

# Evaluation of olive mill wastewater membrane treatment to obtain a final retentate rich in hydroxytyrosol

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## Research Article

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## Abstract

Olive mill wastewater (OMWW) is an extremely polluting dark liquid generated in large quantities during the extraction of olive oil. OMWW is also an interesting source of high-added value natural compounds such as biophenols, especially hydroxytyrosol (HT), a powerful antioxidant with proven nutritional and pharmaceutical properties. The main objective of this work was to explore the use of membrane technology for the treatment of OMWW in order to obtain a retentate stream rich in hydroxytyrosol and a permeate with low chemical oxygen demand (COD). Pretreatment was firstly performed by a combined centrifugation and acidification process. Then, several membrane operations were tested and phenolics rejection, HT content, COD removal and membrane fouling were examined. Best results were obtained using a single ultrafiltration process with a 10 kDa ST polyethersulfone membrane at 8 bar. Improved results were reached using a two-stage integrated system with 100 kDa US100 ultrafiltration and 200 Da NF90 nanofiltration membranes, at 5 and 10 bar, respectively. Volume reduction factor of 1.9 in ultrafiltration and 1.6 in nanofiltration yielded to  $234.49 \pm 26.08$  mg/L of HT in the retentate (9-fold the HT content in the raw OMWW) and 94.15% COD removal.

## Highlights

- OMWW was pretreated with a centrifugation and acidification combined process
- OMWW treatment was performed by membrane operations using different conditions
- Phenolic content, COD removal and membrane fouling were analyzed in all tests runs
- Optimal results correspond to an integrated ultrafiltration-nanofiltration process

## 1. Introduction

Olive mill wastewater (OMWW) is a foul-smelling acidic dark liquid that is generated during the extraction of olive oil and has a significant negative impact on the environment (Dhaouadi and Marrot 2008). The amount and physico-chemical properties of the OMWW depend on the method used for the olive oil extraction. Today, centrifugal systems have replaced traditional pressure method. The three-phase centrifugal system generates a solid husk, oil, and OMWW while the two-phase centrifugal system releases a wet olive husk and oil. Relative to the two-phase centrifugation, the three-phase system utilizes 0.6–1.3 m<sup>3</sup> of additional water during decantation that eventually increases the amount of OMWW (Gebreyohannes, Mazzei, and Giorno 2016).

In recent years, many management options have been proposed for the treatment of OMWW. Most of these methods aim to reduce the phytotoxicity of OMWW for reuse in agricultural applications, or alternatively to recover the biophenolic fraction due to its interesting pharmacological properties (Dhaouadi and Marrot 2008; El-abbassi, Khayet, and Hafidi 2011; Gebreyohannes et al. 2016; Servili et al. 2011). It is worth quoting that wastewater from olive oil mills can be interesting biological source of high added value compounds, such as hydroxytyrosol (3,4-di-hydroxyphenyl-ethanol) or other antioxidant phenolic compounds. In fact, the phenolic compounds are known as important natural antioxidants with nutritional and pharmaceutical properties (Tuck and Hayball 2002). Hydroxytyrosol and tyrosol are the main phenolic compounds in extra virgin olive oil. Hydrophilic extracts of olive oil contain many phenolic compounds and phenyl-alcohols. Oleuropein, hydroxytyrosol and their derivatives are found in large quantities in olive leaves and olive fruits (Kashaninejad et al. 2020, 2021).

Several scientists have demonstrated that olive phenols, especially hydroxytyrosol, are effective in preventing and curing some important diseases (Visioli et al. 1995). Nevertheless, hydroxytyrosol is not commercially available in large quantities like other food additives. Several methods have been proposed for the production of hydroxytyrosol by means of chemical or enzymatic synthesis (Tuck, Tan, and Hayball 2000). Such protocols are usually slow and expensive, resulting in few numbers of commercially available products containing pure hydroxytyrosol (El-abbassi et al. 2011).

Membrane separation processes are useful technologies for recovery, fractionation and concentration of phenolic compounds from aqueous and alcoholic streams coming from byproducts and biomass processing (Conde et al. 2013). Among these processes, pressure driven membrane operations have been successfully employed over the last three decades in food and beverage industries. According to the required transmembrane pressure (TMP) and the membrane pore sizes, pressure driven membrane operations can be divided in: microfiltration (MF, 0.1–5 µm, 1–10 bar), ultrafiltration (UF, 0.5–100 nm, 1–10 bar), nanofiltration (NF, 0.5–10 nm, 10–30 bar) and reverse osmosis (RO, <0.5 nm, 35–100 bar) (Strathmann, Giorno, and Drioli 2011).

Membrane processes have a range of inherent limitations e.g., a membrane system designed to treat OMWW may be limited by the OMWW's high concentration of suspended solids, viscosity, osmotic pressure, and temperature to attain a target quality. Therefore, the optimal separation process in many cases may be a membrane-based hybrid process. The term hybrid or integrated membrane process here refers to integration of membrane with membrane or membrane with traditional unit operations such as centrifugation (Singh 2005). MF and UF are mostly employed as a pre-treatment step in hybrid system to remove suspended particles and colloids while allowing the polyphenols and other soluble compounds to pass through (Coskun, Debik, and Demir 2010; El-Abbassi et al. 2013).

The major drawback in the development and large-scale application of membrane processes for OMWW treatment is the loss of membrane flux due to concentration polarization and membrane fouling. Generally, the membrane fouling problem is more pronounced in large pore membranes as MF and UF. Pressure driven membrane operations typically exhibit a high percentage of flux loss within a short operating period. Therefore, fouling limits productivity and can lead to the need for a larger membrane surface, which translates into higher capital and operating costs (Gebreyohannes et al. 2016).

