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# INNOVATIVE TECHNOLOGIES BASED ON MEMBRANES FOR CONCENTRATING EXTRACTS FROM OLIVE CAKES

**SIMION Demetra[1], GAIDAU Carmen[1], PAUN Gabriela[2], STANCA Maria[1], STOICA Tonea[3]**

[1]The Research Development National Institute for Textiles and Leather, Division Leather and Footwear Research Institute, 93 Street Ion Minulescu, 031215, Bucharest, Romania, E-mail: demetra.simion@gmail.com

[2]National Institute for Research and Development of Biological Sciences, Center of Bioanalysis, 296 Splaiul Independentei, Bucharest 6, 060031, Romania, E-mail: gpaunroman@gmail.com

[3]SC Taro Comimpex SRL, Int. Giurgiului, 28A, Jilava, Ilfov, 077120, Romania, tarocomimpex@yahoo.com

Corresponding author: Simion Demetra, E-mail: demetra.simion@gmail.com

***Abstract:*** *The aim of the paper is to concentrate extracts from olive cakes using innovative technologies based on membranes. Among the modern methods of purification and concentration of biologically active compounds (polyphenolic) in extracts are membrane technologies (microfiltration and ultrafiltration). These technologies, compared to all the other classics, have the advantage of separating, purifying or concentrating a certain compound in a single phase, cold, without the intervention of chemical reagents, with very low energy consumption. Concentrated extracts from olive cakes, obtained by membranes technologies, can be used as tanning and retanning agents, alternative to petroleum origin materials in leather industry.*
*Olive cake is rich in polyphenolic compounds, fats, tannins, non-tannins, possible to be extracted, concentrated, chemical processed in view of developing a new tanning product. The use of new renewable materials from oil industry as biobased tanning material for leather industry represents an important step in lowering carbon footprint of both sectors and complies with circular economy principles. The antioxidant and antimicrobial properties of olive oil cake can be exploited in view of increasing the efficiency of the new product.*
*The paper presents the concentration by membrane technologies and characterisation of five kinds of olive cake water and water-organic solvent extracts as future tanning-retanning materials.*

***Key words:*** *membrane technologies, olive cake extracts, polyphenols, concentrate extracts, retanning materials in leather industry*

## 1. INTRODUCTION

The European Union is the main producer, consumer and exporter of olive oil. The EU produces about 67% of the total quantity worldwide (M.D. Niculescu, C. Gaidau; 2014). There are eight different categories of olive oil: extra virgin olive oil; virgin olive oil; lampante olive oil; refined olive oil; olive oil composed of refined and virgin oil; olive cake oil; crude olive oil; refined olive cake oil. Not all of these categories can be sold to consumers. At retail, they can only buy extra virgin olive oil, virgin olive oil, olive oil composed of refined and virgin olive oil and olive cake oil. The different categories of olive cakes are classified according to quality parameters, in terms of: physicochemical characteristics, such as acidity level, peroxide index, fatty acid content and sterol content; organoleptic (sensory) characteristics, lack of organoleptic defects (C. Gaidau, M. Niculescu, E. Stepan, D.-G.

Epure, M. Gidea; 2014). The purpose of the exploitation of olive cakes is to make extracts from them and use as a source of bioactive compounds with added value. There are several extraction methods: ultrasonic cold maceration (fig.1), microwave-assisted extraction, microwave-assisted gravitational hydrodiffusion. The best known is ultrasonic cold maceration. It is possible to extract and concentrate some biological compounds from extracts by unconventional membrane technologies (Antunes, A.P.M., Attenburrow, G., Covington, A.D., Ding, J, 2008).

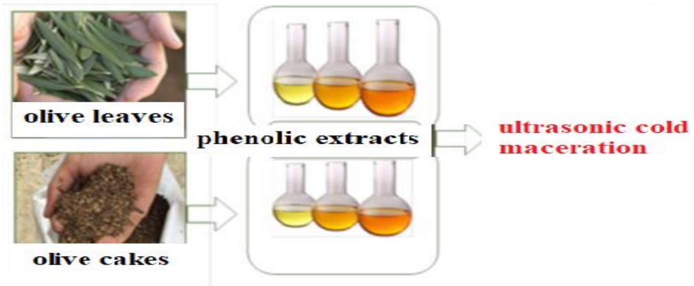



***Fig. 1***. *Ultrasonic assisted maceration extraction method [3]*

The aim of this paper is to concentrate by membrane technologies and characterise five kinds of olive cake water and water-organic solvent extracts as future tanning-retanning materials.

