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Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration

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ABSTRACT

Membrane separation technology, for bioactive compounds separation, gained great attention lately. Our main goal in this work was to produce an oleuropein concentrate. The water extract of olive leaves has been subjected to a screening on the basis of molecular size. First microfiltration process (0.2 μm) allowed large particles removal, a following step of ultrafiltration permitted the removal of molecules larger than 5 kDa, finally a nanofiltration process (300 Da) allowed the concentration of polyphenols mainly oleuropein. Permeate fluxes of ultrafiltration and nanofiltration were investigated and analyzed.

Results revealed that a large portion of phenolic compounds were recovered in the permeate fraction of the UF process. The nanofiltration retentate showed high polyphenol and flavonoid contents. Based on the content of solute in feed and retentate fractions of NF membrane, oleuropein was concentrated approximately 10 times to reach 1685 mg/100 g extract. In addition, this fraction demonstrated high antioxidant capacities monitored by total antioxidant capacity and ferric-reducing ability power. High antibacterial activity was observed against *S. enterica* and *K. pneumonieae* (25 and 28 mm, respectively).
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1. Introduction

In recent years, there has been a growing interest in obtaining biologically active compounds from natural sources. The protective effects of diets rich in fruits and vegetables against cardiovascular diseases and certain cancers have been attributed partly to antioxidants contained therein. Olive tree is one of the potential natural antioxidant sources because of its phenolic contents in fruits [1], oil [2] and leaves [3,4].

Olive leaves are regarded as a cheap raw material which can be used as a good source of high-added value bioactive compounds [5].

Several reports have demonstrated that olive leaf extract has the capacity to lower blood pressure in animals and increase blood flow in the coronary arteries, to relieve arrhythmia and prevent intestinal muscle spasms [5–9].

Oleuropein, the major constituent of the secoiridoid family in olive leaves, has been shown to be a potential antioxidant endowed with anti-inflammatory and antithrombotic properties [3,10,11]. Oleuropein is also suggested to support hypotensive and radical scavenging activities and its hydrolysis leads to antimicrobial compounds [3]. Consequently, there is a growing interest in extracting and separating oleuropein from olive leaves because natural active compounds are safer for human health than synthetic chemicals.

In this context, several techniques have been adopted for recovering olive polyphenols. These techniques involve mainly extraction, centrifugation, precipitation and chromatographic procedures. However, in these processes, complexities result from both their cost and operational characteristics and from the toxicity and flammability of organic solvent required in large amounts [12,13].

Recently, there has been an increasing interest in the application of membrane technologies for separation, purification and concentration of bioactive compounds from aqueous solutions. Membrane processes have been investigated for high quality concentration of phenolic compounds due to their low operating temperature and minimal energy consumption [14,15]. This procedure is based on the principle of selective permeation of the solute molecules through either polymeric or inorganic semi-permeable membranes.

Nanofiltration has been successfully employed for concentrating phenolic compounds extracted from natural products [16]. Indeed, Dammak et al. [17] used nanofiltration to concentrate aqueous oleuropein solution. However, there is no information

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available on the concentration of oleuropein from olive leaves using three different membrane filtration types; micro-, ultra- and nano-filtrations.

The aim of this study was to produce a concentrated fraction of oleuropein from extract of olive leaves. The extract was fractionated using a sequence of different membrane operations: the microfiltration (MF) followed by the ultrafiltration (UF). The ultrafiltration permeate was then subjected to a nanofiltration (NF). Different fractions were evaluated in terms of total phenolics content, flavonoids content, antioxidant capacity and HPLC profiles. Antioxidant and antibacterial activities of the nanofiltration retentate were investigated.

2. Materials and methods

2.1. Raw material

Olive leaves (*O. europaea*, Chemlali variety) were collected from Sfax (Tunisia), dried in a tunnel microwave dryer (Adasen, JN-100, China) (1200 W, 70 °C) for 10 min, then milled and stored in darkness at 4 °C until extraction.

2.2. Equipment and processes

The feed solution was prepared from olive leaf powder dispersion in water at a rate of 2.5% and stirring for 1 h at room temperature (30 °C). The obtained mixture was decanted for 4 h and the supernatant was clarified through linen cotton in order to remove large particles.

