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# Integrated membranes for the recovery and concentration of antioxidant from olive mill wastewater

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ABSTRACT

During olive oil production a great amount of olive mill wastewater (OMW) is produced that is potentially a rich source of bio-phenols with wide array of biological activity. The purpose of the present work is to recover and concentrate the valuable polyphenols from the OMW using integrated membrane systems. Further study was conducted by employing enzymatic hydrolysis using β-glucosidase followed by acid hydrolysis in order to release higher concentration of pure hydroxytyrosol (HT). Meanwhile, efficient treatment of OMW could be achieved for water reuse as well as protecting the environment. The OMW was directly subjected to a microfiltration (MF) where 77.6 and 34.8% reduction of total suspended solids (TSS) and chemical oxygen demand (COD), respectively, were achieved. The Permeate of MF was further subjected to ultra-filtration (UF), where the removal was 44.9% and 100% for the COD and TSS, respectively. Another portion of the MF permeate was also subjected to nano-filtration (NF), where COD was reduced from 33 to 6.9 g/l and 100% TSS removal was achieved. The polyphenols in raw OMW was 2.5 g/l, it was recovered and concentrated in the Retentate of MF, UF and NF to 2.3, 2.0 and 2.4 g/l, respectively. Almost all polyphenols were recovered and concentrated in the NF Retentate solution. By employing the enzymatic hydrolysis using β-glucosidase on NF Retentate followed by acid hydrolysis an amount of 1.3 g/l of pure HT could be released. Based on these results OMW can be used as alternative source of valuable phenolic compound that are useful for industrial and pharmaceutical applications.

*Keyword:* Olive mill wastewater; Antioxidant; Hydroxytyrosol; Polyphenols; Integrated membrane; β-glucosidase hydrolysis

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## 1. Introduction

The presence of phenolic compounds has a negative effect on the microbiological treatment of the olive mill wastewater (OMW). In contrast, phenols are widely used by pharmaceutical, cosmetic and nourishment sectors [1]. Their properties, such as anti-inflammatory, anti-microbic and antioxidant activity, the inhibition of oxidative damage and the radical elimination, have been largely studied [1–3]. These compounds are usually synthesized by chemical methods that are responsible of their high price [2].

Fresh OMW is rich in polyphenols especially oleuropein which is the major bioactive compound of this by-product in the early harvest season. The molecule consists of three structural subunits: a polyphenol, namely 4-(2-hydroxyethyl) benzene-1, 2-diol which is also known as hydroxytyrosol (HT), a secoiridoid called elenolic acid and a glucose molecule (Fig. 1) [3].

The hydrolysis of oleuropein, thanks to β-glucosidase action, gives high value-added compounds that are pharmacologically active [4,5]. HT can be found in olive products either as the simple phenol or esterified with elenolic acid to form oleuropein. In addition, HT is the major bioactive metabolite of oleuropein and it is considered as one of the most powerful naturally derived antioxidants [6]. It has been demonstrated that the other structural subunit, elenolic acid exhibits strong antiviral properties [7,8]. However, regarding the beneficial effects of oleuropein and its degradation products especially the HT, several methods have been developed to produce this compound by means of chemical synthesis [9], enzymatic conversion [10], photochemical synthesis [11], and bench scale purification from either olive leaves [12] or olive wastewater [13]. Other biological methods have also been developed to produce HT [14,15].

Hence, if phenols could be recovered from OMW, that will lead to economic benefits. Several researches have evaluated the feasibility and the economic processes for recovering olive phenols from OMW [16] or from olive mill solid wastes [17]. The main systems proposed to recover the phenols from OMW are: resin chromatography; selective concentration by ultra-filtration (UF) and reverse osmosis (RO); solid–liquid or liquid–liquid extraction with solvents and supercritical fluid extraction [18]. In previous investigations [18], it was reported that among all procedures that were taken for natural antioxidants recovery, liquid–liquid solvent extraction represents a simple and convenient alternative. It was widely used in pilot-scale production and in ultimate commercial recovery [16]. The use of integrated membrane system is becoming another real alternative to recover polyphenols as it is established in some recent works [19,20,21]. The OMW may be treated efficiently using UF, nano-filtration (NF) and/or RO to obtain a permeate fraction which can be discharged safely to the aquatic systems or can be used for irrigation. In this case, NF was employed for the separation of the most part of phenols present in OMW fraction [19]. A membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from OMW was also proposed [19]. This was based on the preliminary MF of the OMW, followed by two UF steps realized with 6 and 1 kDa membranes, respectively, and a final RO treatment [19]. The RO Retentate, containing enriched and purified low molecular weight polyphenols, was proposed for food, pharmaceutical or cosmetic industries while MF and UF Retentate can be used as fertilizers or in the production of biogas in anaerobic reactors [20]. The potentialities of an integrated membrane system for the recovery, purification and concentration of polyphenols from OMW were studied [21]. The proposed system included some well-known membrane operations such as microfiltration (MF) and NF [21].