## 2. EXPERIMENTAL

### 2.1. Materials and Methods

Four types of olive waste originated from 2-phase extraction process, by-products of the olive cake: Arbequina, Palomar, Agro Igualada, Polpa d'oliva, in dry condition were received from different Spanish olive oil companies. The characterization of olive solid waste was performed according to standardized methods for tanning materials: dry substance (SR EN ISO 4684:2006), ash (SR EN ISO 4047:2002), total nitrogen and protein content using 6.25 conversion factor (SR EN 5397: 1996), extractible substances (SR EN ISO 4048: 2018), total residuum, total soluble substances, non tannins, tannins (shaking method), insoluble substances, tanning power, binding power (SR 1883: 2008) and pH (STAS 86193/3: 1990). The total phenols were analyzed following Folin-Ciocalteu method. To obtain the phenols content, 15 g of olive waste sample were magnetically stirred for 24 hours in 60 mL solution of 80% methanol. After stirring, the samples were ultrasounded for one hour and then filtered on Whatman paper. The UV-VIS spectra (JASCO V550) were recorded at $\lambda$ =740 nm on a calibration curve made with gallic acid. ATR-FTIR spectroscopy was performed on solid extracts in order to compare the chemical profile of different olive waste products and to understand the differences in their tanning properties by using a Jasco FT-IR 400 equipment from JASCO, Metertech. Four aqueous extracts were made from olive cakes: Arbequina, Palomar, Agro Igualada, Polpa d'oliva, by ultrasound for 1 hour, at pH = 2 (fig.2). Polpa d'oliva was selected for this study.

***Fig. 2.*** *Four aqueous extracts made from olive cakes:1- Arbequina, 2-Palomar,3- Agro Igualada, 4- Polpa d'oliva, by ultrasound for 1 hour, at pH = 2*

The extraction methods were based on the variation of different conditions in aqueous and alcohol medium, at 55°C or by ultrasound (Elmo ultrasound bath, 280 W) and are presented in Table 1 for ground Polpa d'oliva.

**Table 1:** *Extraction methods for olive extracts*

| *Sample* | *Extraction conditions* |
|---|---|
| 1 | water at pH= 2, 1: 40 (w/w), 1 h ultrasound. |
| 2 | water at pH= 2, 1: 40 (w/w), 4 h at 55°C. |
| 3 | water, 1: 40 (w/w), 4 h at 55°C. |
| 4 | water: ethanol = 1:1 (v/v), pH=2, 1:40 (w/w), 1h ultrasound. |
| 5 | water: ethanol = 1:1 (v/v), pH= 2, 1:40 (w/w), 4h at 55°C |

Extracts obtained from olive cakes - 5 extracts (1-5) were processed by membrane methods: microfiltration (MF) followed by ultrafiltration (UF) through a laboratory plant KMS Laboratory Cell CF-1 purchased from Koch Membrane (Germany), fig.3. The microfiltration was performed by microfiltration membranes made of cellulose acetate, with a pore size of 0.45 µm, at a pressure of 3-4 bar resulting in the retention of all coarse particles in suspension. Ultrafiltration was performed through regenerated cellulose membranes with a cut-off of 3,000 Da; the concentration was achieved in the ratio 1: 3, at a pressure of 8 bar.

***Fig. 3.*** *Laboratory installation KMS Laboratory Cell CF-1*

## 3.RESULTS AND DISCUSSIONS

### 3.1. Olive cake powders characterization

The 4 powders of olive cakes: Arbequina, Palomar, Agro Igualada, Polpa d'oliva were studied. The tanning power has the values which are not in agreement with total phenol content. The tanning power was ranked as follows: Arbequina > Palomar > Agro Igualada > Polpa d'oliva.