The main objective of this research was to use membrane technology for OMWW treatment in order to obtain a retentate stream with a high content of biophenols, especially hydroxytyrosol. Different centrifugation and acidification pretreatments of OMWW were tested to achieve suitable feed conditions. Several single and integrated MF, UF and NF operations were tested. In integrated membrane runs, the obtained permeate from UF was used as feed in NF. The

efficiency and optimization of the membrane processes were evaluated by measuring phenolic rejection, hydroxytyrosol content in retentate, chemical oxygen demand (COD) removal and membrane fouling.

## 2. Materials And Methods

### 2.1. Samples and chemicals

Olive mill wastewater (OMWW) used in this study was obtained by means of a three-phase extraction system and was kindly provided by "Mamalan" Agro Industrial Company, Tarom city (Zanjan), Iran. Its physico-chemical composition is provided in Table 1.

All chemicals used in this study were analytical grade and supplied from VWR International Eurolab (Llinars del Vallès, Spain) and Sigma-Aldrich (Darmstadt, Germany).

The chemicals used for each analysis are as follows: Folin–Ciocalteu reagent, gallic acid and sodium carbonate were used for total phenolic content (TPC) measurement. Potassium dichromate ( $K_2Cr_2O_7$ ), sulfuric acid ( $H_2SO_4$ ), ferrous ammonium sulfate titrant (FAS) and ferroin were used for COD measurement. DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used for antioxidant activity (AA) analysis by DPPH assay. Hydrochloric acid (HCl, 37%) was used for the acidification of OMWW. Acetic acid ( $CH_3COOH$ ), acetonitrile ( $C_2H_3N$ ) and ammonium acetate ( $NH_4Ac$ ) were the chemicals used in HPLC for identification and quantification of individual phenolic compounds. Sodium hydroxide ( $NaOH$ , 16 g/L) and nitric acid ( $HNO_3$ , 60%) in 5 g/L aqueous solution were used as alkaline and acid cleaning for ceramic membranes before and after each treatment. Several types of ceramic and polymeric membranes were used and their characteristics are shown in Table 2.

Table 1  
Physico-chemical characteristics of Olive Mill Wastewater (OMWW)

Parameter	Unit	Value
Total phenols	mg/L	$1200.15 \pm 13.05$
Chemical oxygen demand (COD)	mg/L	$19418 \pm 868$
Biochemical oxygen demand (BOD)	mg/L	$8600 \pm 0.0$
Total organic carbon (TOC)	g/L	7.93
Total suspended solids (TSS)	g/L	6.48
Antioxidant Activity (AA)	mg <sub>Trolox</sub> /L	$185.25 \pm 1.21$
pH	-	4.9
Turbidity	NTU	8225
SO <sub>4</sub>	mg/L	170
NO <sub>3</sub>	mg/L	530

Table 2  
Characteristics of ceramic and polymeric membranes used

Treatment	NF	NF	NF	UF	UF	UF	UF	MF
Name	NF270	NF90	NOP10	ST, PES	MK, PES	Ceramic	US100	Ceramic
Type	Flat sheet	Flat sheet	Flat sheet	Flat sheet	Flat sheet	Tubular	Flat sheet	Tubular
Manufacturer	Dow Filmtec™	Dow Filmtec™	Microdyn-Nadir	Synder™	Synder™	Tami Industries	Microdyn-Nadir	Tami Industries
Material	Polyamide	Polyamide	PES	PES	PES	TiO <sub>2</sub>	Polysulfone	TiO <sub>2</sub>
Cut-off	200 ~ 400 Da	200 ~ 400 Da	1000–1200 Da	10 kDa	30 kDa	50 kDa	100 kDa	0.2 μm
pH range	2–11	2–11	0–14	2–11	2–11	0–14	1–14	0–14
Water permeability ( $L m^{-2} h^{-1} bar^{-1}$ )	10.5	6.3	7.5	25	25	50	400	170
MF: Microfiltration; UF: Ultrafiltration; NF: Nanofiltration								

## 2.2. Acidification and centrifugation

The pH value of the raw OMWW measured by means of a glass pH electrode (Crison, Barcelona, Spain) was 4.9. Afterwards, by considering the work performed by Bazzarelli et al. (Bazzarelli et al. 2015), OMWW was acidified with HCl (37%) using 0.003 mL/L content. OMWW samples were centrifuged (Eppendorf centrifuge 5804, Germany) at 4000 rpm for 30 min. The operating conditions for centrifugation in this study were the same as those used by Russo (Russo 2007).

## 2.3. Particle size measurement

Particle size and particle size distribution were measured using a laser light scattering system (Malvern Mastersizer 2000, Malvern Panalytical, UK). The mean particle size was expressed as the volume-weighted mean diameter, D<sub>4,3</sub>. The width of particle size distribution was expressed as a Span number, calculated by the following equation:

$$\text{Span} = \frac{D(0.9) - D(0.1)}{D(0.5)}$$

1

where D(0.1), D(0.5) and D(0.9) are the particle diameters at the 10th, 50th and 90th percentiles, respectively. D(0.5) is also known as the median diameter. The mean particle diameter and Span values were calculated on the basis of five different experimental runs.

## 2.4. Determination of total phenolic content

Total phenolic content (TPC) of extracts were measured using Folin-Ciocalteu standard method (E. HOFF and I. SINGLETON 2006) with some modifications as indicated in the previous works (Niknam et al. 2021, 2022; Niknam, Escudero, and Benito 2020). Briefly, a standard calibration curve ( $R^2 = 0.98$ ) was prepared using gallic acid solution. 100 µl of extracts containing phenolic compounds was added to a test tube. Then 2.8 ml of distilled water, 2 ml of 7.5% sodium carbonate and 100 µl Folin-Ciocalteu reagent added to the test tube. Contents were mixed and after 60 minutes in the dark at room temperature, the absorbance was measured spectrophotometrically at 750 nm (Hitachi U-2000 spectrophotometer). TPC was expressed as milligrams of gallic acid equivalents per liter of OMWW (mg GAE/L).