It has been reported that combination of different membrane technologies was employed for the purpose of reducing the organic load of OMWs. MF was employed to remove the suspended solids [21]. Meanwhile, Khoufi et al. [3] employed the enzymatic hydrolysis successfully to recover phenols from OMW. Such hydrolysis treatments were investigated by using culture broth of *Aspergillus niger* enzyme on wheat bran [3]. One step of ethyl acetate extraction of hydrolyzed OW allowed the recovery of 0.8 g of HT per liter of OW [3]. Mazzei et al. [22] attempted successfully to recover a hydrophobic phenol compound (oleuropein aglycon). They used the combination of both membrane and enzymatic technologies as biocatalytic membrane reactor [22].

The overall goal of the present study is to combine membrane and enzymatic technologies to recover and concentrate valuable phenol compounds from OMWs.

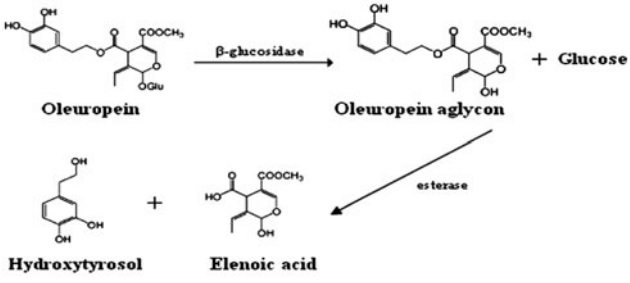


Fig. 1. Oleuropein and its derivatives after enzymatic hydrolysis by β-glycosidase and esterase.

The double advantage is to obtain valuable compounds with promising applications in the pharmaceutical, food and cosmetics industries as well as remediating the wastewater by removing the phytotoxic load. The study employs integrated membrane system, namely MF, UF and NF for the treatment of OMW. Further objective is to examine the β-glucosidase as enzymatic treatment to yield the hydrophobic compound (oleuropein aglycon) out of the highly rich polyphenol Retentate. In addition, acid hydrolysis was carried out in order to release pure low molecular weight phenolic compound with high antioxidant activity (HT).

## 2. Materials and methods

### 2.1. Feed solution

The OMW was delivered by the TTZ-Bremerhaven (Water, Energy and Landscape Management), Germany. The sample came from an evaporation pond of a three phase olive mill plant in Chania region, Greece. The raw OMW was stored in an evaporation pond for four months followed by storing in three closed dark 4,000 l tank for three months. The tank content had been homogenized through pumping. A sample of 25 l was taken in plastic containers and brought to TTZ, where it was kept in a tight dark, container at 4˚C. The physical/chemical characteristics of the OMW before and after treatment were carried out according to APHA [23].

### 2.2. Equipment

#### 2.2.1. Laboratory-scale membrane unit P-28

Laboratory-scale membrane unit P-28 is a universal bench-top unit suited for conducting tests in the fields of MF, UF, and NF systems and RO. The main feature of the unit is the flat sheet membrane cell with a meander-type flow channel for optimum distribution of the mixture that will be separated. The unit consists of a membrane cell, feed tank with heating, pump, pressure indicator as well as pressure control, including safety and discharge valves. The unit is mounted on a stable frame structure. The specification of the unit is summarized in Table 1. The operating parameters of the unit (max. 35 bar, max. 90˚C) allow the tests for the purpose of covering complete range of industrial membrane applications. This enables the cost-effective pre-selection of adequate processes (i.e. membrane technology for industrial application). The schematic diagram of this laboratory-scale membrane plant is illustrated in Fig. 2.

### 2.3. Membrane operations

Treatment of OMW can be summarized and illustrated in (Fig. 3(a) and (b)). All MF, UF and NF experiments were performed by using the pre-described laboratory scale membrane unit.

#### 2.3.1. Microfiltration

OMW was directly subjected to the MF unit without any preliminary centrifugation. The unit was equipped with a MF Nadir MV020 membrane that was made of polyvinyl-fluoride with mean pore size of 200 nm and supplied by Microdyn-NADIR GmbH (Germany). The MF system was operated at a TMP of 1 bar and a temperature of 20 ± 0.01˚C.

The permeate of the MF was divided into two equal portions. The first portion was further treated with UF. The second portion was subjected to the NF treatment system for correlation.

#### 2.3.2. Ultra-filtration

The UF system is a laboratory-scale membrane unit that is equipped with membrane module as follow: (membrane made of ceramic with nominal molecular weight cut-off 20 kD). UF experiments were carried out at an operating temperature of 20 ± 0.01˚C and a TMP of about 8 bars.

#### 2.3.3. Nano-filtration

The NF unit was equipped with a DMS DK membrane (membrane material composite, nominal molecular weight cut-off 150–300 Da) supplied by DMS GmbH. NF experiments were carried out at an operating temperature of 20 ± 0.01˚C and a TMP of 8 bars.

