsions is E3c (having the highest intensity over the entire spectral range) and the maximum absorption specific to lavender oil was found. The lavender oil is fixed better on the leathers than the immortelle oil, with the new bioemulsions created.

KEYWORDS: new bioemulsions, biotechnologies based on lavender extract and surfactants, immortelle, leathers processed

OBȚINEREA UNOR NOI BIOEMULSII PE BAZĂ DE EXTRACT DE LAVANDĂ ȘI SURFACTANȚI

REZUMAT. S-au creat noi bioemulsii utilizând biotehnologii bazate pe extract de lavandă (ulei) și doi agenți tensioactivi: amestec Tween® 20 și Tween® 80: E1 – ulei de lavandă/Tween® 20/apă; E2 – ulei de lavandă/Tween® 80/apă; E3 – ulei de lavandă/Tween® 20 și Tween® 80 (raport 1:1)/apă, pentru diferite concentrații (28,56%, 7,14%) de ulei de lavandă, pentru a îmbunătăți proprietățile suprafeței cu aplicații în industria de pielărie. Emulsiile mai concentrate cu ulei de lavandă (28,56%) s-au notat cu E1c, E2c, E3c. În procesul de finisare a pieilor prin pulverizare cu șase tipuri de emulsii obținute comparativ cu o piele netratată, s-a urmărit îmbunătățirea proprietăților antifungice, antimicrobiene precum și moliciunea, aspectul pieilor. Ordinea introducerii componentelor în biotehnologiile dezvoltate, condițiile de lucru și mai ales alegerea concentrației de surfactanți >CMC sunt esențiale în solubilizarea uleiurilor vegetale și obținerea bioemulsiilor dorite. Comparativ, s-au realizat bioemulsii pentru varianta cu doi surfactanți, dar în loc de lavandă s-a introdus imortelă, rezultând emulsiile: Lemulsion, Iemulsion. Bioemulsiile și pieile prelucrate cu acestea au fost analizate prin spectroscopie FTIR-ATR, DLS și teste microbiologice. Se poate observa că pielea cu cea mai mare cantitate de lavandă după prelucrare cu emulsii concentrate este E3c (având cea mai mare intensitate pe întregul interval spectral) și s-a determinat absorbția maximă specifică uleiului de lavandă. Uleiul de lavandă se fixează mai bine decât cel de imortelă pe piei, cu noile bioemulsii create.

CUVINTE CHEIE: noi bioemulsii, biotehnologii bazate pe extract de lavandă și agenți tensioactivi, imortelă, piei prelucrate

L'OBTENTION DE NOUVELLES BIOÉMULSIONS À BASE D'EXTRAIT DE LAVANDE ET DE TENSIOACTIFS

RÉSUMÉ. De nouvelles bioémulsions ont été créées par les biotechnologies à base d'extrait (huile) de lavande et de deux tensioactifs : Tween® 20 et Tween® 80 mélange : E1 – huile de lavande/Tween® 20/eau ; E2 – huile de lavande/Tween® 80/eau ; E3 – huile de lavande/Tween® 20 et Tween® 80 (rapport 1:1)/eau, pour différentes concentrations (28,56%, 7,14%) d'huile de lavande, afin d'améliorer les propriétés de surface avec des applications dans l'industrie du cuir. Les émulsions plus concentrées en huile de lavande (28,56%) ont été notées : E1c, E2c, E3c. Dans le processus de finition des cuirs par pulvérisation de six types d'émulsions obtenues par rapport à un cuir non traité, le but était d'améliorer les propriétés antifongiques, antimicrobiennes ainsi que la douceur, l'aspect des cuirs. L'ordre d'introduction des composants dans les biotechnologies développées, les conditions de travail et surtout le choix de la concentration en tensioactifs >CMC, sont essentiels à la solubilisation des huiles végétales et à l'obtention des bioémulsions souhaitées. Comparativement, des bioémulsions ont été réalisées pour la version avec deux tensioactifs, mais à la place de la lavande, de l'immortelle a été introduite, ce qui a donné des émulsions : Lemulsion, Iemulsion. Les bioémulsions et les cuirs traités avec celles-ci ont été analysés par spectroscopie FTIR-ATR, DLS et tests microbiologiques. On constate que le cuir qui contient la plus grande quantité de lavande après traitement avec des émulsions concentrées est le E3c (ayant l'intensité la plus élevée sur toute la gamme spectrale) et on a determiné l'absorption maximale propre à l'huile de lavande. L'huile de lavande se fixe mieux que l'huile d'immortelle sur les cuirs, grâce aux nouvelles bioémulsions créées.

