1 Recovery of Polyphenols from **Olive Mill Wastewater** using Drowning-out 2 Crystallization based Separation Process

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Abstract

Olive oil extraction generates a large amount of olive mill wastewater (OMW) (10 million ton/year), requiring specific management to minimize its potential negative impact on the environment. However, OMW is a rich source of polyphenols having a wide range of bioactivities. For that reason, the selective recovery of polyphenols from OMW provides the double opportunity to obtain bioactive compounds and to reduce its phytotoxicity.

The present study relates to a highly efficient and novel method, using green technology, for obtaining a natural bioactive concentrate rich in polyphenols from OMW. The clean technology integrate centrifugation, batch evaporation and drowning-out crystallization-based separation process, for the separation of polyphenols from the different components present in OMW, based on the solubility behaviour changing after addition of ethanol, accepted as food grade. Further, batch evaporation was applied for the regeneration of ethanol for sustainable process. Consequently, the proposed method provides a highly-concentrated polyphenols isolate (up to 75% (w/w)), with up to (510 mg/g) of the polyphenols content being hydroxytyrosol. The recovered polyphenols can be of interest for the food industry, cosmetic industry, or pharmaceutical industry.

Keywords: Drowning-out, Olive mill wastewater, Polyphenols, Concentration, Recovery efficiency.

Chemical compounds studied in this article

Oleuropein (PubChem CID: 5281544); Hydroxytyrosol (PubChem CID: 82755); Gallic acid (PubChem CID: 370); Tyrosol (PubChem CID: 10393); *p*-Hydroxyphenyl acetic acid (PubChem CID: 127); Raffinose (PubChem CID: 439242); Maltose (PubChem CID: 6255);

Mannitol (PubChem CID: 6251); Glucose (PubChem CID: 5793); Xylose (PubChem CID: 135191)

1. Introduction

Valorization of food processing by-products is a challenging opportunity for the sustainable and competitive development of several relevant industrial sectors. One of the examples is olive mill wastewater (OMW), which represents serious problems for the olive oil manufacturing industry, due to their toxicity and high volumes processed (Ntaikou et al., 2009). Instead of causing serious environmental problems, OMW could represent an appreciable source of potentially valuable molecules.

Olive polyphenols have been identified as potent natural antioxidants, and have been proven as very effective in the prevention of various diseases (Bertin et al., 2011; Widmer et al., 2013). Consequently, a number of reports have appeared in the literature endeavoring the recovery of olive polyphenols with the aim to reduce its toxicity (Justino et al., 2010; Khoufi et al., 2009) and recover its polyphenols yields (Galanakis et al., 2010; Cerretani et al., 2005).

Solvent extraction is the most widely used technique to recover polyphenols from OMW despite its high cost resulting from the requirement of large amounts of organic solvents. A large number of solvents have been used including methanol (Cardoso et al., 2005), ethyl acetate (Allouche et al., 2004), and less commonly n-butanol, propanol, and tert-butyl methyl ether. High yield (85.5%) recovery of hydroxytyrosol from OMW has been achieved using a three-stage continuous counter-current liquid-liquid extraction unit (Allouche et al., 2004).

The disadvantages of organic solvents, such as flammability and toxicity, have recently been overcome using supercritical fluids extraction, notably, supercritical CO₂. Thus, following the method described by Lafka et al., (2011), OMW's polyphenols have been extracted using CO₂ supercritical fluid. In terms of yield, supercritical CO₂ was found to be a

1 more efficient solvent than ethyl acetate, but it was surpassed by polar solvents (methanol,
2 ethanol, *n*-propanol).

3 In the same manner, membrane separation technology has been developed to avoid the
4 use of solvents during extraction and concentration. Garcia-Castello et al., (2010) used an
5 integrated membrane system to recover and concentrate polyphenols from OMW. In this
6 case, 78% of polyphenols was recovered in the permeate stream. Membrane separation
7 technology is generally assumed as being safe and cheap. However, the cost of the process is
8 governed directly by fouling and restrictions in the cleaning procedure specially in the case of
9 OMW containing high fouling materials (Tsagaraki and Lazarides, 2012).