All membranes; namely MF, UF and NF; were cleaned after operation using concentrated basic solution of 0.5% strength cleaning solution (P3-ultrasil 53).

### 2.4. Enzymatic hydrolysis using β-glucosidase

For Enzymatic hydrolysis: 2.5 ml of the NF Retentate was dissolved in mixture of 40 ml acetate buffer and 4 ml of Dimethyl sulfoxide in a round-bottomed flask. The hydrolysis was initiated by the addition of β-glucosidase enzyme to the mixture and incubated at 25˚C for 24 h with stirring. Due to the resistivity of enzyme at high speed, the stirring was conducted at slow speed. This experiment was carried out in duplicate. The blank was also prepared in duplicate with distilled water and enzyme. The

Table 1
Specification of the laboratory-scale membrane unit

| | |
|---|---|
| *Membrane* | |
| Membrane disk | 75 mm outside diameter |
| Active membrane surface | 28 $cm^2$ |
| *Parameters* | |
| Maximum filling volume | 500 ml |
| Maximum operating pressure | 35 bar g |
| Maximum operating temperature | 90°C |
| Maximum pump delivery rate | 1.8 l/min |
| Operating < 8 bar | Setting pressure control valve |
| Operating > 8 bar | Nitrogen blanketing |

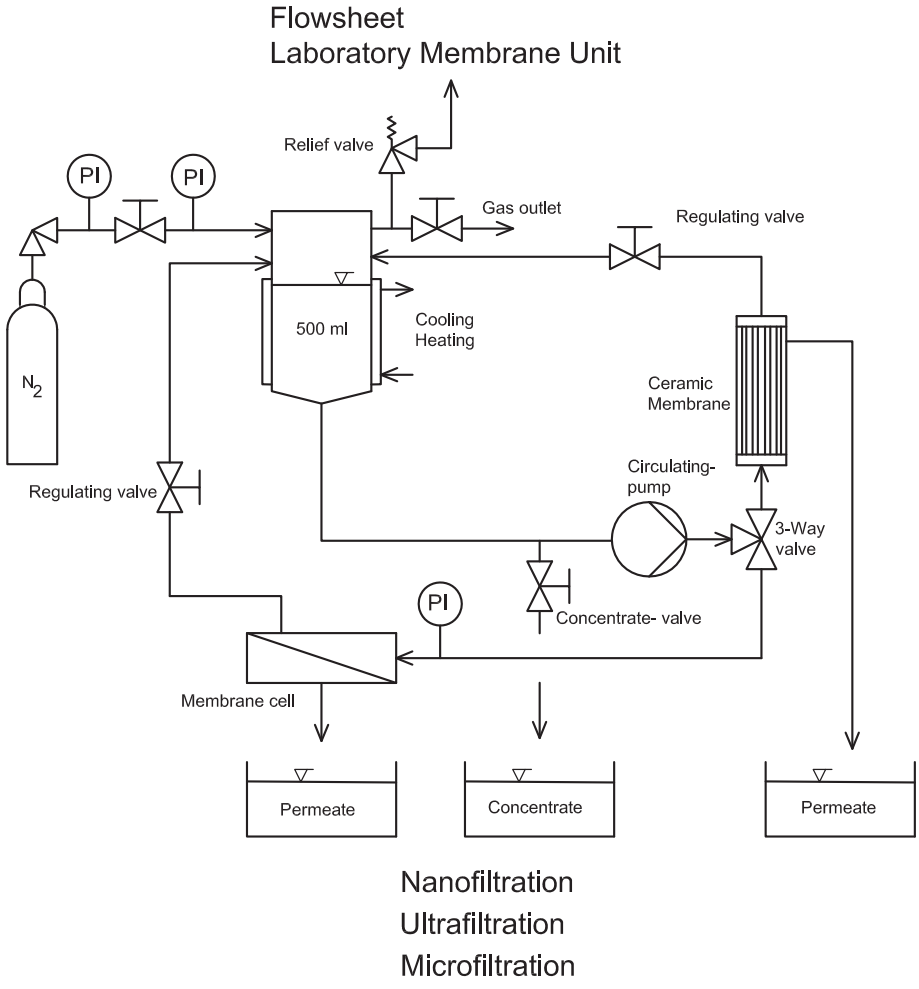


Fig. 2. Schematic diagram for laboratory-scale membrane plant.

hydrophobic fraction was extracted by separating funnel using tetra-butyl methyl ether. For this purpose, 10 ml tetra-butyl methyl ether was added to the mixture followed by vigorous shaking for 10 min to achieve the equilibrium state. The mixture was then allowed to settle for 1 h. Two phases were separated and the extraction process was repeated successively twice. Tetra-butyl methyl ether was subsequently removed by evaporation. Dry residue was obtained.