MOTS CLÉS : nouvelles bioémulsions, biotechnologies à base d'extrait de lavande et de tensioactifs, immortelle, cuirs traités

* *Correspondence to: Demetra SIMION, National Research and Development Institute for Textiles and Leather (INCDTP) – Division: Leather and Footwear Research Institute (ICPI), 93 Ion Minulescu st., 031215, Bucharest, Romania, demetra.simion@yahoo.com*

INTRODUCTION

This paper presents biotechnologies to create new bioemulsions, based on lavender extract (oil) for different concentrations (28.56%, 7.14%) and two surfactants: Tween® 20 and/or Tween® 80 [1], in order to improve surface properties with applications in leather industry.

Lavender oil is an essential oil obtained by distillation from the flower spikes of certain species of lavender. There are over 400 types of lavender worldwide with different scents and qualities. Two forms of lavender oil are distinguished, lavender flower oil, a colorless oil, insoluble in water, having a density of 0.885 g/mL; and lavender spike oil, a distillate from the herb *Lavandula latifolia* (Figure 1), having a density of 0.905 g/mL. Like all essential oils, it is not a pure compound; it is a complex mixture of phytochemicals, including linalool and linalyl acetate.

Figure 1. Image of *Lavandula latifolia*

The phytochemical composition of lavender oil varies from species to species, consisting primarily of monoterpeneoid and sesquiterpeneoid alcohols [2-6]. Linalool (20-35%) and linalyl acetate (30-55%) dominate, with moderate levels of lavandulyl acetate, terpinen-4-ol and lavandulol, 1,8-cineole, camphor, limonene and tannins. Lavender oil typically contains more than 100 compounds, although many of these are at negligible concentrations. Lavender oil has been used for perfume, aromatherapy and skincare applications, but these uses have no clinical benefit. Lavender oil is used in massage therapy as a way of inducing relaxation through direct skin contact.

Tween 80 and Tween 20 are biocompatible surfactants [1-6]. Tween 80 is a polyethylene sorbitol ester, also known as Polysorbate 80, PEG (80) sorbitan monooleate, polyoxyethylenesorbitan monooleate. 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 is a group of non-volatile surfactant derivatives derived from glycerol esters. Tween-20 and Tween-80 vary in chemical and physical properties very much but 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 like as a thermosetting agent in the process of manufacturing thermosetting plastics and as an adhesive for repairing paper materials. It also helps in the manufacture of plastic parts and even metal parts.

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.

In this research the new bioemulsions created and leathers processed with them were analyzed by FTIR-ATR spectroscopy, DLS and microbiological tests.

EXPERIMENTAL

Materials and Methods

In order to obtain new bioemulsions the following materials have been used: Tween 20 and Tween 80 from Sigma-Aldrich; lavender oil from “VIORICA” company.

The experimental techniques used in this paper consist in:

- “MALVERN” zetasizer-nano equipment, with measuring range between 0.3 nm-60.0 microns and zeta potential determination with an accuracy of +/-2%;

- JASCO FTIR-ATR spectrophotometer.

A number of four samples of bioemulsions were prepared in the following working conditions: Tween 20 or/and Tween 80 at 1:1 ratio, temperature=45°C for 60 minutes with lavender or immortelle oil – c=7.14%, Figure 2. More concentrated emulsions with oil lavender (28.56%) were created and marked: E1c, E2c, E3c. Comparatively, bioemulsions were made for the version with two surfactants, but instead of lavender, immortelle was introduced, resulting in emulsions: Lemulsion, Iemulsion.