The fractionation process of olive leaf extract performed through the combination of three membrane operations was presented in Fig. 1. The feed stream was pre-treated in a microfiltration system through 0.2 μm pores size membrane (Microporous membrane TOPER Model, diameter 300 mm, CN). The microfiltration permeate was submitted to a cross-flow ultrafiltration system of molecular weight cut-off (MWCO) 5 kDa (GE Power & Water, ZeeWeed 1500 Minnetonka, MN). The UF membrane can retain colloidal substances, protein and macromolecular pigments in the feed liquid and it permitted to recuperate the larger polyphenols contained in the extract. Finally, the ultrafiltration permeate feed a cross-flow nanofiltration System of MWCO 300 Da (GE Osmonics, HL2540TF, Minnetonka, MN). The cross-flow nanofiltration system retentate delivered a concentrated extract rich in oleuropein. Manometers before and after the MF,UF and NF membranes were used to measure the inlet and the outlet pressure so as to control the trans-membrane pressure (ΔP) which was equal to 1 bar for MF and UF. However, the NF was operated at a trans-membrane pressure of 9 bar. Fig. 2 shows a photo of permeate and retentate samples of the olive leaves extract of each filtration process.

The permeate flux J (L/h m^2) was measured during UF and NF processes and calculated according to the following equation:

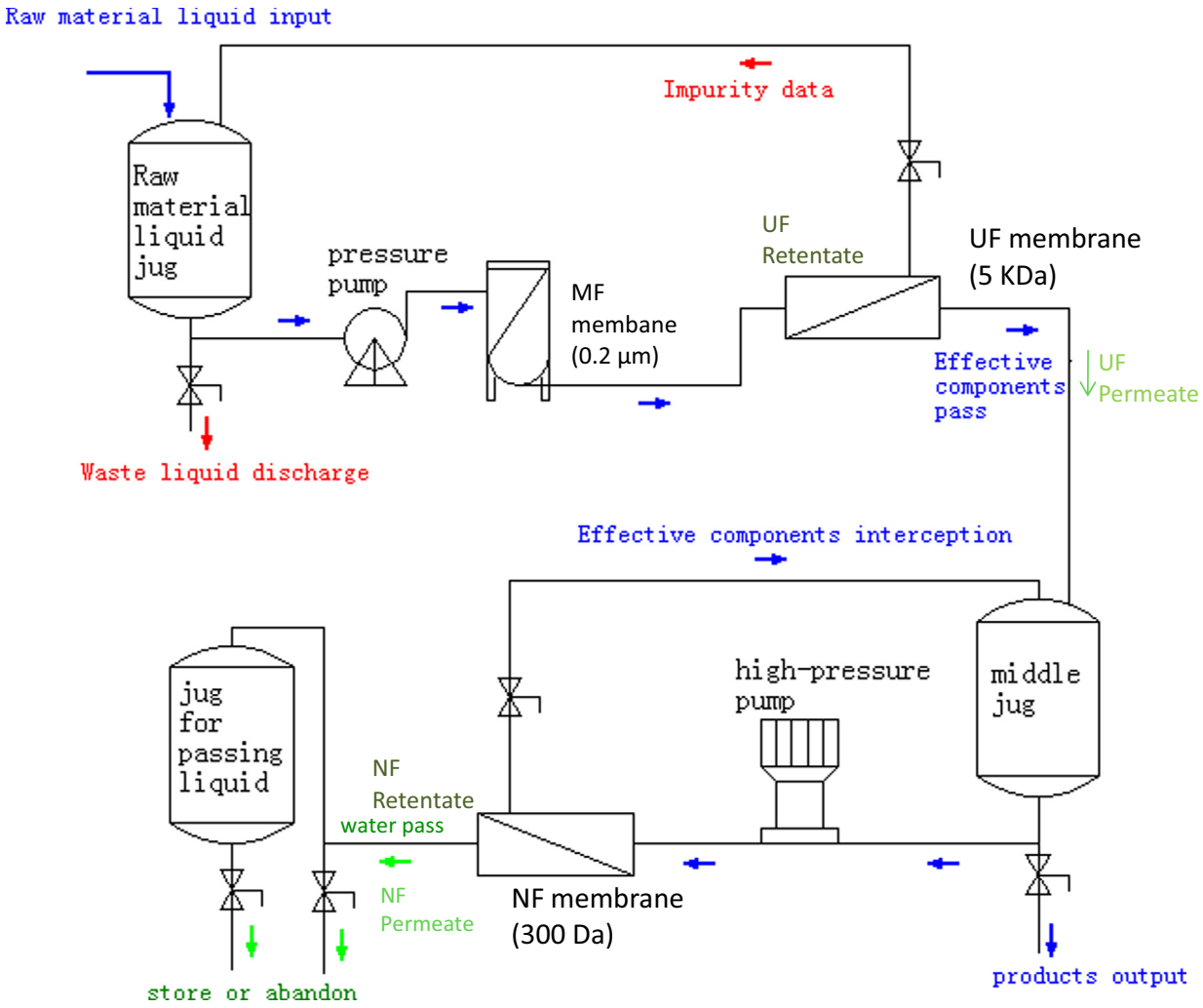

Fig. 1. Experimental set up used to perform filtration process.