### 2.5. Acid hydrolysis

In order to obtain HT rich extract: the resulted dry residue from enzymatic hydrolysis was dissolved in

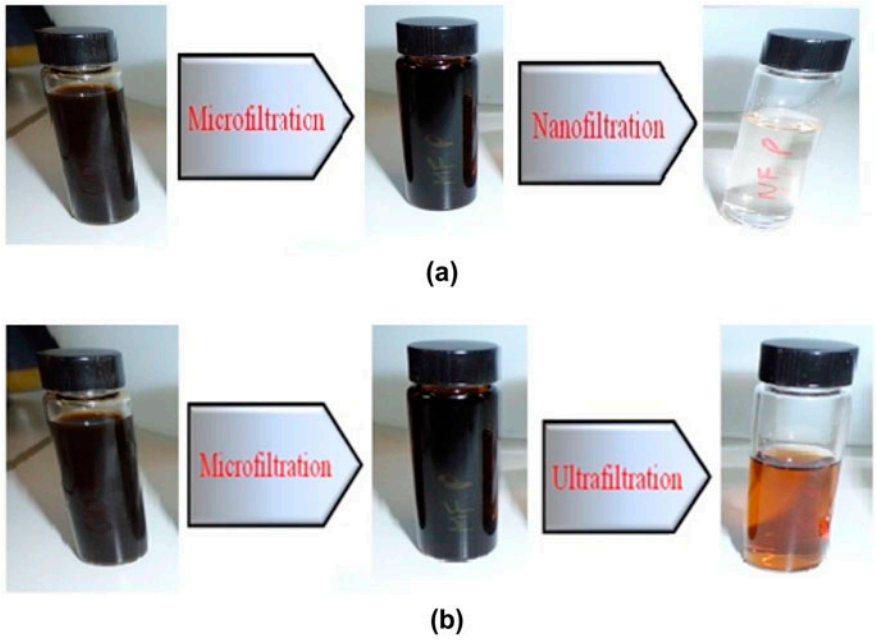


Fig. 3. Filtration of raw mill wastewater by: (a) MF followed by NF (b) MF followed by UF.

10 ml of MeOH/$H_2O$ mixture in a sealed vial. The solution was hydrolyzed at 100˚C for 1 h using 5 ml of HCl (2 M). The sample was left 1 h for cooling, and then diluted with 10 ml water. The hydrophobic fraction was extracted by a separating funnel for three successive times with 25 ml of ethyl acetate. The later was subsequently removed by evaporation. Identification, quantification of HT was monitoring by HPLC.

### 2.6. Chemicals

The chemicals used in this study are: Folin Ciocalteu-phenol reagent (Merck), acetone 99%, methanol, hydrochloric acid and tannic acid 99.5% (Sigma-Aldrich). The β-glucosidase from almond (lyophilized powder, one unit liberates 1.0 μ mole of glucose from salicin per min at pH 5.0 and 37˚C) was obtained from Sigma-Aldrich. Acetic acid and sodium acetate (Sigma-Aldrich) were used to prepare 80 mM acetate buffer at pH 6.6.

### 2.7. Analytical methods

The pH, total suspended solids (TSS) and the chemical oxygen demand (COD) were determined in all samples before and after operation according to the Standard Methods [23]. The polyphenols were determined by photometric method [24].

HPLC analysis was performed using an Agilent technologies series 1200 system equipped with an automatic injector, a column oven, a diode array UV detector at 278 nm and Agilent Eclipse XDB-$C_{18}$ RRLC column, 50 × 4.6 mm id (1.8 μm pore size). The mobile phase consisted of (A) 0.1% formic acid aqueous solution and (B) acetonitrile. The solvent program was initially 2 min isocratic with 90% A and 10% B, then from 2 to 5 min linear gradient to 30% B, finally at 6.5 min linear gradient to 10% B and re-equilibrium for additional 1.5 min for subsequent analysis. The flow rate was 2.0 ml/min. The injection volume was 5 μl. The column temperature was set at 30˚C.

Table 2
Characteristics of the raw OMW

| Parameters | *N* | Value ± RSD (%) |
|---|---|---|
| pH | 3 | 5.6 ± 0.12 |
| EC (mS) | 3 | 15.16 ± **1.04** |
| TSS (mg/l) | 3 | 3,800 ± 0.9 |
| COD (g/l) | 3 | 56 ± 1.1 |
| COD dissolved (g/l) | 3 | 46.7 ± **0.98** |
| Polyphenols (g/l) | 3 | 2.50 ± 0.86 |

Notes: *N* = number of measurement.
RSD (%) = Relative standard deviation (%).

## 3. Results and discussion

The characteristics of the raw OMW are given in Table 2. The level of EC, TSS and COD indicates high concentration namely 15.16 ms, 3,800 mg/l and 67.3 g/l, respectively. The pH was slightly acidic and the polyphenols was 2.5 g/l.