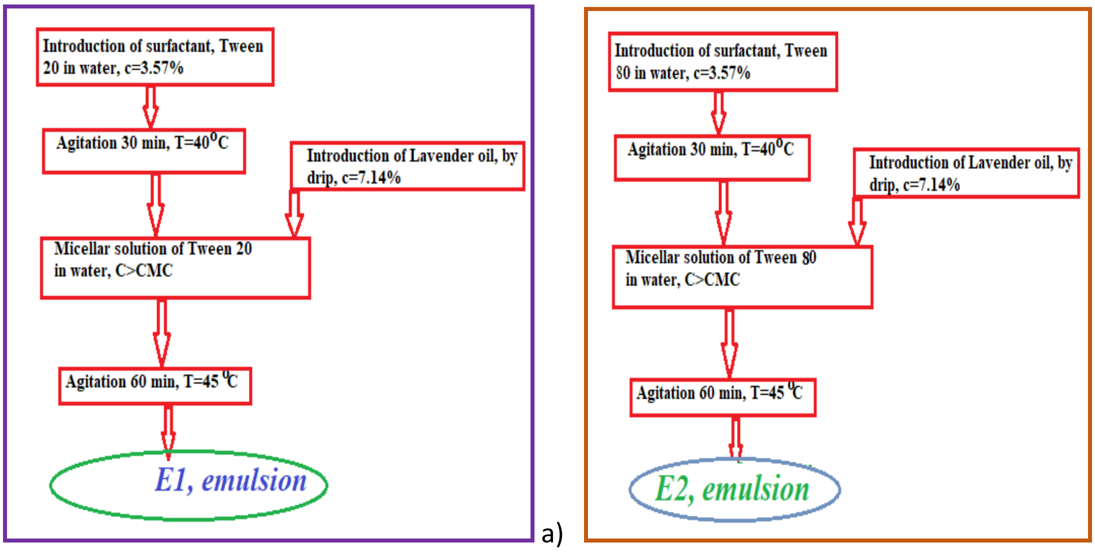
a) b) c) d)

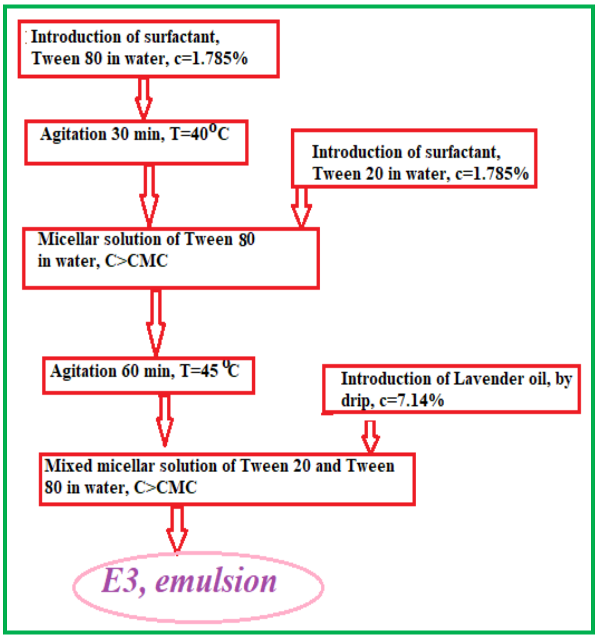
Figure 2. Image of bioemulsions: a) 1 – E1 – lavender oil/Tween® 20/water; 2 – E2 – lavender oil/Tween® 80/water; 3 – E3 – lavender oil/ Tween® 20 and Tween® 80 (ratio 1:1)/water; 4 – Iemulsion – immortelle oil/ Tween® 20 and Tween® 80 (ratio 1:1)/water; b) Lavender oil; c) Tween 20; d) Tween 80

RESULTS AND DISCUSSIONS

Obtaining New Bioemulsions Based on Lavender or Immortelle Oil and Surfactants

Aqueous emulsions were obtained using two surfactants with different hydrophobic chains, Tween 20 and Tween 80, in which oil plant extracts (lavender or immortelle oil) were introduced. According to original innovative biotechnologies in Figure 3, three types of lavender-based bioemulsions were made: E1, E2, E3. The preparation biotechnologies of bioemulsions were optimized, introducing a larger amount of lavender, c=28.56%, four times higher than c=7.14%. The new emulsions were marked: E1c, E2c, E3c and the leathers processed with them: E1l, E2l, E3l. The antimicrobial and antifungal effect was increased with the increase in the amount of lavender.





c)

Figure 3. Biotechnologies for obtaining three types of bioemulsions with lavender oil: a) E1; b) E2; c) E3