10 Chromatographic separation is generally considered to be the most effective for the
11 recovery of polyphenols. For instance, the recovery yield was 60% by using Amberlite
12 XAD16 resin as the adsorbent and ethanol as the biocompatible desorbing phase (Scoma et
13 al., 2011). Considering the study conducted by Ferri et al., (2011), the highest polyphenols
14 recovery (76%) was achieved using IRA96 polar resin.

15 While all the above-mentioned separation processes often depend on several property
16 differences for their overall success (distribution between immiscible liquid phases in case of
17 solvent extraction, surface sorption in case of chromatography, molecular size and diffusivity
18 in case of membrane separation technologies). Solubility is a particularly important property
19 for creating separation of various organic compounds specially used as main property
20 difference during crystallization process (Chiou, 2012). Solubility can be modified by the
21 presence of miscible solvent in the solution, which changes the physical and chemical
22 properties of solvent. Indeed, this process is often referred in the literature as drowning-out
23 crystallization-based separation process (Berry et al., 1997; Holmbäck and Rasmuson, 1999;
24 Kim et al., 2012). Drowning-out process is widely used for the separation of pharmaceutical
25 products and biomaterials in industrial processes (Myerson, 2002). Unlike other

crystallization processes, drowning-out process is particularly suitable for the separation of heat sensitive component because crystallization can often occur at or near ambient temperature (Berry et al., 1997). Moreover, this process allows the achievement of high product yields in narrow ranges of temperature. It also enables the control of impurities in the product (Berry et al., 1997).

For our knowledge, there are no studies referring to the application of this process for the recovery of polyphenols from OMW. Ethanol possesses various advantages: it is cheap, recoverable, as well as nontoxic, and the corresponding extracts could be utilized directly in beverages, foods, and cosmetics (Galanakis et al. 2010).

In this work, we have proposed to investigate drowning-out crystallization-based separation process for the recovery of polyphenols from OMW. For that purpose, model solutions of organic compounds and real OMW were treated in hydro-ethanolic mixtures, and the effect of process parameters was studied by monitoring the recovery and concentration of polyphenols extract. This information was then used to propose a sustainable recovery of polyphenols from OMW.

2. Material and methods

2.1. Chemicals

Caffeic acid ($C_9H_8O_4$, MW=180.15, >98% pure), gallic acid ($C_7H_6O_5$, MW=170.12, >98% pure), tyrosol ($C_8H_{10}O_2$, MW=138.16, >98% pure), oleuropein ($C_{25}H_{32}O_{13}$, MW=540.51, >80% pure), *p*-hydroxyphenyl acetic acid (*p*-HPA) ($C_8H_8O_3$, MW=180.15, >98% pure), raffinose ($C_{18}H_{32}O_{16}$, MW=504.43, >80% pure), mannitol ($C_6H_{14}O_6$, MW=82.17, >98% pure), glucose ($C_6H_{12}O_6$, MW=180.15, >98% pure), xylose ($C_5H_{10}O_5$, MW= 150.13, >98% pure), maltose ($C_{12}H_{22}O_{11}$, MW=342.3, >98% pure), potassium chloride

(KCl, MW=74.55, >99% pure) and quercetine ($C_8H_{16}O_2$, MW=302.23, >98% pure) were purchased from Wako (Japan).

2.2. Solvent selection

Various desired properties (miscibility, solubility, molecular structure, economic, and toxicity) were considered to determine the appropriate solvent for drowning-out separation process. **Table 1** lists the tested solvents, molecular structure, relative permittivity, dipole moment, polarity constant, water solubility and the viscosity of the solvents. Most solvent were selected as it was economical, safe, and common.

2.3. Solubility experimental procedure

Basic experimental arrangement for solubility measurement is given in **Fig. 1**. For the solubility measurements: distilled water (conductivity around 1.5 μ S/cm) and ethanol of analytical grade (C_2H_6O , 95% pure) was supplied by Wako (Japan).