### 3.1. MF system

By subjecting the OMW to MF, the initial permeate flux of 0.77 l/m$^2$ h was reduced by the end of the experiment to about 44.4%, the time-course of permeate fluxes during the entire experiment is presented in Table 3. The outlet (permeate) characteristics is given in Table 4. Basically, pH was slightly changed during the overall process. The MF achieved 77.6 and 34.8% reduction in the TSS and COD, respectively (Figs. 4 and 5). Only 10% of the polyphenols was removed (i.e. permeate contained 90% of polyphenols).

### 3.2. UF system

Permeate of MF that is rich in the polyphenols was subjected to UF. The initial permeate flux of 6.1 l/m$^2$ h was reduced to about 51% by the end of the experiment (time-course is presented in Table 3). The removal rate of the TSS, COD and the polyphenols in permeate was increased to 100, 44.9 and 37.8%, respectively, Table 4. The TSS and COD decreased in the permeate from 850 to 120 mg/l, from 36.5 to 20.1 g/l, respectively Table 3. The polyphenols decrease in the permeate from 2.25 to 1.40 g/l, where the difference was concentrated into a smaller volume of the Retentate (Fig. 6). The permeate solution was not clear yet indicating the presence of polyphenols in reasonable amount (Fig. 3).

### 3.3. NF system

When the MF permeate was subjected to the NF, the initial permeate flux of about 0.21 l/m$^2$ h was reduced to about 60% by the end of the experiment (time-course is presented in Table 3). High removal rate was obtained for TSS, COD and polyphenols; namely 100, 81.1 and 55.6%, respectively, Table 4. The outlet (permeate) of the NF parameters reached 6.9 g/l for COD and zero for TSS (Figs. 4 and 5). The polyphenols decreased in the permeate from 2.25 g/l in the inlet to 1.0 g/l in the outlet (permeate) (Fig. 6). The difference was concentrated into a smaller volume of the Retentate. The permeate solution became clear indicating high removal of the polyphenol compounds (Fig. 3).

In this respect, Garcia-Castello, et al. [21] reported that NF Permeate can be further treated via RO to obtain extra amount of the polyphenols.

### 3.4. Recovering of polyphenols

Polyphenols were detected in the Retentate in all the studied membrane filters and the results are given in Table 5. The polyphenols concentration in the Retentate of MF, UF and NF was 2.3, 2.0 and 2.4 g/l, respectively. Almost all polyphenols were recovered and concentrated in the NF Retentate solution. HPLC was employed for monitoring the simple phenolic compounds that were recovered through NF process (Fig. 7). The HPLC chromatograms (Fig. 7) indicate that the polyphenol compounds are: gallic acid, HT, protocatechuic acid, tyrosol, p-coumaric acid and oleuropein. However, the highest detected compound was oleuropein (Fig. 7).

### 3.5. Enzymatic and acid hydrolysis

Further study was carried out using the enzymatic hydrolysis followed by acid hydrolysis for the purpose of recovering and concentrating the polyphenols out of the Retentate. Therefore, β-glucosidase was employed for enzymatic hydrolysis of the NF Retentate that is highly concentrated with polyphenols. In this respect, the enzyme breaks the bond between oleuropein and glucose to yield oleuropein aglycon. The lipophilic molecule oleuropein aglycon was extracted into the organic phase while the hydrophilic

Table 3
Time course of the permeate flux

| | Permeate flux Jw (l/m$^2$ h bar) | | | |
|---|---|---|---|---|
| Filtration system | Initial flux | After 10 min | After 20 min | After 30 min (final flux) |
| Micro | 0.77 | 0.70 | 0.6 | 0.45 |
| Ultra | 6.1 | 5.0 | 4.9 | 3.1 |
| Nano | 0.21 | 0.15 | 0.13 | 0.084 |

Table 4
Characteristics of the raw OMWs, after MF, UF and NF membrane filtration

| Sample | N | pH ± RSD (%) | COD | | TSS | | Polyphenols | |
|---|---|---|---|---|---|---|---|---|
| | | | g/l ± RSD (%) | %R | mg/l ± RSD (%) | %R | g/l ± RSD (%) | %R |
| Raw OMW | 3 | 5.6 ± 0.12 | 56 ± 1.1 | – | 3,800 ± 0.9 | – | 2.50 ± 0.86 | – |
| MF permeate | 3 | 5.7 ± 0.21 | 36.5 ± 0.85 | 34.8 | 850 ± 0.78 | 77.6 | 2.25 ± 0.68 | 10 |
| UF permeate | 3 | 6.2 ± 0.16 | 20.1 ± 0.59 | 44.9 | 120 ± 0.54 | 85.9 | 1.40 ± 0.37 | 37.8 |
| NF permeate | 3 | 6.5 ± 0.19 | 6.9 ± 0.28 | 81.1 | 0 | 100.0 | 1.00 ± 0.21 | 55.6 |

Notes: OMW = olive mill wastewater, %R = percentage of removal, MF = micro filtration, UF = ultra filtration, NF = nano filtration relative, RSD (%) = relative standard deviation (%).