## 2.5. Determination of chemical oxygen demand

Chemical oxygen demand (COD) determination was performed on raw and treated OMWW samples according to the protocols described in the Standard Methods for the examination of water and wastewater (Carranzo 2012). Briefly, OMWW samples were diluted 1:50 or 1:100, depending on their prior treatment. Then 5 mL of diluted sample were poured in a glass tube along with 3mL of  $K_2Cr_2O_7$  and 7mL of  $H_2SO_4$ . Also, blank and title samples were prepared by using distilled water instead of OMWW. The mixtures in all glass tubes were sealed and heated at 160°C for at least two hours except the title sample. After getting cold and adding ferroin as indicator, contents of each tube were titrated with standard ferrous ammonium sulfate titrant (FAS). COD value was measured by the application of Eq. (2):

$$\text{COD} = f \times \frac{(B - M)}{T} \times D.F$$

2

where f equals to 960 and D.F is the dilution factor. B, T and M are consumed volumes in titration processes for blank, title and OMWW samples, respectively. All samples in the COD analysis were prepared with two replicates.

## 2.6. Determination of antioxidant activity

Antioxidant activity (AA) of the OMWW samples was measured by following DPPH method described by Shen et al. (Shen et al. 2010). Briefly, 1mg of DPPH reagent (2, 2-Diphenyl-1-picrylhydrazyl) was dissolved in 50 ml methanol and stored in the dark at 4°C for at least 4 hours. In order to perform DPPH assay, 60 µl of sample extract were mixed with 2940 µl of DPPH reagent solution. For preparing the control solution, 60 µl of distilled water were mixed with 2940 µl of DPPH reagent solution. The mixtures were allowed to stand in the dark at room temperature for 60 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer model (Hitachi U-2000 spectrophotometer). AA was expressed as milligrams of Trolox (mg<sub>Trolox</sub>/L<sub>OMWW</sub>). A standard calibration curve ( $R^2 = 0.995$ ) was prepared using Trolox solution. Also, the capability of scavenging the DPPH radical was calculated by using Eq. (3):

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where  $A_0$  is the absorbance of the control reaction, and  $A_1$  is the absorbance of the samples. All tests in this study were performed in duplicate and the results were averaged.

## 2.7. Identification and quantification of hydroxytyrosol by HPLC-DAD

Identification and quantification of hydroxytyrosol was carried out by reverse phase HPLC at 280 and 330 nm wavelengths. Analytical-scale HPLC analysis of the OMWW and fractions were performed with a high efficiency liquid chromatograph (HPLC-DAD Agilent 1100, CA, USA) equipped with a Kinetex® 5 µm Biphenyl 100 Å column of 250 × 4.6 mm (Phenomenex, Inc. CA, USA). Separation was achieved using a linear gradient of two solvents: solvent A (ammonium acetate 5 mol/m<sup>3</sup> with 1% (v/v) acetic acid in water), and solvent B (ammonium acetate 5 mol/m<sup>3</sup> with 1% (v/v) acetic acid in acetonitrile). The program involved a linear increase of solvent B: 2% of B for 7 min; 2–8% of B in 13 min; 8–10% of B in 15 min; 10–18% of B in 20 min; 18–38% of B in 10 min; 38–65% of B in 10 min; 65–80% of B in 5 min; 80% of B for 10 min at a flow rate of 0.8 mL/min and a column temperature of 25 °C. The injection volume was 10–100 µL depending on the sample content. All tests were performed in duplicate and the results were averaged. The evaluation of each phenolic compound was based on comparison of peak sequence and intensity with standard solutions. Results were expressed as milligrams of hydroxytyrosol per liter of OMWW (mg/L).

## 2.8. Pretreatments and membrane processes

Three pretreatment types were considered for OMWW before using it as feed in membrane treatment. In first type (feed A), only centrifugation was conducted. In second type (feed B), acidification to pH = 1.9 followed by centrifugation were carried out. In third type (feed C) centrifugation followed by acidification to pH = 1.9 were conducted. Centrifugation process was performed at 30 min and 4000 rpm for all feed types. It may seem that feed B and feed C are similar, however, preliminary light scattering tests showed that mean particle diameter for feed B and feed C were considerably different. Therefore, this difference could be the cause of the different results obtained in the subsequent membrane treatments.

OMWW membrane treatments were performed using two different membrane modules. The first one was a tubular module supplied by Tami Industries (Nyons, France) operating in concentration mode where permeate was collected in a separate vessel and concentrate stream was circulated back to the feed tank. Ceramic TiO<sub>2</sub> MF or UF membranes, shown in Table 2, with a membrane area of 47 cm<sup>2</sup> were used. A positive displacement pump (Masterflex, L/S EasyLoad II, IL, USA) and a 500 mL capacity feed tank were used. Feed temperature was kept constant by means of a circulating bath, and pressure was measured by means of two manometers located at the inlet and outlet of membrane module. Tubular membrane experiments were performed at 40°C and at a transmembrane pressure of 1–2 bar.

Once experiment was finished, membrane was rinsed with distilled water for 10 min, cleaned with an alkaline aqueous solution (16 g/L NaOH) at 50°C for 30 min, rinsed again with distilled water for 15 min, and cleaned with an acid solution (5mL/L HNO<sub>3</sub> (60%)) at 50°C for 30 min. Finally, the system was rinsed with distilled water for 15 min until neutrality and water permeate flux was checked.