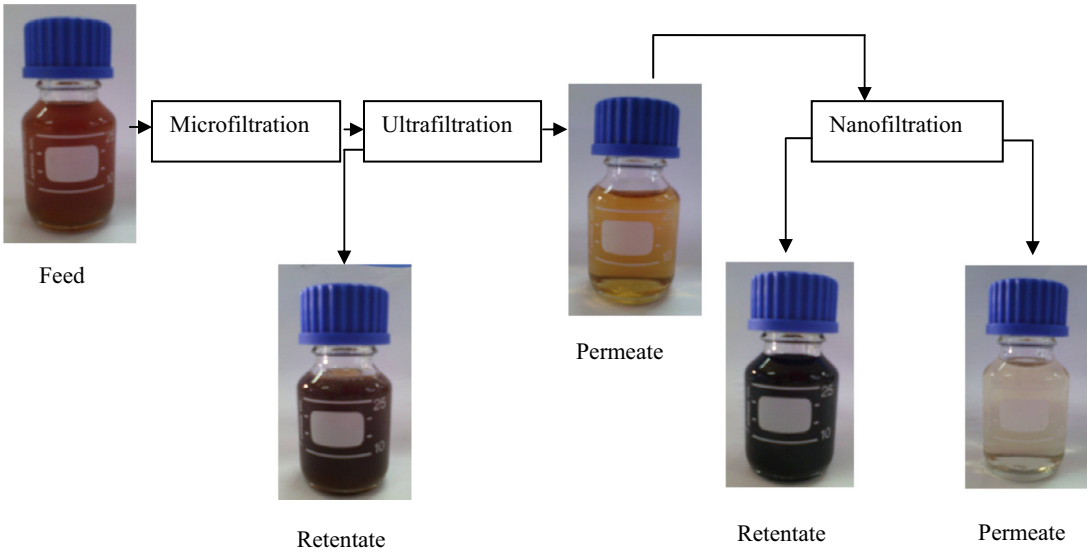

Fig. 2. Permeate and retentate samples obtained in the purification and the concentration of olive leaves extracts by integrated membrane.

$$J = Vp/(t \times S) \quad (1)$$

where V_p (L) is the amount of permeate collected during the period of time t (h) and the permeation surface area of membrane S (m^2).

2.3. Analytical methods

During filtration process, feed solution, permeate and retentate of UF and NF systems were analyzed. The rejection (R) of each membrane towards specific compounds was determined by the following equation:

$$R = (1 - Cp/Cf) \times 100 \quad (2)$$

where *Cp* and *Cf* are the concentration of a specific compound (total phenolic content, flavonoids content, antioxidant capacity and oleuropein content) in the permeate and feed solution, respectively.

The volume reduction factor (VRF) is an important factor in UF and NF processes as it relates to the concentration degree.

$$VRF = \frac{Vf}{Vr} \quad (3)$$

where *Vf* is the feed volume and *Vr* is the retentate volume of each process.

2.3.1. Total Phenolic Content

Total Phenolic Content (TPC) was determined by the Folin–Ciocalteu method as described by Singleton et al. [18]. The absorbance was measured at 765 nm using a spectrophotometer (UVmini-1240, Shimadzu, Japan). Results were expressed in terms of Oleuropein Equivalent (mg OE/100 g of extract). Presented values are the average of three measurements.

2.3.2. Flavonoids Content

Flavonoids Content (FC) of each sample was determined using spectrophotometric method as described by Hajji et al. [19] with some modifications. 1 mL of distilled water and 150 μL of sodium nitrite solution (5%) were added to 250 μL of each sample. After 5 min, 75 μL of aluminum chloride (10%) solution was added. After 5 min, 1 mL of NaOH solution (1 M) was added. The mixture was immediately made up to 2.5 mL with distilled water and mixed well. The absorbance was then measured at 510 nm by spectrophotometer (UVmini-1240, Shimadzu, Japan). Flavonoids content was expressed as mg Rutin Equivalent (RE)/100 g extract. Presented values are the average of three measurements.

2.3.3. HPLC analysis

The quantitative analyses of polyphenols in olive leaves extracts were made by high performance liquid chromatography (Agilent 1260 Infinity quaternary LC, Germany). Phenolic compounds were separated on a C_{18} column (4.6 mm × 250 mm) and then analyzed using a Shimadzu SPD6AUV detector measuring the optical density at 254 nm during 50 min.

The mobile phase was a mixture of A and B solutions: (A) 70% acetonitrile in water and (B) 0.1% phosphoric acid in water with the percentage by volume of (A) solution varying linearly along the time as follows: from 10 to 25% for the first 25 min then from 25 to 80% up to 35 min and finally from 80 to 100% up to 50 min. The column temperature was maintained at 40 °C and the mobile flow rate was fixed at 0.6 mL/min. Oleuropein, hydroxytyrosol and syringic acid were identified and quantified using external standards and calibration curves.