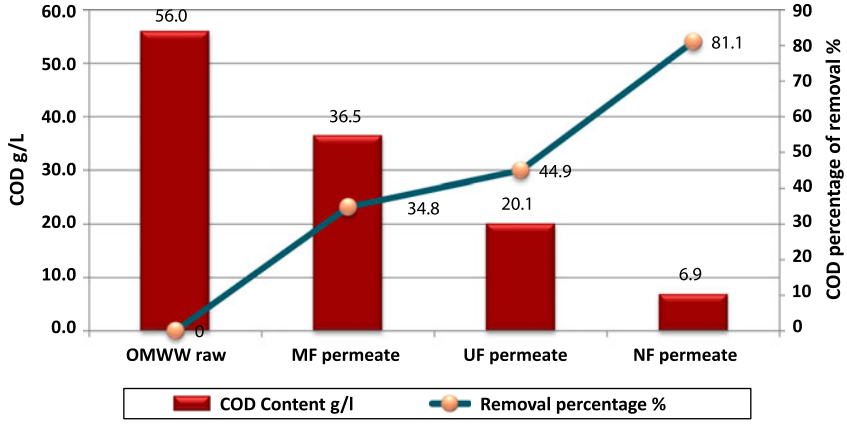


Fig. 4. Level of COD and percentage of removal via different membrane filters.

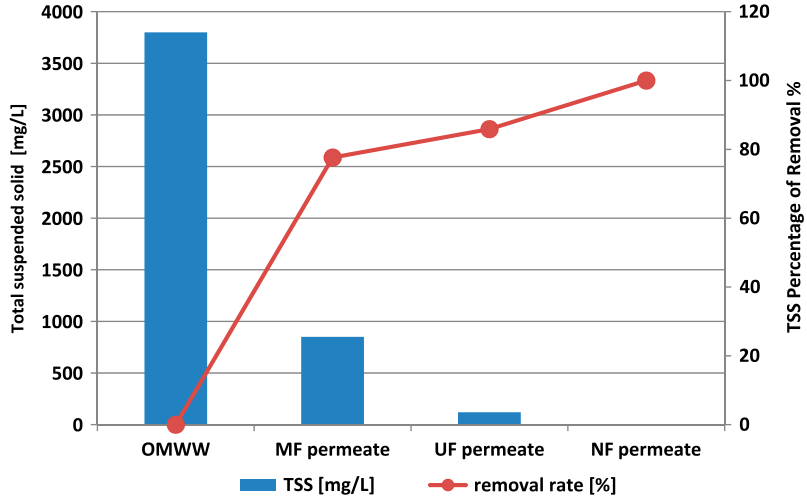


Fig. 5. Level of TSS and percentage of removal via different membrane filters.

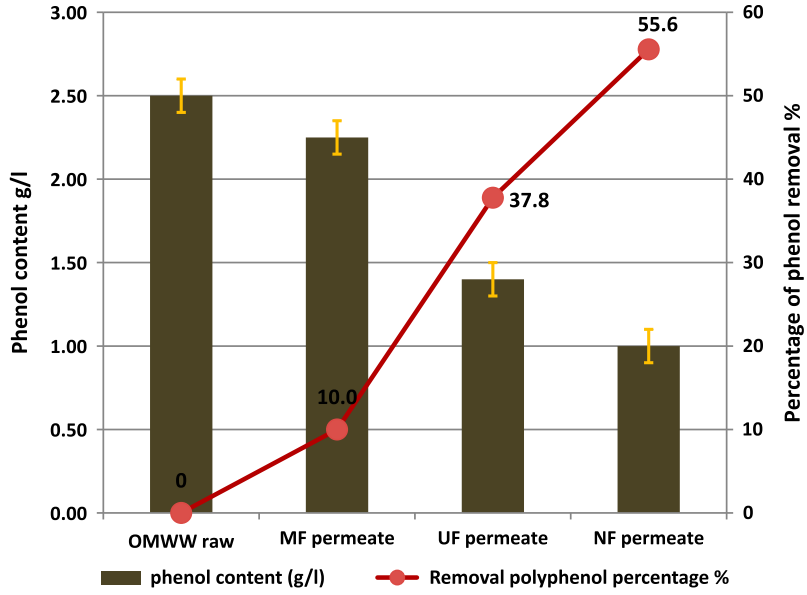


Fig. 6. Level of polyphenol content and percentage of removal via different membrane filters.

Table 5
Polyphenols recovery from OMWs processed by different membrane filters

| Sample | *N* | Polyphenols (g/l) ± RSD (%) |
|---|---|---|
| Feed | 3 | 2.50 ± 0.86 |
| Retentate MF | 3 | 2.30 ± 0.56 |
| Retentate UF | 3 | 2.00 ± 0.04 |
| Retentate NF | 3 | 2.40 ± 0.10 |

Notes: MF = micro filtration, UF = ultra filtration, NF = nano filtration, *N* = number of measurement, RSD (%) = relative standard deviation (%).