The physical-chemical characteristics of olive powders of olive cakes are presented in Table 2.

***Table 2.*** *Physical-chemical characteristics of olive powders*

| Characteristics | Arbequina | Palomar | Agro Igualada | Polpa d'oliva |
|---|---|---|---|---|
| Dry substance, %, | 89.31 | 86.08 | 90.24 | 92.24 |
| Ash, % | 2.65 | 1.74 | 2.40 | 6.18 |
| Total nitrogen, % | 0.76 | 0.70 | 1.17 | 2.23 |
| Protein, % | 4.75 | 4.38 | 7.31 | 13.93 |
| Extractible substances % | 14.48 | 10.90 | 11.71 | 7.92 |
| Total residuum, %, | 29.49 | 34.73 | 25.00 | 20.72 |
| Total soluble substances, % | 18.54 | 9.31 | 14.22 | 13.98 |
| Non tannin, % | 15.14 | 6.88 | 12.06 | 11.94 |
| Tannin, % | 3.40 | 2.43 | 2.16 | 1.77 |
| Insoluble substance, % | 10.95 | 25.42 | 10.78 | 8.51 |
| pH (1:10), pH units | 5.10 | 5.05 | 5.52 | 6.15 |
| Tanning power, % | 3.10 | 1.77 | 1.97 | 1.52 |
| Binding power, % | nd | nd | nd | nd |

The main components of olive cake powders in ATR-FTIR spectras (fig.4) can be recognized from the main functional groups vibrations: polyphenols, alcohols and carboxylic compounds (3258-3311.18 $cm^{-1}$), cellulose (2922.59-2925.48 $cm^{-1}$ and 2853.15-2854.43 $cm^{-1}$), hemicelluloses in Agro Igualada and Arbequina (1740-1743 $cm^{-1}$), oleuropein (1022.05-1078 $cm^{-1}$), proteins (1631.48-1637.27 $cm^{-1}$ and 1598 $cm^{-1}$), phenols (1371.14-1375.96 $cm^{-1}$) and ß-glycoside links (1151-1156.12 $cm^{-1}$). The transmittance in ATR-FTIR spectras at polyphenol wave number was recorded for Arbequina olive powders as the most intense, compared to the others, which is in agreement with tanning affinity.