For immortele oil, the method of obtaining emulsions is similar to the one in Figure 3, but the lavender oil is replaced with the immortele oil. Only the E3 emulsion was made for comparison, in which the lavender oil was introduced once and then the immortelle oil, obtaining the emulsions marked: Lemulsion, Iemulsion. The E3 emulsion variant with the two surfactants, Tween 20 and Tween 80, in a 1:1 ratio was selected because it is the most stable over time (1 month). The way of introducing surfactants and vegetable oil in obtaining emulsions is very important. The surfactant micellar solution is always made in water at a concentration above the micellar critical concentration – CMC, and then the vegetable oil is added drop by drop and mixed. The chosen temperature is 40-45°C for a good solubilization of the vegetable oil in the surfactant micelles. When there are two surfactants, micellar solutions in water are made separately for them, then the two solutions are mixed and mixed micelles in water are obtained. In the solution of mixed micelles, the vegetable oil is introduced drop by drop, stirring at the appropriate temperature. In the end, the emulsion is obtained with lavender oil solubilized in the mixed micelles. The yield of multiple drop formation decreases rapidly as the homogenization time increases. Bioemulsions are formed and the properties derive from the surfactants used, as well as the conditions and working parameters. This phenomenon is controlled by the concentration of lavender or immortelle oil, surfactants, temperature, pH=4.

Mechanism of Lavender Oil Solubilization in Surfactant Micelles

In order to solubilize the lavender oil, many media are adopted, among which surfactant solubilization is important. In this research, the interaction of lavender oil with two nonionic tween surfactants, Tween 20 and Tween 80, was investigated. A mechanism for the solubilization of lavender oil in surfactant micelles was proposed, Figure 4.

The effect of the length of the carbon chain on the interaction was analyzed by FTIR-ATR spectroscopy. The experimental results suggested that Tween 80 was most efficient out of the two surfactants taken for the study. The order of stability is given as Lavender oil – Tween 80 > Lavender oil – Tween 20.

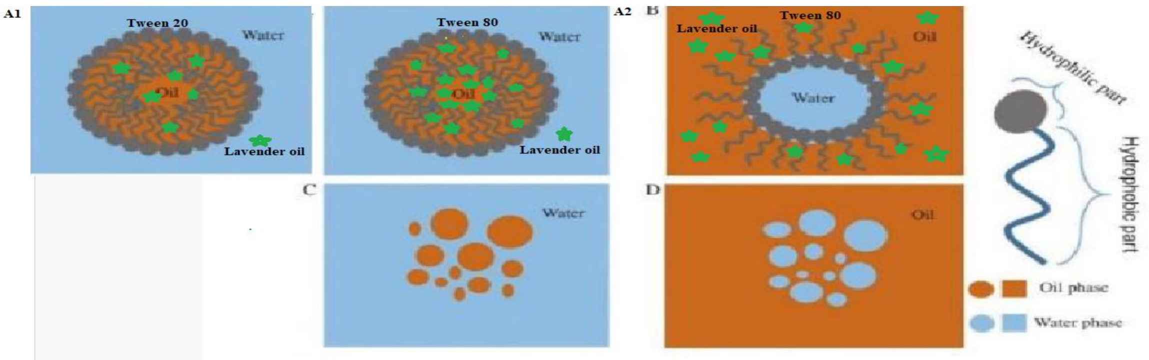


Figure 4. Proposed mechanism for solubilization of lavender oil in Tween 20 or Tween 80 surfactants. Schematic representation of oil-in-water (O/W)-A1-A2; water-in-oil (W/O)-B; emulsions, oil droplets in water-C; and water droplets in oil-D

Lavender oil is hydrophobic and gets stuck in the core of the micelles but also on the alkyl ends of the hydrophobic chains. For Tween 80, the amount of solubilized lavender oil is higher than in the case of Tween 20, because it has a larger hydrophobic chain. Interaction forces are responsible. The mechanism is similar for immortelle oil.

Characterization of the Emulsions Obtained and the Leathers Processed with Them

Characterization by FTIR-ATR Spectroscopy

The FTIR-ATR spectroscopy is part of the nondestructive analytical methods whose objective is to determine the functional groups of the analyzed sample by absorbing IR radiation at characteristic frequencies, thus obtaining an individual spectrum. FTIR-ATR spectrometers being coupled to computers equipped with specialized software can process the spectra and also store databases with a rich content of IR spectra of known substances that can be used in the automatic identification of a new, unknown substance. Emulsions obtained based on surfactants and lavender or immortele oils were used in the processing of leathers in different variants. The emulsions and leathers processed with them were analyzed spectrophotometrically by FTIR-ATR. Lavender oil has the following characteristic absorption maxima at the wavenumbers: 2966 cm^{-1}, 2927 cm^{-1}, 1735 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, 1240 cm^{-1}. The spectrum of immortelle oil is also shown in Figure 5.

The overlap of the FTIR-ATR spectra for the obtained emulsions E1, E2, E3 and lavender oil are illustrated in Figure 6.