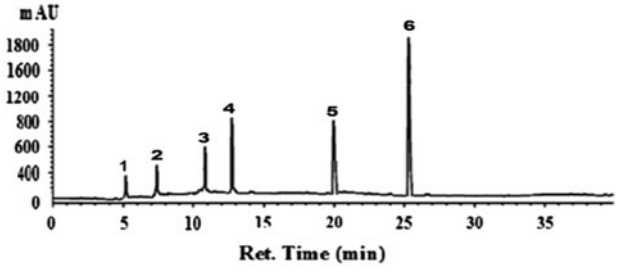


Fig. 7. HPLC chromatograms of the polyphenols in NF Retentate, (1) gallic acid; (2) hydroxytyrosol; (3) protocatechuicacid; (4) tyrosol; (5) p-coumaric acid; (6) oleuropein.

components (i.e. glucose and other polyphenols) were present in water phase. The process exhibits the possibility to extract the available oleuropein aglycon in a solvent to be used as a source of pure HT.

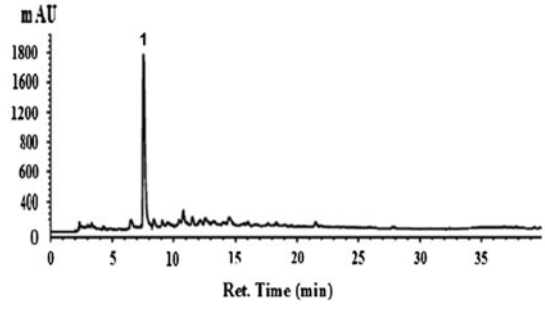


Fig. 8. HPLC chromatograms for enzymatic hydrolysis of NF Retentate: (1) HT.

Acid hydrolysis of oleuropein aglycon was carried out for the purpose of extracting pure HT. In this respect, acid hydrolysis induced breakdown of the more complex phenolic molecule (oleuropein aglycon) to HT. The HPLC profile (Fig. 8) showed that HT was the main compound in the hydrolysis extract.

HPLC standard curve related to HT standard was employed for calculating the amount of HT released via enzymatic and acid hydrolysis. The calculated amount was found to be 1.3 g/l which represents 54.2% of original value in the Retentate.

## 4. Conclusion

The overall results reveal that MF was able to remove 34.8% of the COD, 77.6% of the TSS and 10% of the polyphenols. Further treatment by UF increased

the total removal to 64.1, 96.8 and 44.0% of COD, TSS and polyphenols, respectively. By employing NF, the removal efficiency reached 87.7, 100 and 60.0%, respectively.

The polyphenols was concentrated in the Retentate of MF, UF and NF to 2.3, 2.0 and 2.4 g/l, respectively. Oleuropein was the major phenolic compound that was detected in NF Retentate. When oleuropein was subjected to hydrolysis reaction large quantities of HT was obtained. By employing β-glucosidase enzymatic hydrolysis followed by acid hydrolysis, 1.3 g/l of HT was released representing 54.2% of original value in the Retentate. This study demonstrates that the hydrolysis of NF Retentate is a potential source of the bioactive phenolic compounds with promising applications in food and pharmaceutical industries.

The present study demonstrates a successful method for the treatment OMW for the purpose of recovering and concentrating the polyphenols that can offset the cost of WW treatment with the profit made from the commercialization of valuable bioactive molecules. This could improve the economic stability of olive mills, convert a problematic waste into a resource, and encourage environmental best practices in the wastewater treatment sector as well as in the food and pharmaceutical sectors.

## Recommendation

It is recommended that the permeate of NF can be further treated via RO to recover extra amount of the polyphenols. At this point the treated permeate can be reused safely for irrigation.

## Acknowledgement

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## References

[1] E. De Marco, M. Savarese, A. Paduano, R. Sacchi, Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters, Food Chem. 104 (2007) 858–867.

[2] H.K. Obied, M.S. Allen, D.R. Bedgood, P.D. Prenzler, K. Robards, R. Stockmann, Bioactivity and analysis of biophenols recovered from olive mill waste, J. Agric. Food. Chem. 53 (2005) 823–837.

[3] S. Khoufi, M. Hamza, S. Sayadi, Enzymatic hydrolysis of olive wastewater for hydroxytyrosol enrichment, Bioresour. Technol. 102 (2011) 9050–9058.

[4] M. Servili, S. Esposto, R. Fabiani, S. Urbani, A. Taticchi, F. Mariucci, R. Selvaggini, G.F. Montedoro, Phenolic compounds in olive oil: Antioxidant, health and organoleptic activities according to their chemical structure, Inflammopharmacology 17 (2009) 76–84.