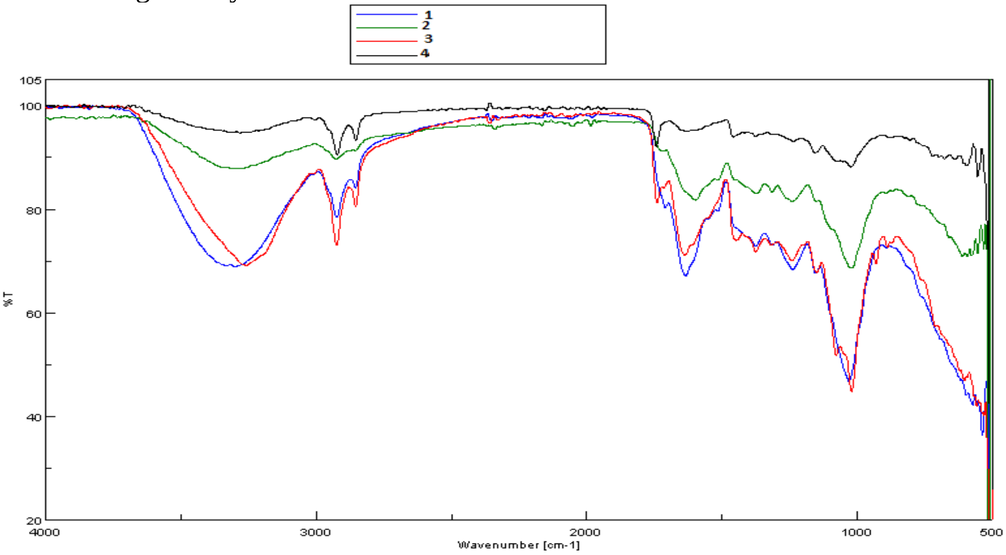



***Fig. 4.*** *ATR-FTIR of olive powders: 1- Palomar, 2- Polpa d'oliva, 3- Agro Igualada, 4- Arbequina*

Polyphenols are indicated in the FTIR-ATR spectra as being specific to the wave number: 3400 $cm^{-1}$. It is observed quantitatively in the initial samples, depending on the values of the transmittance in the spectra, the following order, the largest amount of polyphenols in: Remolta Palomar (1)> Agro Igualada (3)> Polpa d'oliva (2)> Arbequina (4). In this study Polpa d'oliva was selected. In fig.5 is the ATR-FTIR spectra for OBE tanning agent.

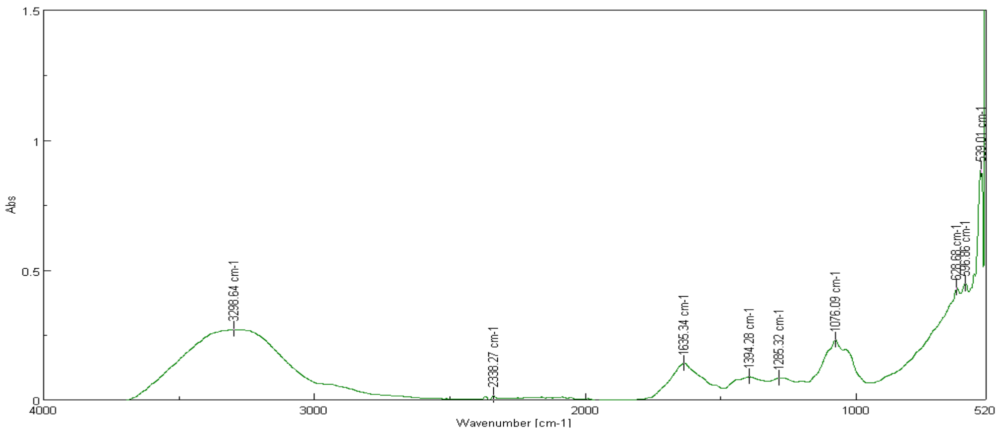



***Fig. 5.*** *ATR-FTIR spectra for OBE tanning agent*

### 3.2.Olive Extracts Characterization

For the four extracts in figure 2, the total polyphenol content was determined to select which of the 4 types of olive cake is the best for the study. The principle of the method used is based on the property of phenols to form with the complex phosphowolframic reagent (Folin-Ciocâlteu reagent) blue complexes, which can be dosed spectrophotometrically at $\lambda$=760 nm. Basically, 5 ml of the extract sample is treated with 5 ml of Folin-Ciocalteu reagent, stirred and then filtered. 1 ml of filtrate is made up to a 10 ml graduated flask with. 20% sodium carbonate solution, and after about 2 minutes read the absorbance of the sample at 660 nm compared to the 20% sodium carbonate solution used as a control. The polyphenol content was calculated using a standard curve made with chlorogenic acid (CA), in the range of concentrations 0-800 mg/L, the concentration of polyphenols in the extracts being expressed as mg CA / L, according to the table 3.

***Table 3.*** *Experimental results for the polyphenol content of the samples with: 1- Arbequina, 2-Palomar,3- Agro Igualada,4- Polpa d'oliva, by ultrasound for 1 hour, at pH = 2*

| ***Sample*** | ***Dilution*** | ***Absorbance at $\lambda$=760 nm*** | ***Concentration., µg ACE/mL*** |
|---|---|---|---|
| 1 | - | 0,6170 | 480,2 |
| 2 | - | 0.6813 | 511.2 |
| 3 | - | 0.6441 | 481.7 |
| 4 | 10 | 0.2344 | 1568.7 |

The ATR-FTIR spectras for these four extracts from figure 2 are presented in figure 6.