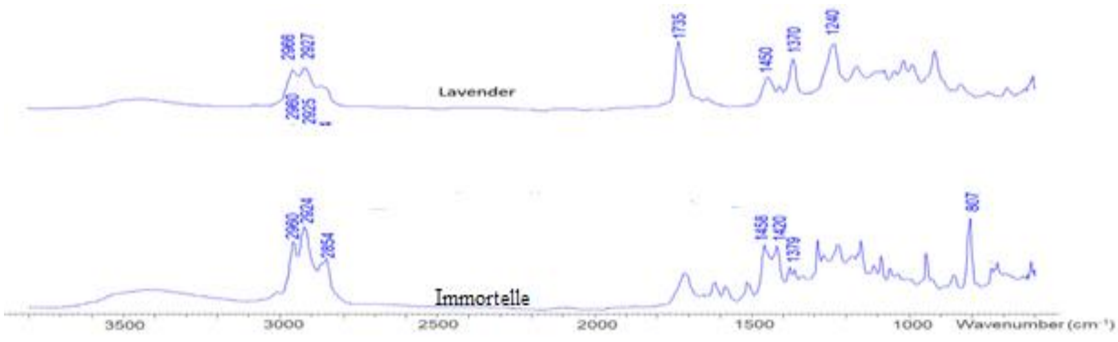


Figure 5. FTIR-ATR spectra for lavender and immortelle oils

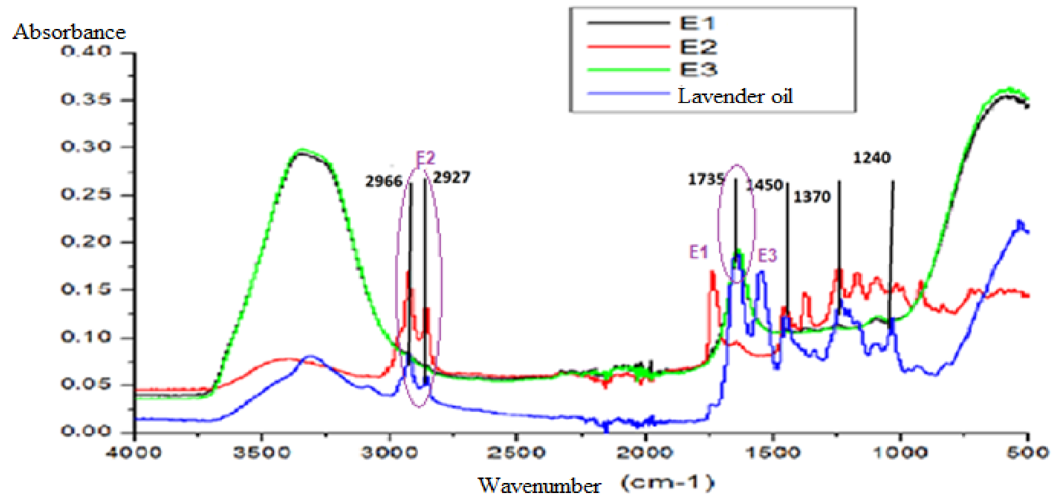
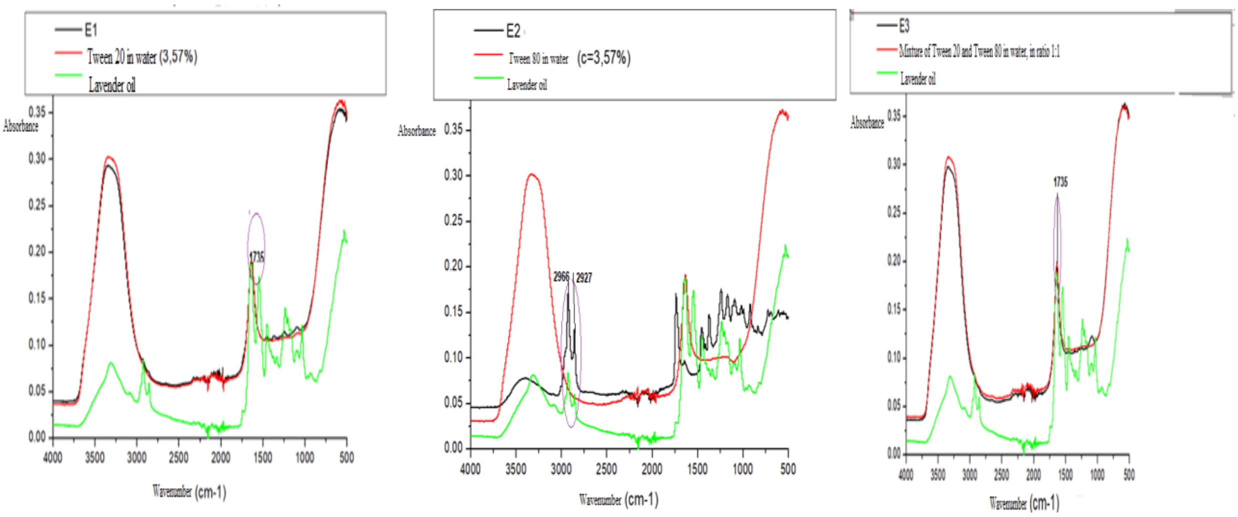