Flat sheet polymeric membranes shown in Table 2 were used in UF and NF experiments using a stainless steel HP4750 stirred batch cell supplied by Sterlitech Corporation (Kent, WA, USA). A transmembrane pressure between 5–10 bar was provided through a nitrogen cylinder, which also prevents oxidation of the biocompounds. Membrane surface area was 14.6 cm<sup>2</sup> and experiments were performed at room temperature and 300 rpm stirring speed. Stirred cell modules provide uniform transmembrane pressure and hydrodynamic conditions on the membrane surface; therefore, the effects of operating parameters on efficiency for small-scale process can be very easily determined using these cells. However, advantage of tubular membrane module includes turbulent flow (providing good membrane/solution contact and removing retentate film build-up), relatively easy cleaning, easy handling of suspended solids and viscous fluids and ability to replace or plug a failed tube while the rest of the system runs (El-Abbassi et al. 2012).

The permeate flux was monitored by measuring the permeate volume collected at a specific time described by Darcy's equation (Fried 1997):

$$J = \frac{1}{A_m} \cdot \frac{V_p}{t}$$

4

where A<sub>m</sub> is the effective membrane area, V<sub>p</sub> is the permeate volume, and t is the filtration time.

Volume reduction factor (VRF) is defined as:

$$VRF = \frac{V_F}{V_R}$$

5

where V<sub>F</sub> and V<sub>R</sub> are the initial feed volume and the retentate volume (V<sub>R</sub>= V<sub>F</sub>-V<sub>P</sub>), respectively. This is an important parameter in concentration operating mode.

Percent rejection (%) of feed components is calculated as:

$$R(\%) = \left(1 - \frac{C_p}{C_F}\right) \times 100$$

6

where  $C_p$  and  $C_f$  are the concentration of total phenols in permeate and feed solutions, respectively (Zirehpour, Jahanshahi, and Rahimpour 2012).

Membrane fouling percentage was calculated by measuring the difference between initial membrane flux ( $J_0$ ) and steady state flux ( $J_{ss}$ ). For this purpose, Eq. (6) was used in which  $C_f$  and  $C_p$  were replaced by  $J_0$  and  $J_{ss}$ , respectively.

### 3. Results And Discussion

#### 3.1. Effect of pretreatments on the hydroxytyrosol content of OMWW

Table 3 shows the hydroxytyrosol (HT) content of raw OMWW and samples treated by centrifugation and acidification. It is observed that centrifugation does not affect the HT content; however, the acidification effect was very significant. HT concentration in raw OMWW was  $25.30 \pm 4.34$  mg/L whereas after acidification was  $115.10 \pm 6.68$  mg/L, which represents an increase in HT concentration of more than 350%. This agrees with Mateo (Mateo 2002) who discovered that adding acid to the vegetation water up to pH between 2–4, and its subsequent incubation for a period of two months, allows reaching that at least 75% of the oleuropein originally present in the vegetation water becomes HT.

#### 3.2. Effect of pretreatments on the OMWW particle size

The centrifugation effect on raw and acidified OMWW samples was also studied. For this, particle sizes and their distribution were measured by light scattering in a Mastersizer 2000 apparatus (Malvern Instrument Ltd., UK). Also, zeta potential of samples was measured in a Nanoziser Nano ZS of the same company. Results are depicted in Table 4. It is observed that, as expected, centrifuged sample of raw OMWW (natural pH = 4.9) has lower particle size than the uncentrifuged one. Specifically, the volume weighted mean diameter decreases from  $D[4,3] = 4.580$   $\mu\text{m}$  to  $(D[4,3] = 0.247$   $\mu\text{m}$  after 30 min centrifugation. However, centrifugation of acidic OMWW samples at pHs of 1.5 and 3 increases  $D[4,3]$  for about 68%. This surprising result is related to the decrease in zeta potential with acidification that leads to increased instability. The zeta potential of the centrifuged and non-centrifuged OMWW samples at their natural pH is very similar, with values of -26.4 and -27.1 mV, respectively, in this case centrifugation does not affect the zeta potential which is related to particle charge. As the pH of OMWW decreases, the zeta potential approaches zero and consequently flocculation and sedimentation of particles could occur. These results can affect membrane fouling and viability of membrane processes for OMWW treatment. The use of three different pretreatments on the behavior of membranes will be discussed in next section in order to optimize the OMWW pretreatment before feeding the membrane processes.

Table 3  
Hydroxytyrosol content in raw and pretreated OMWW

Sample type	Hydroxytyrosol content (mg/L)
Raw OMWW	$25.30 \pm 4.34$
Centrifuged OMWW	$34.76 \pm 6.65$
Acidified OMWW to pH = 1.9	$115.10 \pm 6.68$
Centrifuged and acidified OMWW to pH = 1.9	$103.84 \pm 4.37$

Table 4  
Effect of centrifugation on OMWW samples at natural pH of 4.9 and acidified at pH 3 and 1.5. Results of total phenol analysis, zeta potential and light scattering.

pH	Centrifugation time (minute)	Total phenols (mg/L)	Zeta potential (mV)	D[4,3] ( $\mu$ m)	Span	D(0.9) ( $\mu$ m)
1.5	0	1146.3	+ 0.067	4.372	1.138	3.156
1.5	30	1072.5	- 0.176	7.324	1.364	3.137
3.0	0	1066.7	- 4.35	4.695	1.140	3.116
3.0	30	1032.5	- 4.91	7.908	1.434	3.135
4.9	0	1200.1	- 26.4	4.580	1.201	2.362
4.9	30	931.3	- 27.1	0.247	1.345	0.120