2.3.4. Antioxidant Capacity

Total Antioxidant Capacity (TAC) was performed according to the procedure described by Kumaran and Karunakaran [20]. Briefly, 300 μL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. The absorbance was read at 695 nm by spectrophotometer (UVmini-1240, Shimadzu, Japan). Results were expressed as mg ascorbic acid/100 g extract.

Ferric-Reducing Ability Power (FRAP) was performed according to Benzie and Strain [21], with some modifications. The FRAP reagent was freshly prepared from 2.5 mL of a 10 mM TPTZ (2,4,6-Tripyridyl-s-Triazine) solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl3{\cdot}6H_2O$ and 2.5 mL of 0.3 M acetate buffer pH 3.6. In short, 900 μL of FRAP reagent were mixed with 30 μL of distilled water and 30 μL of sample or water used as an appropriate reagent blank. The mixture was kept at 37 °C for 30 min. The absorbance was read at 595 nm using a spectrophotometer (UVmini-1240, Shimadzu, Japan) and it was expressed in terms of trolox equivalent (mol trolox/100 g extract).

2.3.5. In vitro antibacterial activity of the NF retentate

The antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome [22]. The evaluation of the antimicrobial activity of NF retentate is based on the diffusion agar method. Antibacterial activity of

the concentrated fraction was tested against the Gram-positive bacteria, *Bacillus cereus* (ATCC 11778) and *Micrococcus luteus* (ATCC 4698) and the Gram-negative bacteria, *Salmonella enterica* (ATCC 43972, *Escherichia coli* (ATCC 25922), *Enterobacter* and *Klebsiella pneumonieae* (ATCC 13883). Succinctly put, The Luria-Bertani (LB) agar plates of media were prepared by spreading culture suspensions of the tested microorganisms and wells were made in the plate. 25 μL of NF retentate were loaded into wells (7 mm depth, 6 mm diameter). All the inoculated plates were kept at 4 °C for 1 h and, after that, they were incubated at 37 °C for 24 h. The antibacterial activities were evaluated by measuring the diameter (in millimeters) of the inhibition zone around the wells.

2.4. Statistical analysis

The results were reported as the mean (±standard deviation) of three experiments. Statistical analyses were performed using SPSS statistics 17.0 and significance was declared at $P \leqslant 0.05$. The correlation between the determined antioxidant activity applying the two independents tests (FRAP and TAC) and the oleuropein content and TPC were performed using Pearson's correlation test by SPSS statistics 17.0.

3. Results and discussion

3.1. Permeate flux of UF and NF processes

Permeate flux of UF and NF processes were measured with time. Fig. 3 presents permeate flux (J) decline in terms of liters of permeate produced per unit time and area (L/h m^2) in ultrafiltration (a) and nanofiltration (b) system processes up to VRF of 7 and 43, respectively. The initial permeate flux of UF system was 39.35 L/h m^2. It decreased gradually over time and finally tended to be constant (27.50 L/h m^2). A similar trend was observed for the NF system. These results are entirely consistent with that of Cassano et al. [14] and Benedetti et al. [23]. This behavior could be linked to the increase of the thickness of gel layer over the membrane surface with time leading to an increase in resistance to solvent flow and, therefore, a decline in permeate flux [24]. However, it is clear from Fig. 3b that permeate flux of NF system decreased very rapidly during filtration. The reduction of permeate flux in NF process was about 82%. Cassano et al. [14] have attributed this decline to the increase of the solute concentration in feed, concentration polarization and fouling phenomenon. The increase of the solute concentration may be due to the high pressure applied to NF process ($\Delta P = 9$ bar). The applied pressure is the driving force for transport solute across the membrane. Indeed, a high pressure leads to the increase of polyphenols at the membrane barrier. It induces a highly concentrated layer of polyphenols near the membrane surface which resists the mass transfer of permeate [17].