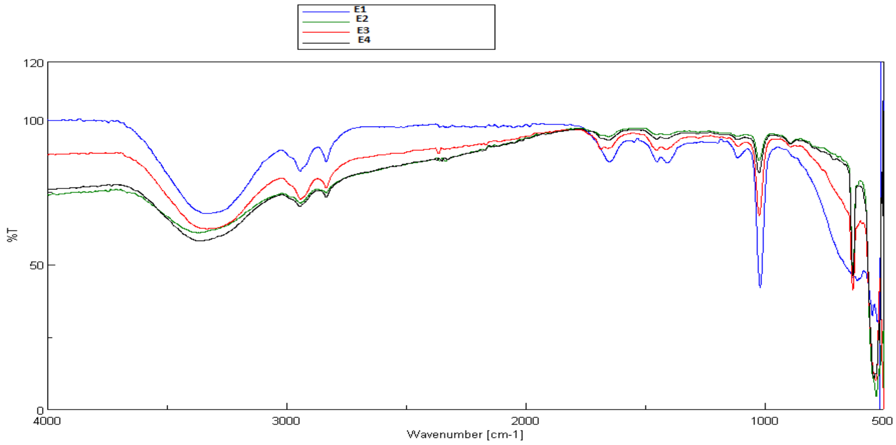
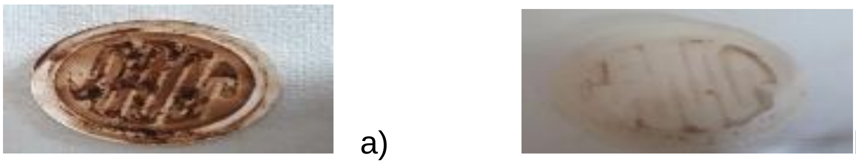



***Fig. 6.*** *ATR-FTIR of olive extracts: E1- Palomar, E2- Polpa d'oliva, E3- Agro Igualada, E4- Arbequina*

There are significant differences in the spectra for the initial powders compared to the extracts obtained. The total phenols of tested extracted methods performed on Polpa d'oliva cakes presented in table 1 conclude that the extraction in water and assisted by ultrasound was the most efficient (15.32 mg GAE/g from olive cakes). Similar phenol concentrations, between 7.72-9.42 mg GAE/g from olive cakes, were extracted in water or in water heated at 55°C for 4 hours, or in water by ultrasound for 1 hour. Further research will be carried out for high molecular weight phenols identification (tannins, anthocyans, catechol-metaninic polymers etc. in correlation to extraction methods and tanning properties. *All these 5 extracts from table 1 were concentrated by membrane technologies.*

### 3.3.Membrane technologies

Microfiltration (MF) is the process that most closely resembles ordinary filtration and is used to separate particles with dimensions between 0.1–10 µm, such as suspended solid particles, viruses, bacteria, yeast cells, etc. Ultrafiltration (UF) is a process of separating colloidal substances and compounds with a molecular weight between 1000 and 100,000,000 Da, using filtration through membranes at low pressures. The separation through ultrafiltration membranes is due to the sieving mechanism, after which the solvent and the smaller molecules of the solute are transported through the pores of the membrane, which retains only the molecules of some solutions with dimensions larger than the pore size. Five extracts obtained from olive cakes (from table 1) were processed by membrane technologies: microfiltration (MF) followed by ultrafiltration (UF) through a laboratory plant KMS Laboratory Cell CF- 1 purchased from Koch Membrane (Germany), figure 3.The microfiltration was performed by microfiltration membranes made of cellulose acetate, with a pore size of 0.45 µm, at a pressure of 3-4 bar (figure 7). Ultrafiltration was performed through regenerated cellulose membranes with a cut-off of 3,000 Da at a pressure of 8 bar (figure 7).



a) b)

***Fig. 7.*** *Membranes: a) microfiltration membrane (MF) after passing the extract; b) ultrafiltration membrane (UF) after extract concentration*

The technological flow is shown in figure 8.