### 3.3. Evaluation of operating conditions on the performance of single membranes

The influence of three types of OMWW pretreatments consisting in centrifugation (feed A), acidification until pH 1.9 following centrifugation (feed B), and centrifugation following acidification to pH = 1.9 (feed C) on the performance of several UF and NF single membranes was analyzed. The ST-PES, 10 kDa membrane (UF4, UF5 and UF6 in Table 5) and the NP010, 1000 Da membrane (NF1, NF2 and NF3) were operated with all types of feeds under the same operating conditions (8 bar and 10 bar for UF and NF experiments, respectively). The TiO<sub>2</sub>, 50 kDa membrane was run with feeds A (UF2) and B (UF1) at 2 bar TMP, while the US100, 100 kDa membrane was run with feed C (UF7) at 5 bar TMP. Main results are shown in Table 5. The total polyphenols content (TPC) rejection, COD removal, membrane fouling and steady state permeate flux ( $J_{ss}$ ) are depicted in Fig. 1.

A membrane process for OMWW treatment can be considered functional and effective if yields high values of TPC rejection, COD removal, steady state flux, and low membrane fouling. Figure 1a shows that the highest polyphenol rejections were obtained for UF4, NF3 and UF6 with 70.79%, 70.16% and 69.57%, respectively. Furthermore, the highest COD removal values were 80.06% and 76.16% for UF6 and NF3 experiments, respectively (Fig. 1b), both working with feed C. For these two experiments, membrane fouling and  $J_{ss}$  show reasonable and acceptable values.

Regarding the antioxidant activity (AA), the objective is to obtain a final permeate with low AA values that assures minimum loss of phenolic compounds in permeate. As reflected in Table 5, the lowest AA values of the final permeates in single membrane treatments correspond to the UF6 (VFR = 1.8) and NF3 (VFR = 1.6) experiments with  $148.93 \pm 0.91$  and  $115.17 \pm 0.30$  mg Trolox/L, respectively.

Furthermore, for a complete analysis of the experimental results in accordance with the main objective of this work, the content of hydroxytyrosol (HT) in the final retentates was analyzed. At this regard, Table 5 shows that the UF6 and NF1 experiments with  $131.30 \pm 13.73$  and  $104.03 \pm 1.88$  mg HT / L are the optimal runs in UF and NF, respectively, following by NF3 with  $50.29 \pm 3.20$  mg HT / L. Despite the notable higher concentration of HT in NF1 with respect to NP3, it should be noted that the TPC rejection and COD removal values are significantly lower in NP1. Thus, the global assessment of the operating variables discussed in this section leads us to consider that the operation conditions of UF6 and NF3 are the optimal among those studied, with an OMWW pretreatment type C in both cases

**Table 5**  
Operating conditions and final results of single and integrated membrane experiments for OMWW treatment.

Module type	code	Feed type*	Membrane / Cut-off	TMP (bar)	Steady state flux ( $\text{L m}^{-2} \text{h}^{-1}$ )	Membrane fouling (%)	TPC of permeate		COD of permeate		Antioxidant activity of permeate		Hydro conte reten (mg/
							mg/L	Rejection (%)	mg/L	Removal (%)	mg/L	Inhibition (%)	
Tubular stainless steel Cell	MF1	Feed B	TiO <sub>2</sub> (0.2 μm)	1	9.6	55.6	1086.31 ± 2.18	9.49	11676 ± 931	39.87	188.24 ± 0.0	96.07 ± 0	97.26 (VRF)
	UF1	Feed B	TiO <sub>2</sub> (50 kDa)	2	5.9	51.2	842.46 ± 17.41	29.80	9668 ± 81	50.21	185.68 ± 1.21	94.77 ± 0.62	95.75 (VRF)
	UF2	Feed A	TiO <sub>2</sub> (50 kDa)	2	6.0	70.0	774.00 ± 4.35	35.51	9535 ± 134	50.90	179.48 ± 0.30	91.60 ± 0.15	43.94 (VRF)
HP4750 Stirred Cell	UF3	Feed A	MK, PES (30 kDa)	8	8.2	55.7	528.23 ± 4.90	55.99	8931 ± 244	54.01	181.41 ± 1.81	92.58 ± 0.93	25.13 (VRF)
	UF4	Feed A	ST, PES (10 kDa)	8	8.2	38.8	350.59 ± 5.98	70.79	8305 ± 231	57.23	179.27 ± 4.23	91.49 ± 2.16	77.83 (VRF)
	UF5	Feed B	ST, PES (10 kDa)	8	6.2	52.4	453.62 ± 12.51	62.20	6873 ± 359	64.61	166.66 ± 3.93	85.06 ± 2.0	89.18 (VRF)
	UF6	Feed C	ST, PES (10 kDa)	8	6.1	59.5	365.15 ± 7.07	69.57	3872 ± 118	80.06	148.93 ± 0.91	79.57 ± 0.48	131.3 (VRF)
	UF7	Feed C	PS, US100 (100 kDa)	5	13.9	75.9	1014.0 ± 40.25	15.51	14149 ± 431	27.13	160.89 ± 0.91	93.19 ± 0.53	103.6 (VRF)
	NF1	Feed B	PM-NOP10 (1000 Da)	10	4.3	51.8	452.46 ± 1.09	62.30	5898 ± 421	69.63	157.69 ± 0.60	84.25 ± 0.32	104.0 (VRF)
	NF2	Feed A	PM-NOP10 (1000 Da)	10	8.3	56.8	517.08 ± 41.34	56.91	5516 ± 39	71.59	172.86 ± 2.11	92.35 ± 1.13	25.99 (VRF)
NF3	Feed C	PM-NOP10 (1000 Da)	10	6.9	45.2	358.10 ± 9.56	70.16	4630 ± 39	76.16	115.17 ± 0.30	61.53 ± 0.16	50.29 (VRF)	
NF4	MF1 permeate	PM-NOP10 (1000 Da)	10	6.7	52.3	532.85 ± 10.33	50.95	5433 ± 53	53.47	166.02 ± 4.53	88.70 ± 2.42	162.7 (VRF)	
NF5	UF1 permeate	NF270-PA (270 Da)	10	5.6	65.6	178.23 ± 2.72	78.84	2412 ± 410	75.05	138.03 ± 3.02	73.74 ± 1.61	145.4 (VRF)	
NF6	UF2 permeate	NF270-PA (270 Da)	10	15.2	63.4	102.46 ± 4.35	86.76	1741 ± 0	81.74	145.08 ± 2.72	77.51 ± 1.45	89.5 (VRF)	
NF7	UF7 permeate	NF90-PA (200Da)	10	4.5	77.2	56.31 ± 5.44	94.45	828 ± 91	94.15	33.97 ± 0.91	19.68 ± 0.53	234.4 (VRF)	