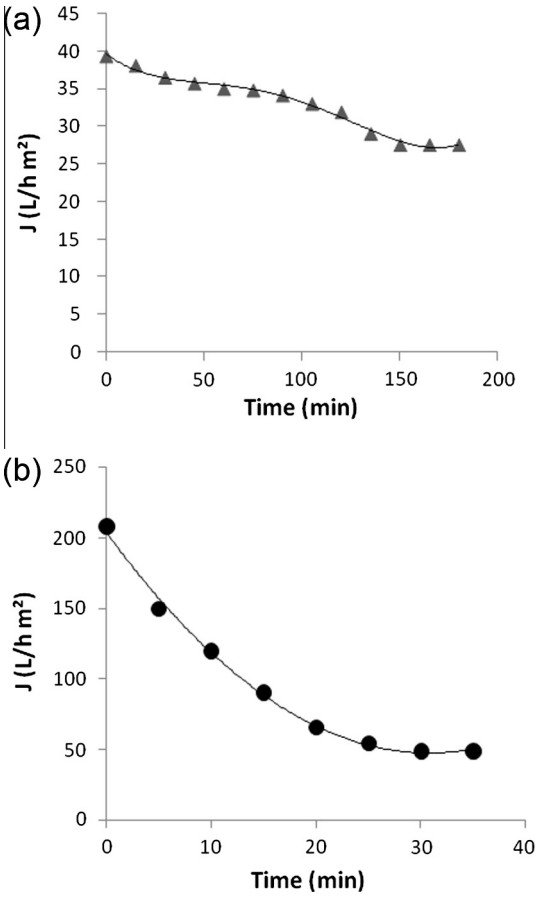

Fig. 3. Evolution of the permeate flux (J) during UF (a) and NF (b).

3.2. Total phenolic and flavonoid contents

TPC of samples coming from UF and NF membranes are illustrated in Table 1. TPC analyses displayed a rejection coefficient of 37.02% and 95.07% for the UF and the NF membranes, respectively. The variation of rejection coefficient between these two membranes was caused by the difference of their MWCO, which was 5 kDa and 300 Da for UF and NF, respectively. Indeed, the most important mechanism of solute rejection is physical sieving of solutes larger than the membrane MWCO [25]. UF membrane permitted to recover polyphenols in permeate fraction (182.19 mg OE/100 g). Cassano et al. [14] said that phenolic compounds are supposed to be recovered in the permeate stream of the UF processes. However, the large polyphenols were maintained in retentate fraction yielding a quantity of 261.88 mg OE/100 g.

The high rejection value of NF membrane is due to the fact that this process was supposed to concentrate polyphenols in olive leaves extract. The major mechanism of solute rejection by NF is physical sieving of molecules larger than the MWCO of membrane [25]. All phenolic compounds having a MWCO higher than 300 Da were retained at more than 95%. This result was similar to that obtained by Cassano et al. [14] who have revealed that the NF membrane (90 Da) used to concentrate purified olive mill waste water has a rejection coefficient of 93%. Besides, Brás et al. [26] demonstrated that phenolic compounds from *Cynara cardunculus* var. have a rejection near 100% for the NF270 membrane with a molecular weight cut-off (MWCO) of 400 Da.

The experimental data, displayed in Table 1, indicate that phenolic compounds were concentrated 14 times using NF membrane to reach 2623.77 mg OE/100 g. On the other hand, the NF permeate was a clear aqueous solution as shown in Fig. 2. This finding was in agreement with that obtained by Cassano et al. [14] who have used the UF and NF processes in order to fractionate olive mill wastewaters.

The FC in different fractions revealed a rejected coefficient of 29 and 98% for UF and NF membranes, respectively. Indeed, quantified flavonoids have, in their majority, a molecular weight above the MWCO of the NF membrane; therefore, they are retained by NF membrane. Results presented in Table 2 show that the feed and the retentate extracts of NF membrane contained 128.45 and 477.58 mg/100 g extract, respectively. This result indicates that flavonoids were concentrated approximately 4 times. The concentration increase of flavonoids was less than that of total phenolic compounds. This effect may be due to various parameters such

Table 1
Total phenolics content in feed stream, retentate and permeate of cross-flow ultrafiltration system and cross-flow nanofiltration system processes.

Membrane type	Total phenolics content (mg OE/100 g extract)			R (%)
	Feed	Permeate	Retentate	
UF 5000 Da	289.57 ± 3.16[c]	182.19 ± 1.87[a]	261.88 ± 5.24[b]	37.02
NF 300 Da	182.19 ± 1.87[b]	9.01 ± 0.21[a]	2623.77 ± 25.98[c]	95.07

[a–c] Different letters in the same line indicate significant differences ($p < 0.05$) between fractions of the same membrane.

Table 2
Flavonoids content in feed stream, retentate and permeate of cross-flow ultrafiltration system and cross-flow nanofiltration system processes.

Membrane type	Flavonoids content (mg RE/100 g extract)			R (%)
	Feed	Permeate	Retentate	
UF 5000 Da	180.92 ± 1.64[b]	128.45 ± 0.17[a]	255.58 ± 4.37[c]	29.00
NF 300 Da	128.45 ± 0.17[b]	5.46 ± 0.32[a]	477.58 ± 17.78[c]	97.86

[a–c] Different letters in the same line indicate significant differences ($p < 0.05$) between fractions of the same membrane.

as the increase of temperature during filtration process and the existence of interactions between flavonoids and NF membrane.