The solubilities in the different model solutions were determined according to Smith and Vaz, (1993).

Ethanol (95%) and water mixtures were prepared by weighing the desired amount of each solvent using an analytical balance (UX620H Shimadzu) with an accuracy of ± 0.001 mg. An excess of solid compound was added to the liquid phase, and the solution was continuously stirred with a magnetic stirrer (700 rpm) to reach equilibrium. Several equilibrium times were tested (from 6 to 48 h), and 24 h was found suitable to reach liquid-solid equilibrium for the tested compounds. To analyze solubility, a sample from the liquid phase was filtered using a 0.2 μ m pore syringe filter (cellulose acetate) and the concentration was measured using HPLC. Two independent experiments were realized to determine the solubility. In the case of polyphenols, HPLC (JASCO, Japan) was used, composed of a PU-2089 plus pump and an UV-2075 plus UV/Visible detector. The column was a C-18 (4.6×150 mm; Shim-pack VP-ODS), and its temperature was maintained at 40°C. The

mobile phase used was 0.1% phosphoric acid in milli-Q water (A) versus 70% acetonitrile in water (B) for a total running time of 40 min, and the following proportions of solvent B were used for the elution: 5–25 min, 0–40%; 25–35 min, 25%; and 35–40 min, 40–50%. The flow rate was 0.5 mL/min, and the injection volume was 20 µL. To analyze saccharides and organic acid solutes, we used another HPLC system JASCO equipped with a pump (PU-2080) and a refractometer detector (model RI-2031, JASCO). The column was Shodex SUGAR KS-804 (300 × 8.0 mm) from Denko America, Inc. (New York), and the temperature was maintained at 50°C. The mobile phase used was distilled water, and the flow rate was 1 mL/min.

The solubility of solute (x_a) was expressed as the influence of ethanol mass fraction defined as:

$$x_a = \frac{\text{mass of dissolved solute}}{\text{mass of ethanol} + \text{mass of water} + \text{mass of dissolved solute}} \quad (1)$$

2.4. Characterization of OMW

OMW sample was collected from an olive oil producing plant located in Sfax (southern Tunisia), during November 2012. A three-phase continuous cycle modular machinery (Pieralisi system, Italy) was used for extracting olive oil in situ. The composition of OMW sample is shown in **Table 2**. The samples were stored frozen at (-20°C) in dark prior to analysis, in order to keep its chemical composition.

A conductivity meter (CM-117S model, Kyoto Electronics) was used to measure the conductivity of the samples. A pH-meter (Metrohm, model 827 pH lab) was used to measure pH of the sample. An electronic densitometer (KEM Co., DA-130 N, Japan) was used to measure the density of the samples.

Total dissolved solids (TDS) were determined by drying in aluminum pans at 105°C for 24 h, the amount of TDS in (g/L) was calculated as follows:

$$\text{TDS} = (W_2 - W_1) \times 100 / V \quad (2)$$

1 where

2 W_1 : mass of empty pans [g]

3 W_2 : mass of filled pans after drying at 105°C [g]

4 V : volume of sample [L]

5 **2.5. Total polyphenol content**

6 Total polyphenol (TP) contents were analyzed using the Folin-Ciocalteu assay according
7 to the method of Singleton and Rossi (1965). Absorbance was measured at 725 nm by a V-
8 530 UV/VIS spectrophotometer (JASCO, Japan).

9 **2.6. Total saccharides content**

10 The method of Dubois et al. (1956) was used to determine the total saccharides content
11 in the sample.

12 **2.7. Pre-treatment of OMW**

13 As pretreatment, OMW sample was submitted to centrifugation operation at 6,000×g,
14 and 5°C to reduce suspended solids and colloidal material using Beckman Coulter preparative
15 centrifuge Avanti-J25 (Brea, California). Pre-treated OMW sample was concentrated using
16 rotary evaporator with following conditions: operating pressure: 0.15 bar; temperature, 42°C;
17 time, 60 min. Rotary evaporator was also used for the separation of ethanol, after the
18 drowning-out process; the condenser and reboiler temperature was 10 and 45°C, respectively;
19 the operating pressure was 0.15 bar. Batch distillation column was used for the regeneration
20 of ethanol (95%) in order to be further used in separation batches.