\* Feed A: OMWW centrifugated; Feed B: OMWW pH adjusted to 1.9 and then centrifugated; Feed C: OMWW centrifugated and then pH adjusted to 1.9.

In order to elucidate the effect of the pretreatments, the variation of permeate flux with time for comparable UF and NF experiments with the three feed types, is shown in Figs. 2a and 2b, respectively. It is observed that the higher permeate flux are for UF4 and NF2 experiments, both working with feed type A consisting in OMWW centrifugation at its natural pH. Differences between feeds A and B are indistinguishable for the UF experiments, but are highly significant in the NF experiments.

Membrane processing of OMWW is highly dependent on concentration polarization and membrane fouling (Tsagaraki and Lazarides 2010). Concentration polarization is a reversible phenomenon caused by increased transport resistance in the boundary layer. Whereas, membrane fouling is an irreversible phenomenon that affects surface and pore fouling through different mechanisms including adsorption, gel formation, plugging, partial blocking, or cake

formation. Permeate flux profiles under fouling typically shows an initial stage sudden drop followed by a smoother but continuous decay (Gebreyohannes et al. 2016).

Figure 2b shows that the steady state permeate flux of NF3 (feed C) is approximately 60% higher than that of NF1 (feed B). Taking into account the results discussed in section 3.2, in our opinion, a possible explanation could be that the OMWW pretreatment type C, which is the most effective and involves centrifugation followed by acidification, causes the formation of porous flocs in the material accumulated in the boundary layer that facilitate the permeation. In contrast, in type B pretreatment, acidification is performed first, followed by centrifugation. In this case, the larger flocs formed by acidification are separated by centrifugation and the layer of accumulated solids on the membrane surface is more compact and more resistant to permeation.

### 3.4. Optimization of OMWW treatment by membrane processes

Previous analysis on materials rejection, HT content and membrane fouling indicates that the optimal results for single membrane processes were achieved in the UF6 experiment. Therefore, it can be concluded that the OMWW treatment can be optimally carried out by means of a centrifugation pretreatment followed by acidification to pH 1.9 and then its treatment with a ST-PES (10 kDa) membrane. The results obtained using a stirred membrane module at a TMP of 8 bar up to a VRF of 1.8 yielded values of 69.57% TPC rejection, 80.06% COD removal,  $131.30 \pm 13.73$  mg HT / L in the final retentate, with a stable permeate flux of 6.1 L / h m<sup>2</sup> and 59.5% membrane fouling. Comparison of the final UF6 retentate and the initial raw OMWW (untreated) shows a more than 5-fold increase in HT content and a 54% volume decrease from the initial feed volume. However, these results can be improved working under optimal operating conditions of the membrane process that will be the object of study in a later work.

Systematic analysis of the effect of TMP, feed rate, temperature, membrane material and its molecular weight cut-off has shown that all parameters have a significant effect on permeate flux and material rejections (Tsagaraki and Lazarides 2010). Akdemir and Ozer (Akdemir and Ozer 2009) using a 100 kDa polymeric membrane achieved a 18% permeate flux enhancement by increasing the TMP from 1 to 3 bar at constant feed flow rate, or alternatively increasing the feed flow from 100 to 200 L/h keeping TMP constant. It should be noted that high pressure could lead to irreversible fouling and a consequent decrease in the rate of COD removal and TOC rejection of the MF or UF membranes.

Fouling caused by OMWW is also highly dependent on type and characteristics of the membrane (composition, pore size and thickness). Cassano et al (Cassano, Conidi, and Drioli 2011) carry out a comparative study on the effect of fouling on two polymeric membranes (UF regenerated cellulose and UF PES). Results showed that regenerated cellulose membranes exhibited lower rejections toward phenolic compounds; higher permeate fluxes and lower fouling index compared to PES membranes.

With relation to the feed pretreatments, the study carried out by Coskun et al. (Coskun et al. 2010) highlights. In this study, the using of centrifugation, UF, and the combination of both pretreatments on the performance of a NF process is compared. Centrifugation of OMWW at 3750 rpm for 30 min or the use of a 10 kDa UF Nadyr membrane at 2 bar yielded comparable COD removal of 30–36%, while their combination increased the COD removal efficiency to 56%, with a UF permeate flux of 70 L / m<sup>2</sup> h. The NF permeate flux obtained with the UF pretreated feed was 30% higher than the permeate flux obtained for the non-pretreated feed. Therefore, this study establishes the suitability of using UF as a pretreatment to increase permeate flux during NF.