3.3. HPLC analyses

HPLC analyses of permeate and retentate fractions obtained by UF and NF processes are presented in Table 3. Chromatograms corresponding to Feed, UF permeate, UF retentate, NF permeate and NF retentate are shown in Fig. 4a –e, respectively. Results presented in Table 3 and Fig. 4 revealed that oleuropein is the most representative polyphenol detected in feed solution with a concentration of 265.24 mg/100 g extract. UF membrane exhibited lower rejection values towards hydroxytyrosol, syringic acid and oleuropein due to their low molecular weight (154.16, 198.17 and 540 g/mol, respectively). This result substantiates previous report regarding the lower rejections of UF membrane obtained towards low molecular weight polyphenols [14]. The role of the UF membrane was to retain relatively large dissolved materials and suspended solids while allowing smaller organic dissolved compounds (for example low molecular weight polyphenols) to permeate. Oleuropein was found in the retentate fraction of UF membrane at a rate of 72.22 mg/100 g extract. This effect could be attributed to the severe fouling produced by the adsorption of organic matter from olive leaf extract onto the membrane material [27]. On the other hand, a rejection coefficient of 100% was obtained by the NF membrane (300 Da). The present result is in agreement with previous studies which have reported that NF membrane of 90 Da exhibited a 100% rejection towards low molecular weight polyphenols of olive mill waste water [14]. In our study, the main role of the NF membrane is to concentrate a dilute extract of olive leaves. The retentate steam of the NF process can be considered a fraction enriched in oleuropein, containing 1685.00 ± 5.00 mg/100 g extract.

Table 3 reveals that hydroxytyrosol, syringic acid and oleuropein were concentrated approximately 20, 50 and 10 times using the NF membrane to reach, 111.55, 265.23 and 1685 mg/100 g extract, respectively. Indeed, the NF retentate can find useful applications as high added value extract in the pharmaceutical, cosmetic or functional food sector.

3.4. Antioxidant capacity

Antioxidant capacity of each extract from the UF and the NF membranes was determined via FRAP and TAC methods (Table 4). The retentate fraction of NF membrane presented the strongest antioxidant capacities (438.75 mg vit C/100 g extract and 28.70 mol trolox/100 g extract for TAC and FRAP analyses, respectively). These results are in agreement with the researches of Benedetti et al. [23] and Cassano et al. [14] who have revealed that the NF process enhanced the antioxidant activity of soybean extract and olive mill waste water, respectively. The improvement of the antioxidant capacity might be related to the high content of polyphenols, mainly the oleuropein in olive leaf extract. For this reason, the FRAP and the TAC values of all fractions were correlated with polyphenols and oleuropein contents. A strong correlation ($p < 0.01$) was found between phenolic contents and antioxidant activity ($r^2 = 0.997$ and $r^2 = 0.949$, respectively, for FRAP and TAC tests). As regards oleuropein contents, a significant correlation ($p < 0.01$) with the FRAP and TAC values ($r^2 = 0.990$ and $r^2 = 0.924$, respectively) was highlighted. Ben Othman et al. [1] studied the correlation between the antioxidant activity and both total phenolic content and simple phenolic compound of table olives. They reported that there was no correlation between total phenolic content ($r^2 = 0.2813$) and simple phenolic compounds ($r^2 = 0.6176$) of the olive samples of 7 cultivars and their antioxidant activity. However, Altiok et al. [28] showed that the linear

Table 3
Analysis of identified polyphenols in feed stream, retentate and permeate of cross-flow ultrafiltration system and cross-flow nanofiltration system processes.

Membrane type	Sample	Hydroxytyrosol (mg/100 g extract)	R_H (%)	Syringic acid (mg/100 g extract)	R_{SA} (%)	Oleuropein (mg/100 g extract)	R_O (%)
UF 5000 Da	Feed	8.45 ± 0.17[c]	35.38	5.78 ± 0.26[c]	6.57	265.24 ± 2.03[c]	32.81
	Permeate	5.46 ± 0.26[b]		5.40 ± 0.11[b]		179.80 ± 3.03[b]	
	Retentate	2.17 ± 0.02[a]		1.99 ± 0.02[a]		72.22 ± 0.10[a]	
NF 300 Da	Feed	5.46 ± 0.26[b]	100	5.40 ± 0.11[b]	100	179.80 ± 3.03[b]	100
	Permeate	0[a]		0[a]		0[a]	
	Retentate	111.55 ± 1.00[c]		265.23 ± 3.02[c]		1685.00 ± 5.00[c]	

[a–c] Different letters in the same column indicate significant differences ($p < 0.05$) between fractions of the same membrane.