21 **2.9. Drowning-out procedure**

22 Drowning-out separation consisted of two steps: mixing and crystallization, for the
23 separation based on the solubility behaviour changes, after addition of ethanol. These
24 operations occur at ambient temperature. The liquid phase obtained was then submitted to
25 batch distillation for the regeneration of ethanol (Berry et al. 1997).

A series of experiments was carried out, in batch mode, in order to study the influence of ethanol mass fraction on **polyphenol concentration**. In these experiments, **polyphenol concentration** was kept constant at 1.83 g/L, while the ethanol mass fraction in the system was varied. OMW was submitted to centrifugation step using Beckman centrifuge at 6000×g, at room temperature. Liquid supernatant was then analyzed quantitatively for total polyphenols and total dissolved solids. Subsequently, **polyphenol concentration** was determined by the following expression:

$$\text{Polyphenol concentration} = (C_1/C_2) \times 100 \quad (3)$$

where C_1 and C_2 are the concentrations of TP and TDS, respectively, given with the same unit (g/L).

2.10. Analysis of crystal morphology

To investigate the structure and morphology of crystals in the solid phase, samples were withdrawn from the slurry at a desired crystallization time. The crystals were filtered and dried for 24 h at 50°C for analysis. The surface morphology of the agglomerated crystals was analyzed using a scanning electron microscope, miniscope, TM-1000 (Hitachi, Japan).

2. Results and discussion

3.1. Solubility of model solutions in (ethanol + water) mixtures

The application of drowning-out process requires knowledge of solid-liquid equilibrium for model organic compounds present in OMW. To make clear the effect of ethanol on **polyphenol concentration**, solubility of organic compounds in pure and mixed solvents plays an important role in their separation industrial, pharmaceutical, and environmental applications (Hamdi, 1993). In some recent works, the solubility of polyphenols in several pure solvents have been measured (Queimada et al., 2009; Cháfer et al., 2007; Mota et al., 2008).

Model solutions of six polyphenols with different characteristics (tyrosol, oleuropein, quercetine, caffeic acid, gallic acid and *p*-HPA), monosaccharides (glucose and xylose), disaccharide (maltose) and trisaccharide (raffinose), mannitol and potassium chloride (KCl), generally present in OMW, were studied in this work at $T = 25^{\circ}\text{C}$ using different (ethanol + water) mixtures (**Fig. 2**).

Gallic acid is a naturally occurring phenolic compound present in OMW (Eusébio et al., 2007). The experimental data which present the isothermal liquid solid equilibrium showed an opposite effect of ethanol addition on the solubility of gallic acid and raffinose (**Fig. 3**). The saccharides model molecule (raffinose) was drastically affected by ethanol addition; the solubility of raffinose in water was 17%, and decreased to 0.05% in the ethanol. In the other hand, in case of gallic acid, the model molecule for polyphenols, was more solubilized by the addition of ethanol. The solubility in water was 0.77%, and that in ethanol was 20%.

From **Fig. 2**, results showed a decrease in the solubility of saccharides (glucose, xylose, maltose, raffinose), salts (potassium chloride) and polyalcohols (mannitol), caused by the increasing amount of ethanol. The conceptual phenomena for the ethanol effect related to the low polarity of ethanol molecule compare to water molecule (Trindade et al., 2007). In fact, as the concentration of ethanol increases, electrical attraction between different OH^- groups inter and intra-chain, present in the saccharides model molecules, become strong enough to form stable H-bonds and precipitate molecules.

Water molecule is highly polar which reflected by the high value of its dielectric constant (80.1) at 25°C , ethanol is much less polar than water, its dielectric constant three time lower than water (24.5) (**Table 1**). This means that adding ethanol to an aqueous solution disrupts screening of charge by water. If enough ethanol is added electrical attraction between charged group in molecule and any charged compounds present in solution become strong enough to form stable ionic-bonds and precipitate.