Based on aforementioned results, several integrated membrane experiments were performed. In these experiments, the membranes with the largest pore size were used in the pretreatment stage. Thus, the final permeates obtained in MF1, MF2, UF1, UF2 and UF7 runs were used as feed in NF4, NF8, NF5, NF6 and NF7 experiments, respectively. Results are shown in Table 5. It is observed that NF7 experiment has the highest TPC rejection (94.45%) and HT content (234.49 mg / L) in the final retentate (VFR = 1.6), and noticeably high COD removal in permeate (94.15%), which makes NF7 as the optimal integrated membrane treatment. The results also corroborate the convenience of applying a type C pretreatment on OMWW.

However, the low steady state flux and high membrane fouling of NF7 compared to NF4, NF5 and NF6 runs is also observed in Table 5. This may be due to the high organic material content of NF7 feeding as UF7 experiment used a PS-US100 (100 kDa) membrane which barely yields 15.51% TPC rejection and 27.13% COD removal.

Comparison of the NF7 final retentate and the initial raw OMWW (untreated) shows an increase of 9-fold the HT content. Taking into account the VFR achieved in each step, the volume of the final retentate was 29% of that used as feed for the integrated membrane process. Better results can be achieved if the process operates under optimal operating conditions that must be studied for practical application purposes. Figure 3 shows pictures of samples of raw OMWW and pretreated by type C method, along permeates and retentates obtained in the optimal single and integrated membrane treatments, UF6 and NF7 shown in Table 5, respectively. It is highlighted that NF7 permeate stream is colorless and practically free of phenolic compounds suggesting a potential use for irrigation or disposal in aquatic streams.

## 4. Conclusion

The use of membrane technology for the treatment of olive mill wastewater (OMWW) was explored in this work to obtain a permeate stream with low COD and simultaneously a retentate stream rich in biophenols, especially hydroxytyrosol (HT), the main phenolic compound in OMWW. Several membranes with different cut-off and materials were tested on pretreated OMWW. A combination of centrifugation (30 min, 4000 rpm) and acidification (pH adjustment to 1.9) was selected for OMWW pretreatment before feeding the membrane. Optimal results for pretreated OMWW in single ultrafiltration process was obtained using a 10 kDa ST PES membrane at 8 bar TMP working in concentration mode, reaching a volume reduction factor (VRF) of 1.8 and yielding  $131.30 \pm 13.73$  mg/L of hydroxytyrosol in retentate and 80.06% COD removal in permeate.

Integrated membrane systems were also tested consisting of a first stage of microfiltration (MF) or ultrafiltration (UF) followed by a second stage of nanofiltration

(NF), which was fed with the permeate obtained in the first stage. Best results were obtained on pretreated OMWW using a 100 kDa US100 UF membrane and a 200 Da NF90 NF membrane, working at 5 and 10 bar TMP, respectively. Results for 1.9 VRF in the UF stage and 1.6 VRF in the NF stage yields a hydroxytyrosol content of  $234.49 \pm 26.08$  mg/L in the final retentate and 94.15% COD removal in the final permeate. This means a 9-fold increase in hydroxytyrosol content in the final retentate compared to that of the raw OMWW (untreated) with 29% of the initial feed volume. Furthermore, the final NF permeate obtained in the optimal integrated membrane process is colorless and practically free of phenolic compounds, suggesting its potential use for irrigation or discharge into water streams.

## Declarations

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### Author Contributions

**Seyed Mehdi Niknam:** Investigation, Validation, Formal analysis, Writing - Original Draft.

**Mansoore Kashaninejad:** Investigation, Review & Editing.

**José Manuel Benito:** Supervision, Conceptualization, Formal analysis, Methodology, Funding acquisition.

**Isabel Escudero:** Conceptualization, Methodology, Validation

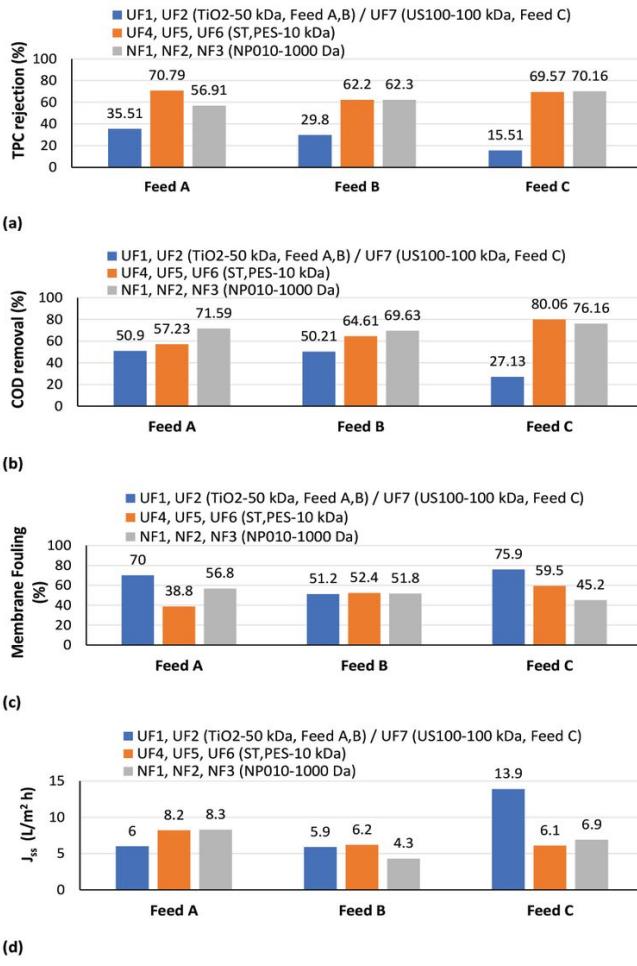
**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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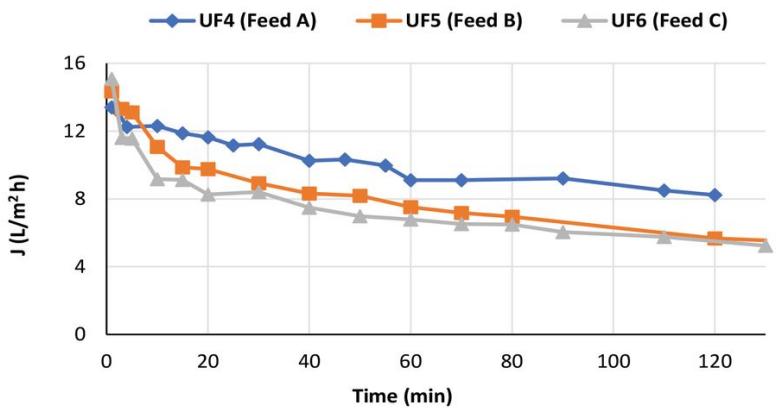
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## Figures