[5] A. Bendini, L. Cerretani, A. Carrasco-Pancorbo, A.M. Gómez-Caravaca, A. Segura-Carretero, A. Fernández-Gutiérrez, G. Lercker, Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade Alessandra, Molecules 12 (2007) 1679–1719.

[6] V. Ziogas, G. Tanou, A. Molassiotis, G. Diamantidis, M. Vasilakakis, Antioxidant and free radical-scavenging activities of phenolic extracts of olive fruits, Food Chem. 120 (2010) 1097–1103.

[7] V. Micol, N. Caturla, L. Perezfons, V. Mas, L. Perez, A. Estepa, The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV), Antiviral Res. 66(2–3) (2005) 129–136.

[8] K. Yamada, H. Ogawa, A. Hara, Y. Yoshida, Y. Yonezawa, K. Karibe, V.B. Nghia, H. Yoshimura, Y. Yamamoto, M. Yamada, K. Nakamura, K. Imai, Mechanism of the antiviral effect of hydroxytyrosol on influenza virus appears to involve morphological change of the virus, Antiviral Res. 83(1) (2009) 35–44.

[9] K.L. Tuck, H.W. Tan, P.J. Hayball, Synthesis of tritium-labeled hydroxytyrosol, a phenolic compound found in olive oil, J. Agric. Food. Chem. 48(9) (2000) 4087–4090.

[10] R. Briante, F. La Cara, F. Febbraio, R. Barone, G. Piccialli, R. Carolla, P. Mainolfi, L. De Napoli, M. Patumi, G. Fontanazza, R. Nucci, Hydrolysis of oleuropein by recombinant β-glycosidase from hyperthermophilic archaeon *Sulfolobus* solfataricus immobilised on chitosan matrix, J. Biotechnol. 77 (2000) 275–286.

[11] S. Azabou, W. Najjar, A. Ghorbel, S. Sayadi, Mild photochemical synthesis of the antioxidant hydroxytyrosol via conversion of Tyrosol, J. Agric. Food. Chem. 55 (2007) 4877–4882.

[12] O.-H. Lee, B.-Y. Lee, Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract, Bioresour. Technol. 101(10) (2010) 3751–3754.

[13] C. Savournin, B. Baghdikian, R. Elias, F. Dargouth-Kesraoui, K. Boukef, G. Balansard, Rapid high-performance liquid chromatography analysis for the quantitative determination of oleuropein in *Olea europaea* leaves, J. Agric. Food. Chem. 49(2) (2001) 618–621.

[14] S.J. Brooks, E.M. Doyle, K.E. O'Connor, Tyrosol to hydroxytyrosol biotransformation by immobilised cell extracts of *Pseudomonas putida* F6, Enzyme Microb. Technol. 39 (2006) 191–196.

[15] Z. Bouallagui, S. Sayadi, Production of high hydroxytyrosol yields via tyrosol conversion by *Pseudomonas aeruginosa* immobilized resting cells, J. Agric. Food. Chem. 54 (2006) 9906–9911.

[16] M.A.T. Reis, O.M.F. De Freitas, L.M. Ferreira, J.M.R. Carvalho, Extraction of 2-(4-hydroxyphenyl)ethanol from aqueous solution by emulsion liquid membranes, J. Membr. Sci. 269 (2006) 161–170.

[17] G. Rodriguez, A. Lama, R. Rodriguez, A. Jiménez, R. Guillén, J. Fernández-Bolaños, Olive stone an attractive source of bioactive and valuable compounds, Bioresour. Technol. 99(13) (2008) 5261–5269.
[18] M.J. Gonzalez-Munoz, S. Luque, J.R. Alvarez, J. Coca, Recovery of phenol from aqueous solutions using hollow fibre contactors, J. Membr. Sci. 213 (2003) 181–193.
[19] C.A. Paraskeva, V.G. Papadakis, E. Tsarouchi, D.G. Kanellopoulou, P.G. Koutsoukos, Membrane processing for olive mill wastewater fractionation, Desalination 213 (2007) 218–229.
[20] C. Russo, A new membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from vegetation waters (VW), J. Membr. Sci. 288 (2007) 239–246.
[21] E. Garcia-Castello, A. Cassano, A. Criscuoli, C. Conidi, E. Drioli, Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system, Water Res. 44 (2010) 3883–3892.
[22] R. Mazzei, E. Drioli, L. Giorno, Biocatalytic membrane reactor and membrane emulsification concepts combined in a single unit to assist production and separation of water unstable reaction products, J. Membr. Sci. 352 (2010) 166–172.
[23] APHA, AWWA and WEF, Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, Washington, DC, 2005.
[24] A. Carrasco-Pancorbo, L. Cerretani, A. Bendini, A. Segura-Carretero, T. Gallina-Toschi, A. Fernandez-Gutierrez, Analytical determination of polyphenols in olive oils, J. Sep. Sci. 28 (2005) 837–858.