Polyphenols include one or more hydroxyl groups (polar part) attached directly to an aromatic ring (non polar part), *i.e.* oleuropein and *p*-HPA are often found as esters or glycosides, rather than as free molecules (Queimada et al., 2009). This stereochemistry distinguishes polyphenols according to their polarity variance. The above property has been utilized for the recovery of polyphenols from natural sources, which is usually accomplished with solvent extraction from solid biomasses. The yield of this process is strongly dependent on the nature of the solvent (Szabo et al., 2010). Subsequently, the extraction is affected by the diffusion coefficient and the dissolution rate of compounds until they reach the equilibrium concentration inside the solvent (Pan et al., 2008). In particular, the recovery of certain phenolic terpenes has been referred to occur preferably with non polar solvents (*i.e.*, hexane, petroleum ether), while flavonoid aglycons (low molecular weight compound) and phenolic acids may effectively be recovered with solvents such as diethyl ether or ethyl acetate. On the other hand, more polar solvents like hydro-alcoholic mixtures have also been reported to extract flavonoid glycosides and higher molecular weight phenols (Oreopoulou and Tzia, 2007). Due to the presence of the aromatic delocalized π -electrons and the electronegative oxygen-atoms, polyphenols are highly polarizable. Furthermore, in most cases, they are conformational flexible. The conformational flexibility may affect the reactivity and solvation of solutes, and is also directly related to the formation of crystal polymorphs in the case of saccharides.

The conceptual phenomena for the ethanol effect relates to the strong tendency of ethanol molecules to form shells of tightly-bound water (hydration shells). As the concentration of ethanol increases, more and more water is bound up in hydration shells depending on the ability to coordinate water. On the other hand, the making/breaking structure can be described by ethanol presence effects on the water structure. The addition of ethanol to the aqueous polyphenols model solutions leads to an arrangement of ordered water

1 molecules around polyphenols molecules. This arrangement could be correlated to the
2 increase of polyphenols solubility (Haslam, 1996).

3 **3.2. Solvent selection**

4 **Fig. 4(a,b)** shows the effect of various tested solvents on TDS content and polyphenol
5 concentration in the resulting liquid phase after drowning-out experiments. The results
6 showed that ethanol (99.5% (w/w)) and ethanol (95% (w/w)) provided the highest polyphenol
7 concentration with 75 and 68% (w/w), respectively. In addition, 2-propanol (> 99.7% (w/w))
8 showed a good separation towards polyphenols, with final polyphenol concentration 63%.
9 For methanol (99.7% (w/w)), ethanol (75% (w/w)), and ethyl acetate (> 99.7% (w/w)),
10 polyphenol concentration increased compared to the control sample, with 47.5, 46 and
11 32.5%, respectively. For hexane (95% (w/w)) and acetic acid (> 99.7% (w/w)), no increase of
12 polyphenol concentration was observed.

13 It is obvious that solubilization is a complicated procedure and its efficiency is governed
14 by a complex interplay of parameters. The solubility tendency can be explained by the
15 stereochemistry of polyphenols (the polar and the non-polar fragments inside their molecules)
16 and the intermolecular forces (mainly hydrogen bonds) occurred between them and the
17 solvents. In particular, the phenols' hydroxyl groups can develop hydrogen bonds with the
18 electronegative oxygen of ethanol, methanol, or acetone. The alcohols' hydroxyl groups can
19 also develop hydrogen bonds with the oxygen atoms occurring inside the polyphenol
20 molecules (such as oleuropein, gallic acid, tyrosol or hydroxytyrosol) and this observation
21 can explain their preference for polar protic (methanol or ethanol), rather than polar aprotic
22 solvents (hexane). The preference of polyphenols between methanol and ethanol can be
23 attributed to their non-polar part and the aliphatic fragment of alcohols. For example, in any
24 dissolution process, the solvent molecules surround the solute molecules. Ethanol contains a
25 flexible aliphatic fragment, thus, surrounds easier polyphenols with substituted carbons inside

1 their aromatic ring. Larger molecules (*i.e.*, oleuropein and rosmarinic acid), phenols with two
2 anti-diametric substituted carbons (such as *p*-hydrobenzoic or *p*-HPA) or longer aliphatic
3 fragments (*i.e.*, tyrosol and hydroxytyrosol) preferred ethanol, as it could “cover” better the
4 gaps between the hydrogen bonds, due to the dipole-dipole interactions developed between
5 the more electronegative fragment of the solvent (oxygen atoms) and the more electropositive
6 fragment of the acid (hydrogen protons) (dipole moment, **Table 1**).