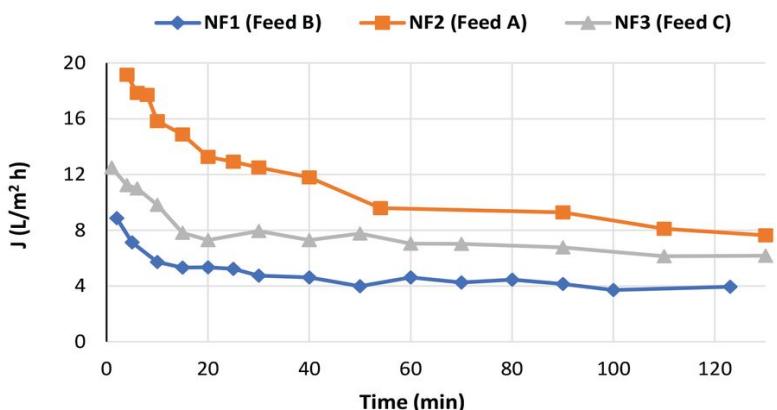


**Figure 1**

Evaluation of feed types on performance of different membranes shown in Table 5 (a) TPC rejection; (b) COD removal; (c) Membrane fouling; (d) Steady state permeate flux.



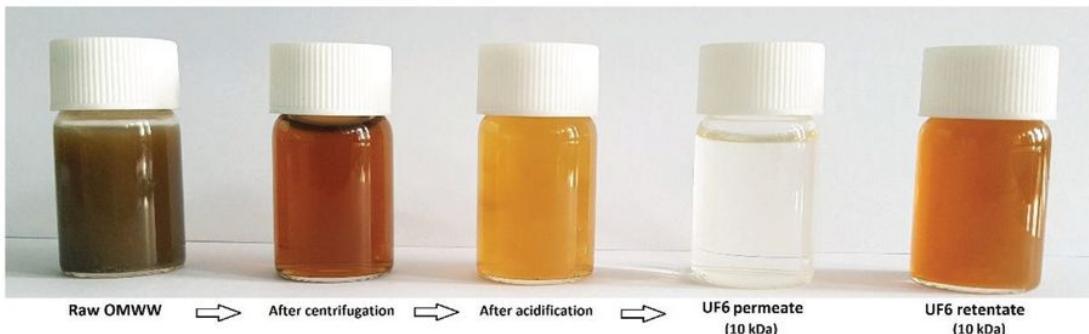
(a): Ultrafiltration (ST, PES-10 kDa)



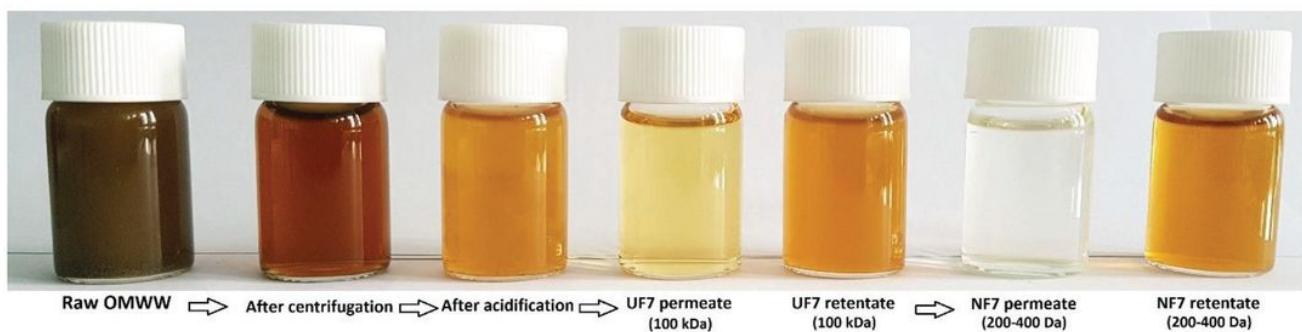
(b): Nanofiltration (NP010-1000 Da)

Figure 2

Variation of permeate flux in time for membrane treatments of UF and NF on several feed types (Feed A: OMWW centrifugated; Feed B: OMWW pH adjusted to 1.9 and then centrifugated; Feed C: OMWW centrifugated and then pH adjusted to 1.9)



**(a): Optimal single membrane treatment**



**(b): Optimal integrated membrane treatment**

**Figure 3**

*Pictures of obtained samples from optimal membrane treatments of OMWW*

## Supplementary Files

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