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Recovery of polyphenols from olive mill wastewater using drowning-out 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 integrates 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 behavior 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.
Industrial relevance: Drowning-out separation process by ethanol was found useful to achieve the main target for the recovery of polyphenols from OMW. Polyphenol concentration increased considerably by the proposed method based on the solubility behavior changing after addition of ethanol, a food grade solvent. Furthermore, batch evaporation was carried out for the regeneration of ethanol, which can be reused in the process. Polyphenols concentrate (up to 75% (*w*/w)) developed in this study have the potential for application in food industry (*e.g.* to develop a premium olive oil containing 1000 ppm of polyphenols, or developing *e.g.* grape juice with enhanced polyphenols content), or into cosmetics (*e.g.* developing a lotion/hand cream with high antioxidant content), or into pharmaceutics (*e.g.* developing capsules high polyphenols content; which could also be considered as food supplement).

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, Ferri, Scoma, Marchetti, & Fava, 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, Aloui, & Sayadi, 2009) and recover its polyphenol yields (Cerretani, Bendini, Biguzzi, Lercker, & Toschi, 2005; Galanakis, Tornberg, & Gekas, 2010).

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, Fki, & Sayadi, 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 overcame using supercritical fluids exatraction, notably, supercritical CO_2. Thus, following the method described by

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Lafka, Lazou, Sinanoglou, and Lazos (2011), OMW's polyphenols have been extracted using CO_2 supercritical fluid. In terms of yield, supercritical CO_2 was found to be a more efficient solvent than ethyl acetate, but it was surpassed by polar solvents (methanol, ethanol, *n*-propanol).

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

Chromatographic separation is generally considered to be the most effective for the recovery of polyphenols. For instance, the recovery yield was 60% by using Amberlite XAD16 resin as the adsorbent and ethanol as the biocompatible desorbing phase (Scoma, Bertin, Zanaroli, Fraraccio, & Fava, 2011). Considering the study conducted by Ferri, Bertin, Scoma, Marchetti, and Fava (2011), the highest polyphenols recovery (76%) was achieved using IRA96 polar resin.

While all the above-mentioned separation processes often depend on several property differences for their overall success (distribution between immiscible liquid phases in case of solvent extraction, surface sorption in case of chromatography, molecular size and diffusivity in case of membrane separation technologies). Solubility is a particularly important property for creating separation of various organic compounds specially used as main property difference during crystallization process (Chiou, 2012). Solubility can be modified by the presence of miscible solvent in the solution, which changes the physical and chemical properties of solvent. Indeed, this process is often referred in the literature as drowning-out crystallization-based separation process (Berry, Dye, & Ng, 1997; Holmbäck & Rasmuson, 1999; Kim, Park, Shim, Kim, & Koo, 2012). Drowning-out process is widely used for the separation of pharmaceutical 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 μS/cm) and ethanol of analytical grade (C_2H_6O, 95% pure) were 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 μm 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 detector of refractive index (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