7 Basically, polyphenols had a solubility preference basically for solvents with
8 intermediate polarity (alcohols and acetone), rather than more polar (*i.e.*, water) or less polar
9 solvents (*i.e.*, hexane, ethyl acetate).

10 Likewise, ethanol (85%) has been referred to preserve the phenolic characteristics of the
11 extracts recovered from OMW and indeed to increase their radical scavenging ability during
12 18 weeks storage (Galanakis et al. 2010).

13 According to the chemical bonds theory, the selective solvation of polyphenols is related
14 to change of solvent polarity after addition of ethanol (99.5% (w/w)) and ethanol (95%
15 (w/w)) at constant temperature (25°C). On this concern, the physical parameters and
16 compositions of the OMW are presented in **Table 2**. Consequently, ethanol (95% (w/w)) was
17 selected for further study.

18 **3.3. Effect of ethanol addition on polyphenols recovery from OMW and its** 19 **concentration**

20 **Fig. 5** indicates the solubility plot for polyphenols after solid-liquid equilibrium, as a
21 function of ethanol mass fraction, after mixing. As shown in this figure, the polyphenol
22 concentration did not increase linearly with ethanol mass fraction. Moreover, maximum
23 polyphenol concentration was attained in 75% ethanol mass fraction in the system. As
24 consequence, the drowning-out of saccharides from water using ethanol, was investigated as
25 it offers the opportunity to achieve high polyphenol concentration.

Fig. 6 shows the HPLC chromatogram representing phenolic monomers in OMW feed of the present upstream process (**Fig. 6a**), and the liquid phase obtained after (**Fig. 6b**), the drowning-out separation step using 90% mass fraction of ethanol in the mixture. The chromatogram showed major peaks of hydroxytyrosol (3), caffeic acid (5), oleuropein (6), and with retention times 4 min, 21 min, and 33 min, respectively. High recovery of phenolic monomers from OMW feed in the final product was obtained after the crystallization process of the present method.

A hydro-alcoholic mixture (50%) was proved to be the best solvent compared to methanol, ethanol, water, and acetone for the extraction of caffeic acid, ferulic acid, and sinapic acid from roots of black colosh (Mukhopadhyay et al., 2006).

In addition, the recovery of phenolic compounds from winery wastes showed that hydro-ethanol mixtures were the most suitable solvents. On the other hand, the use of organic solvents *e.g.*, ethyl acetate and isopropanol was not recommended (Lafka et al., 2007). Ethyl acetate has been also reported to be ineffective for the recovery of syringic and protocatechuic acid from OMW (Obied et al., 2005).

Hydroxytyrosol and tyrosol have been extracted from OMW using ethyl acetate; however, oleuropein was not detected in these extracts (De Leonardis et al., 2007). For the recovery of oleuropein, water-ethanol mixtures have been reported for their advanced recovery of oleuropein, hydroxytyrosol, and tyrosol (Japón-Luján and Luque de Castro, 2007). The use of water-ethanol mixtures for the recovery of polyphenols from olive leaves has also been suggested (Tsakona et al., 2012).

Solvent composition affects the physical properties of the mixture and particularly density, dynamic viscosity and dielectric constant that reflects diffusion and extraction rate (Cacace and Mazza, 2003).

3.4. Crystallization of saccharides during drowning-out separation

Fig. 7 showed the obtained solid phase in the drowning-out step after solid-liquid equilibrium has been reached. The crystals have a lamellar shape and are of a small size (nearly 0.02 mm). Very fine crystal slurries are obtained. Careful control of operating conditions gives crystal products close in filterability to those produced by cooling crystallization.