Figure 6. The overlap of the FTIR-ATR spectra for the obtained emulsions E1, E2, E3 and lavender oil

For the FTIR-ATR spectrum of lavender oil the absorption maxima corresponding to the range of wavenumbers ~1375-1450 cm^{-1} are due to the functional group $=CH_2$, originating from a plane deformation at 1420 cm^{-1}. The presence of the $=CH_2$ grouping increases the intensity of the absorption maxima in the range of wavenumbers: 1330–1410 cm^{-1} specific to the existence of terpenes. The absorption maximum at the wavenumber ~1450 cm^{-1} is the result of the overlap of the CH_2 deformation with the asymmetric CH_3 deformation (the intensity of the absorption maximum being proportional to the number of CH_2 and CH_3 groups present). The range of wavenumbers: 3400–3500 cm^{-1} is specific to oil extracts from the *Lamiaceae* family that have a high content of phenolic compounds and flavonoids. The absorption maximum at the wavenumber: 842 cm^{-1} represents a weak skeletal vibration of isopropyl ($R_1R_2C=CHR_3$), the deformation being out of plane for undeformed, weakly strained systems, i.e. for cyclohexene derivatives. The range of wavenumbers: 1635–1650 cm^{-1} is characteristic of low intensity maxima respectively for $RHC=CH_2$ i.e. linalool and linalool acetate. The absorption maximum specific to the wavenumber: 1745 cm^{-1} is given by the carbonyl group. It can be seen from Figure 6 that the shape of the spectra of E1 and E3 emulsions is similar with higher intensity for E3 emulsion because it contains two surfactants, Tween 20 and Tween 80, compared to E1 which contains only Tween 20. It is found that the spectrophotometric imprint is stronger for Tween 20 than for Tween 80 because the spectrum for E3 which has two surfactants is almost identical to that for E1 which only has Tween 20. For E1 and E3 emulsions the presence of Tween 20 surfactant is given by two characteristic absorption maxima in the range of wavenumbers: 3250-3500 cm^{-1} (given by the phenolic groups) and 2000-2250 cm^{-1} (from the CH_2 and CH_3 groups present), Figure 7. The presence of lavender oil in the two emulsions E1-E3 is given by the significant absorption maximum at 1735 cm^{-1} specific to the carbonyl group but also by the identical spectral allure in the range of wavenumbers: 1450-1240 cm^{-1}. Emulsion E3 is more stable over time (1 month) than E1 (7 days). The presence of two surfactants gives greater stability to the E3 emulsion. Emulsion E2 (compared to E1 and E3) solubilizes lavender oil best, the allure of their spectra being very similar. The Tween 80 surfactant present in E2 emulsion solubilizes lavender oil better than Tween 20. Tween 80 has characteristic absorption maxima at wavenumbers: 3500 and 1750 cm^{-1}. Emulsion E2 has good stability for five days.



a) *b)* *c)*

Figure 7. The overlap of the FTIR-ATR spectra for the samples: a) E1, Tween 20 in water, lavender oil; b) E2, Tween 80 in water, lavender oil; c) E3, mixture of Tween 20 and Tween 80 in water, in ratio 1:1, lavender oil

The leathers were processed by spraying with the three obtained emulsions, E1, E2, E3 and were marked E1l, E2l, E3l (Figure 8) and then analyzed spectrophotometrically using FTIR-ATR.