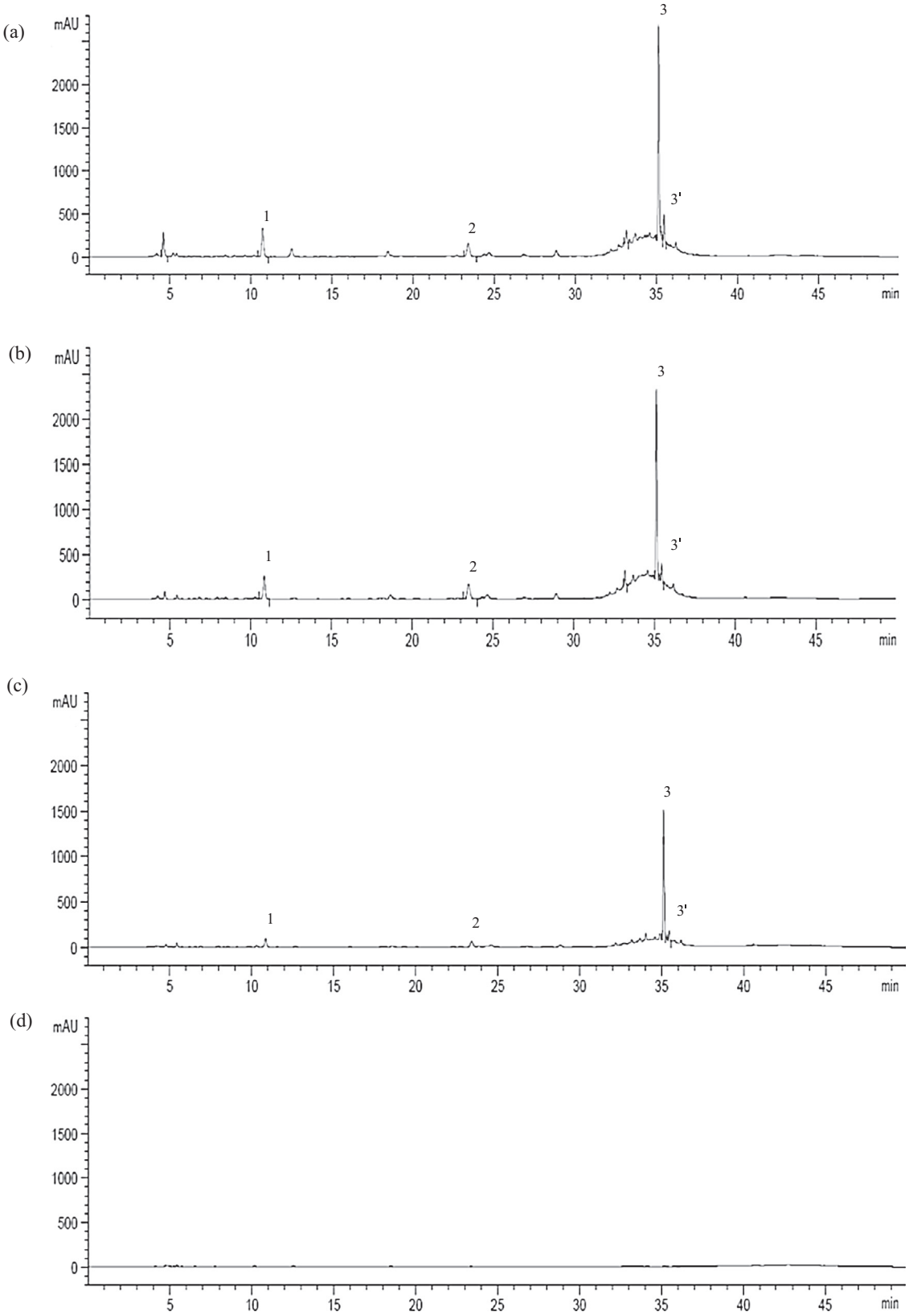

Fig. 4. HPLC chromatograms of polyphenols in feed (a); permeate UF (b); retentate UF (c) permeate NF (d) and retentate NF (e). (1) Hydroxytyrosol; (2) syringic acid; (3) oleuropein; (3') oleuropein isomer.

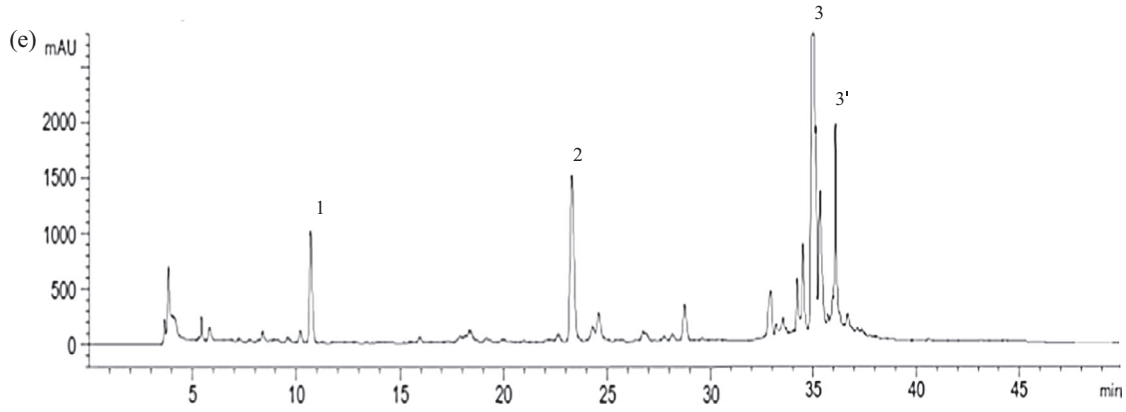

Fig. 4 (*continued*)