3.5. Polyphenols recovery process

Overall, the approach followed in this article provides a two-fold advantage: First, the recovery of polyphenols with high concentration. Second, it is not limited in leading only to qualitative conclusions, but also it offers quantification through the estimation of the different concentration of polyphenols. Therefore, this approach is recommended as a guiding procedure to enable scientists to select the best process for a given application.

Based on the results obtained, a proposed flow sheet scheme for the recovery of polyphenols from OMW is presented in **Fig. 8**. The proposed integrated strategic approach contains different steps of separation for the selective removal of polyphenols from the initial TDS content. A centrifugation step in the upstream allows removing the highest amount of suspended solid in the feed solution. In addition, an evaporation step was conducted before downing-out process, to obtain a concentrated solution up to 60% (w/w) TDS. Mixing with ethanol (95% (w/w)) in the following step and drowning-out step for the splitting of solid phase and liquid phase after solid/liquid equilibrium, recovered polyphenols dissolved in liquid phase, followed by a batch evaporation step for regenerating ethanol, so that rendering the process sustainable.

3.5.1. Centrifugation process

In the proposed method, centrifugation can be primarily used for removing olive cell debris particles and suspended solids from OMW. Centrifugation is also used to separate immiscible residual olive oil droplets that differ in density from the water phase. The

centrifugation was carried out in batches, at a rotation speed of $6000\times g$. After 30 min, three layers of material were separated: a top layer, made-up of an oil droplets, an intermediate rather opaque aqueous solution, and a solid dark deposit that stayed at the bottom of the vessel obtaining a partially clarified liquid, which was then subjected to subsequent batch evaporation operation and a solid residue was obtained, which may be used as fertilizer or compost production line along with the olive residues. The material balance during centrifugation (**Fig. 9**) is based on the removal percentage of particulate components (**Table 3**). More specifically, the removal percentage of particulate components determines the amount of particulate components retained in the concentrate. The concentration factor determines the amount of liquid and solid components retained in the concentrate.

3.5.2. Batch evaporation process

The mass balance in the batch evaporation process was described in **Fig. 10**, the upstream referring to the aqueous phase obtained after centrifugation process, was concentrated under the operating conditions as follows: isothermal condition at 42°C , operating pressure 0.15 bar, water split to vapor was 93% and process time 60 min. The flow rate of vapor outlet and liquid outlet was 6876.5 and 1419.3 kg/batch, respectively.

3.5.3. Drowning-out separation

In **Fig. 11**, the mass balance in mixing step was presented after addition of ethanol to the stream (mass fraction percent 90%), for the recovery of polyphenols from the others components. The residence time of upstream solution in the operation was optimized within 12 h for total recovery of polyphenols from the other soluble solids. The crystallization yield of crystallizable components at this range is mostly 100%. The crystallization was conducted at 25°C . The flow rates of feed, solid and liquid phase were 8277.3, 776.46 and 7500.83 kg/batch, respectively (**Fig. 12**).

3.5.4. Ethanol regeneration

Batch distillation process applied for the regeneration of ethanol, foreseeing economic feasibility of the proposed method, which was operated with condenser temperature 10°C, reboiler temperature 45°C, operating pressure of 0.15 bar, reflux ratio fixed at 10, volume rate 1310 kmol/h and mol fraction of volatile components distilled of 85% with relative volatility of ethanol 2.5 compared to water as reference component. The flow rates of the feed, distillate and concentrate were 7500 kg/batch, 6862 kg/batch and 639 kg/batch, respectively (**Fig. 13**).

4. Conclusion

Experimental data disclosed herein demonstrate evidence of successful application of a novel and efficient method for the recovery of polyphenols from OMW to obtain biomolecules of interest for the food, cosmetic, or pharmaceutical industries. The method provides a highly-concentrated polyphenol isolate (up to 75 % (w/w)) from other components present in OMW, with up to (510 mg/g) of the polyphenols content being hydroxytyrosol. The recovered polyphenols can be applied as antioxidant and antimicrobial agents in food industry; they can be prepared as solid particles, as aqueous solutions, in emulsions, or as lipidic-based nanoparticles.