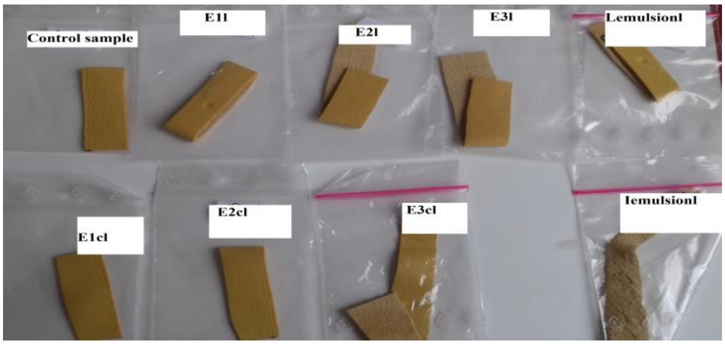


Figure 8. Image of the leathers processed with the three emulsions and oils (lavender and immortelle) and a control sample

To increase the antimicrobial and antifungal effect, the amount of lavender was increased four times in the emulsions. Emulsions marked with E1c, E2c and E3c were made and the leathers processed with these were marked E1cl, E2cl, E3cl. From Figure 9 it can be seen that the largest amount of lavender is found in the leather treated with the E2 emulsion (the spectrum intensity is the highest in the entire spectral range). The order of the lavender on the leather for the three emulsions is as follows: E2>E1>E3.

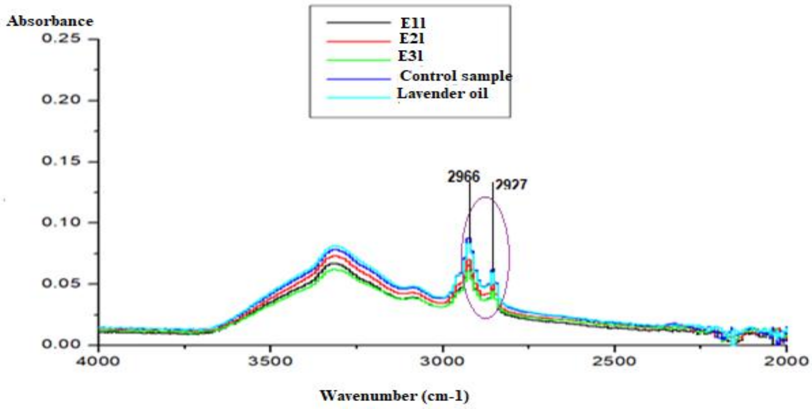


Figure 9. Overlay of FTIR-ATR spectra for leathers processed with emulsions E1, E2, E3, control sample, lavender oil

It can be seen from Figure 9 that all three leathers treated with emulsions contain lavender, the allure of the spectra of the leathers and lavender oil being similar. It can be seen from Figure 10 that the leather with the largest amount of lavender after processing with concentrated emulsions is E3c (having the highest intensity over the entire spectral range) and finding the maximum absorption specific to lavender oil. The order with the amount of lavender fixed on the leathers after processing with concentrated emulsions is E3c>E1c>E2c. The concentrated E3c emulsion is also the most stable >1 month.

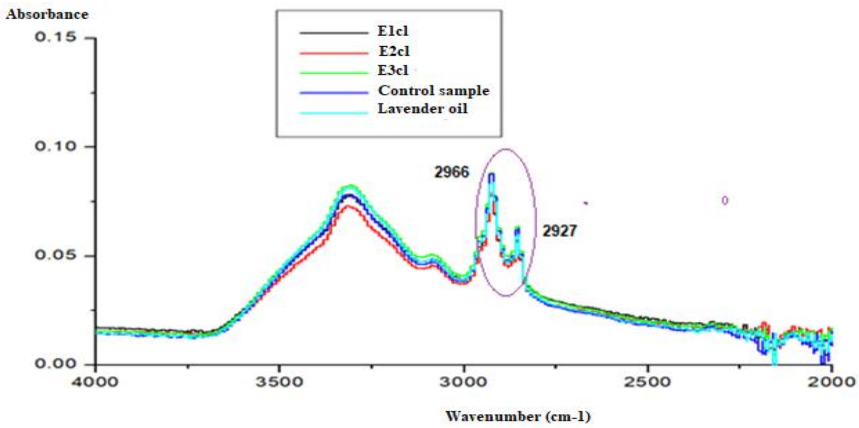


Figure 10. Overlay of FTIR-ATR spectra for leathers processed with emulsions E1cl, E2cl, E3cl, control sample, lavender oil

Characterization by DLS

The three types of emulsions were analysed by dynamic light scattering (DLS), Table 1.