Table 4
Antioxidant capacity (FRAP and TAC) in feed stream, retentate and permeate of cross-flow ultrafiltration system and cross-flow nanofiltration system processes.

Membrane type	Sample	FRAP (mol trolox/100 g extract)	R_{FPAP} (%)	TAC (mg vit C/100 g extract)	R_{TAC} (%)
UF 5000 Da	Feed	0.71 ± 0.01[b]	45.07	98.09 ± 0.53[b]	36.44
	Permeate	0.39 ± 0.02[c]		62.35 ± 0.99[a]	
	Retentate	1.07 ± 0.01[a]		185.98 ± 2.90[b]	
NF 300 Da	Feed	0.39 ± 0.02[b]	84.62	62.35 ± 0.99[b]	90.62
	Permeate	0.06 ± 0.00[a]		5.85 ± 0.10[a]	
	Retentate	28.70 ± 0.59[c]		438.75 ± 7.50[c]	

[a–c] Different letters in the same column indicate significant differences ($p < 0.05$) between fractions of the same membrane.

Table 5
Antimicrobial activity of NF retentate.

Microbial species	Gram-negative				Gram-positive	
	S. enterica	*E. coli*	*K. pneumonieae*	*Enterobacter*	*M. luteus*	*B. cereus*
Diameter (mm)	25.0 ± 1.0	8.0 ± 1.0	28 ± 0.0	–	21.5 ± 0.5	–

correlation between the total phenol content and antioxidant capacities of olive leaf extracts obtained by different ethanol concentrations presented a high regression coefficient as 0.968. These results indicate that the antioxidant activity of olive leaves extract is directly affected by total phenols and oleuropein contents.

3.5. Antibacterial activity of NF retentate

Antibacterial activity of the NF retentate was evaluated using the agar diffusion method and results were displayed in Table 5. Antibacterial activity was exhibited by the inhibitory zone of NF retentate against Gram-negative bacteria (*S. enterica*, *K. pneumonieae*, *E. coli* and *Enterobacter*) and Gram-positive bacteria (*B. cereus* and *M. luteus*). As shown in Table 5, the studied fraction exhibited varying degrees of antibacterial activity against most of Gram-negative bacteria. The inhibitory activities against Gram- negative bacteria were higher than those obtained with Gram-positive bacteria tested. In fact, a strong growth inhibition effect against *K. pneumonieae* (28.5 mm ± 0.5) and *S. enterica* (25.0 mm ± 1.0) was observed. A moderate growth inhibition effect was found against *E. coli* (8.0 mm ± 1.0). However, no antibacterial activity was observed against *B. cereus* and *Enterobacter*. These results are in discordance with those of Lee and Lee [29] who found that olive leaf extract did not show a bactericide effect towards *E. coli*. It is well known that the structure of Gram-positive and Gram-negative cell walls has different compositions. Indeed, the layer of peptidoglycan in Gram-positive bacteria is thicker than in Gram-negative bacteria. Thus, the penetrations of extract to the cell wall of Gram-positive bacteria decrease [30].

4. Conclusion

The combination of MF, UF and NF technologies performs the isolation of biologically-active compounds like polyphenols and flavonoids from olive leaf extract. For industrial applications, membrane separation processes constitute an attractive alternative to the conventional processes. Indeed, these technologies provide unique separation capability, possibilities to scale-up and low energy consumption.

In this study, UF membrane showed low rejection values towards polyphenols, flavonoids, oleuropein, syringic acid and hydroxytyrosol thus allowed these compounds to be transferred in the permeate fraction. However, high rejection values towards the studied compounds were obtained by the NF membrane. Indeed, the main objective of the NF process was to concentrate aqueous solution of polyphenol extract. Similarly, the determined antioxidant capacity applying the FRAP and TAC methods increased after NF, and was mainly correlated to the total phenols and oleuropein contained in the concentrated fraction of olive leaves extract. Besides, the NF retentate exhibited high inhibitory activities against *K. pneumonieae*, *S. enteric* and *M. luteus*.

This fraction presented high oleuropein content which makes this technology useful in the development of new products with

functional properties suitable for cosmetic, food and pharmaceutical industries.

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