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Figure captions

Fig. 1. Schematic representation of the experimental apparatus for conducting drowning-out process experiments. (1) Burette for adding ethanol, (2) Thermometer, (3) Magnetic stirrer, (4) Sample vessel, (5) Water flux inlet at desired temperature, (6) Water flux outlet, (7) Magnetic stirrer.

Fig. 2. Isothermal phase behavior of model solutions, temperature 25°C.

Fig. 3. Isothermal phase behavior of raffinose and gallic acid during drowning-out process, temperature 25°C.

Fig. 4. Effect of the addition of various solvents on total dissolved solids and polyphenol concentration after drowning-out process.

Fig. 5. Experimental equilibrium curve for polyphenol concentration at different ethanol mass fraction (based on 1 kg of olive mill wastewater).

Fig. 6. HPLC chromatogram for olive mill wastewater before (a), and after (b) the drowning-out process.

Fig. 7. Crystal micrographs obtained after the drowning-out process in the obtained solid phase at different time. T_{c1} : after 1 h; T_{c2} : after 3 h; T_{c3} : after 5 h; T_{c4} : after 6 h.

Fig. 8. Flow sheet configuration of the proposed polyphenol recovery process.

Fig. 9. Material balance for centrifugation operation.

Fig. 10. Material balance for batch evaporation.

Fig. 11. Material balance for mixing operation.

Fig. 12. Material balance for drowning-out crystallization process.

Fig. 13. Material balance for batch distillation, for the regeneration of ethanol.

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Table 1. Physicochemical properties of organic solvents applied in this study.

Usual name	Relative Permittivity* ϵ_r at 20°C [-]	Dipole moment μ [debye]	Water solubility at 25°C [%]	Polarity constant $E_T(30)$ [kJ/mol]	Viscosity at 25 °C [mPa.s]	Flash point [°C]	Average Exposure value [ppm]
Water (H₂O)	80,10	1,85	∞	0,900	0.894	-	-
Methanol (CH₄O)	32,66	2,87	∞	0,762	0.544	12	200
2-propanol (C₃H₈O)	19,92	1,66	∞	0,546	2.080	12	400
Acetic acid (C₂H₄O₂)	6.15	1.74	∞	0.648	1.130	39	10
Ethanol (C₂H₆O)	24,55	1,66	∞	0,654	1.074	16	1000
Ethyl acetate (C₄H₈O₂)	6,05	1,82	8,08	0,228	0.426	-4,4	400
Hexane (C₆H₁₄)	1,89	0,08	0,0012	0,009	0.480	-26	50

* Relative permittivity, also known as “dielectric constant”

Table 2. Physical parameters and composition of olive mill wastewater (OMW) before and after pre-treatment.

Parameter	Before pretreatment		After pre-treatment	
	mean value	S.D.*	mean value	S.D.*
pH [-]	4.59	±0.02	5.31	±0.08
viscosity at 25°C [mPa s]	5.30	±0.06	17.3	±0.02
conductivity [mS/cm]	11.1	±0.10	10.3	±.012
fat [g/L]	4.57	±0.21	0.2	±0.18
reducing saccharides [g/L]	32.77	±1.26	31	±1.43
total dissolved solids [g/L]	178.5	±2.84	570	±4.3
total polyphenols [g/L]	4.8	±0.32	4.55	±0.24
density at 25°C [-]	1.04	±0.04	2.15	±0.03

*S.D.: standard deviation.

Table 3. Mass balance during centrifugation.

Removal of solids particulate and oil from the feed		
Components	oil removal %	solid particulates removal %
Minerals	0	0.1
Saccharides	0	64
Oil	100	0
Other solids (<i>i.e.</i> lignin, tannin,...)	0	45.15
Polyphenols	0	0
Proteins	0	81
Total dissolved solids	0	55
Water	0	9