Microbiological Tests of Leathers Processed with Emulsions

The microbiological tests of leathers processed with three emulsions against the attack of *Staphylococcus aureus* ATCC 6538 are presented in Table 2.

Table 1: Results of DLS for three emulsions: E1, E2, E3

Sample at room temperature	Average diameter (nm)	% Intensity	Zeta Potential (mV)
Multiple emulsion based on Tween 20 & Tween 80 mix, E3	27	76.3	-33
	195	19.1	
	351	4.6	
Multiple emulsion based on Tween 20, E1	657	91.7	-52
	22	8.39	
Multiple emulsion based on Tween 80, E2	102	100	-39

Table 2: Results of microbiological tests of leathers processed with three emulsions: E1, E2, E3

Sample	Results	R%	Log_{10}red
Inoculum concentration	T_0=1.5x10^{6}UFC/mL		
Control sample	T_0=1.5x10^{6}UFC/mL T_{24}=6.58x10^{3}UFC/mL	41	1.1
Multiple emulsion based on Tween 20 & Tween 80 mix, E3	T_0=1.5x10^{6}UFC/mL T_{24}=4x10^{3}UFC/mL	87.3	1.3
Multiple emulsion based on Tween 20, E1	T_0=1.5x10^{6}UFC/mL T_{24}=3.89x10^{3}UFC/mL	82.1	1.4
Multiple emulsion based on Tween 80, E2	T_0=1.5x10^{6}UFC/mL T_{24}=3.80x10^{3}UFC/mL	93	1.45

CONCLUSIONS

The conducted research has led to the following results:

1. The innovation consists in biotechnologies for obtaining new bioemulsions based on: lavender/(and/or) Tween® 20, Tween® 80/water and their use in leather finishing.

2. The order with the amount of lavender fixed on the leathers after processing with concentrated emulsions is: E3c>E1c>E2c. The lavender oil is fixed better than the immortelle oil on the leathers, in the created bioemulsions, as shown by the spectral allure and the higher intensity in the case of the leather with lavender compared to the one with immortelle.

3. Tween 20 and Tween 80 are non-ionic surfactants that play a critical role in emulsion preparations, especially in forming stable water/water emulsions and solubilizing hydrophobic substances in aqueous solutions. Their versatility, compatibility and stability-enhancing properties make them valuable ingredients in a variety of industries including the leather industry.

4. A mechanism of solubilization of lavender oil in surfactant micelles was proposed. Lavender oil is hydrophobic and gets stuck in the core of the micelles but also on the alkyl ends of the hydrophobic chains. For Tween 80, the amount of solubilized lavender oil 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.

5. The changes in the aggregation process were observed for each type of emulsion (E1, E2, E3), the solubilization of lavender oil by dynamic light scattering.

6. In the process of finishing the leathers by spraying with three types of emulsions obtained compared to an untreated leather, the antifungal and antimicrobial properties, as well as the softness and appearance of the leathers were improved.

Acknowledgments

This research was funded by the Romanian Ministry of Research, Innovation and Digitalization, under the Nucleus Program, project 6N/2023, TEX-PEL-CHALLENGE 2026, component project PN 23 26 03 02, BIO-LEATHER and project 4 PFE/2022.

REFERENCES

1. Vărăşteanu, D.S., Protein-based Surfactants – Obtaining and Uses, PhD Thesis, "Politehnica" University of Bucharest, **2014**.
2. Balchin, M.L., *Lavender: The Genus Lavandula*, CRC Press, ISBN 978-0-203-21652-1, **2002**.
3. Masango, P., Cleaner Production of Essential Oils by Steam Distillation, *J Clean Prod*, **2005**, 13, 8,

833–839, https://doi.org/10.1016/j.jclepro.2004.02.039.

4. Chanamai, R., Horn, G., McClements, D.J., Influence of Oil Polarity on Droplet Growth in Oil-in-Water Emulsions Stabilized by a Weakly Adsorbing Biopolymer or a Nonionic Surfactant, *J Colloid Interface Sci*, **2002**, 247, 1, 167–76, https://doi.org/10.1006/jcis.2001.8110.
5. Mihala, L., *Bulgarian Lavender: The Sweet Smell of Success*, BBC News, **2020**.
6. Giray, H., An Analysis of World Lavender Oil Markets and Lessons for Turkey, *J Essent Oil-Bear Plants*, **2018**, 21, 6, 1612–1623, https://doi.org/10.1080/0972060X.2019.1574612.