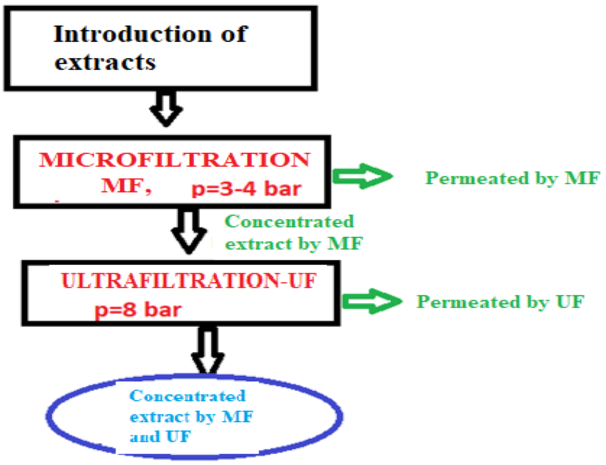



***Fig. 8.*** *Technological flow of concentration through membrane processes*

The results of the ultrafiltration process were measured by permeate flow and polyphenol retention. The permeate flow is calculated according to equation (1):

$$J = \frac{V}{A \cdot t} \quad \textbf{(1)}$$

where: V = permeate volume, A = effective membrane surface, t = time required to collect volume V of permeate. Retention, % R, is calculated according to equation (2)

$$R = \left[1 - \frac{C_p}{C_f}\right] \times 100 \quad \textbf{(2)}$$

where $C_p$ and $C_f$ =concentration of the monitored component in the permeate and in the feed solution, respectively.

### 3.4.Concentrated Extracts Characterization

Five extracts obtained from olive cakes (from table 1) were concentrated by technological flow presented in figure 8. The determination of the polyphenols content of the 5 concentrated extracts by membrane technologies (from table 1) was performed using the Folin-Ciocalteu method. The principle of the method used is based on the property of phenols to form complexes with blue phosphowolframic reagent (Folin-Ciocalteu reagent), which can be dosed spectrophotometrically at a wavelength of 760 nm. The total polyphenol content was expressed in µg equivalents of chlorogenic acid (ACE) / mL extract. At microfiltration, flow values of 297,5÷556,3 L/m$^3$h, were obtained for the samples taken, the lowest value being recorded for sample 1. At the ultrafiltration of the 2 sample, the value of the permeate flow was 8.23 L/ m$^3$h, and the retention degree of the total polyphenols was 51.6% ($C_f$ = 847.02 µg ACE / mL; $C_p$ = 410.01 µg ACE / mL). At the ultrafiltration of the 5 samples concentrated from table 1, the degree of retention of the total polyphenols was presented in table 4:

***Table 4.*** *The degree of retention of the total polyphenols from 5 extracted concentrated*

| *Sample concentrated* | *The degree of retention of the total polyphenols from 5 extracted concentrated by membrane technologies, %* |
|---|---|
| 1 | 59,9 |
| 2 | 51,6 |
| 3 | 42 |
| 4 | 46 |
| 5 | 49 |

The best results were obtained for sample 1 with extract conditions: water at pH= 2, 1: 40 (w/w), 1 h ultrasound. Research is in progress regarding to create new retanning materials.

## 4. CONCLUSIONS

1. In this research were concentrated extracts from olive cakes using innovative technologies based on membranes. Among the modern methods of purification and concentration of biologically active compounds (polyphenolic) in extracts are membrane technologies (microfiltration and ultrafiltration). These technologies, compared to all other classical ones, have the advantage of separating, purifying or concentrating a certain compound in a single phase, cold, without the intervention of chemical reagents, with very low energy consumption.

2.Concentrated extracts from olive cakes, obtained by membranes technologies, can be used as tanning and retanning agents, alternative to petroleum origin materials in leather industry.

3.Olive cake is rich in polyphenolic compounds, fats, tannins, non-tannins, possible to be extracted, concentrated, chemical processed in view of developing a new tanning product. The use of new renewable materials from oil industry as biobased tanning material for leather industry represents an important step in lowering carbon footprint of both sectors and complies with circular economy principles. The antioxidant and antimicrobial properties of olive oil cake can be exploited in view of increasing the efficiency of the new product.

4.This paper presents the concentration by membrane technologies and characterisation of five kinds of olive cake water and water-organic solvent extracts as future tanning-retanning materials.

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