Recovery of Phenolic Antioxidants from Olive Mill Wastewater

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Received: April 17, 2009; Accepted: June 24, 2009; Revised: July 14, 2009

Abstract: Olive mill wastewater (OMW), generated by the oil extraction industry, represents a severe environmental problem due to its highly polluting organic load arising from polyphenol content with low biodegradability. However, with its more than 30 phenolic compounds, OMW is also regarded as a potent source of natural antioxidants. Consequently, during recent years, various reports have appeared in the literature following such benefit. In this article, patent publications between the years 2005 and 2009 for the recovery of phenolic antioxidants from OMW and olive processing solid by-products are reviewed: solvent extraction and membrane separations individually or integrated with some other separation and concentration procedures, such as centrifugation, chromatographic separation and evaporation are the fundamental processes described. In the publications reviewed, hydroxytyrosol, tyrosol, oleuropein and phenolic acids, which possess more powerful antioxidant properties than other phenolic compounds in OMW, are given the most prominence for their recovery. The interest in membrane technologies in processing olive oil by-products indicates that the future direction of the processes for the recovery of antioxidants from OMW is presumably toward the utilization of membranes in a sequential design.

Keywords: Olive mill wastewater, olive vegetation water, olive processing by-products, phenolic antioxidants, hydroxytyrosol, recovery of polyphenols.

INTRODUCTION

Olive processing has been an important and traditional industry for Mediterranean countries since ancient times. The growing interest in the consumption of olive oil as an integral ingredient of the "Mediterranean diet" increases the importance of olive oil sector over the last decades. Worldwide olive oil production for the years 2002/2003-2008/2009 is reported to be 2 775 800 t [1]. The highest olive oil production is in European Union (EU) with 76.6% of total production (Spain: 50.4%, Italy: 29.8%, Greece: 17.7%, Portugal: 1.7%) for the same period. Tunisia (6.2%), Syria (4.8%), Turkey (4.3%), Morocco (2.6%) and Algeria (1.2%) are other significant olive oil-producing countries [1]. Olive oil production involves one of the following extraction processes: i) press olive oil extraction, ii) three-phase centrifugal olive oil extraction, and; iii) two-phase centrifugal olive oil extraction. Each process generates waste products of different characteristics. Pressure and three-phase centrifugation systems produce considerably more liquid effluent which is so-called olive mill wastewater (OMW), vegetation water, or alpechin in Spanish than two-phase centrifugation process. Although the liquid waste is reduced in two-phase centrifugation system, large amounts of semi-solid or slurry waste -commonly referred to as two-phase pomace or alperujo in Spanish- are discharged [2,3]. The production process of olive oil usually yields next to 20% olive oil, a 30% semi-solid waste and 50% aqueous liquor [2]. The liquid effluent of olive oil process, which is OMW, amounts to 0.5–1.5m^3 per 1000kg of olives depending on the process used [4]. Although the properties of OMW vary enormously with type of the process, climatic conditions and region of origin, it represents a severe environmental problem due to its highly polluting organic load arising from polyphenol content with limited bio-degradability. However, OMW can provide a cheap source of phenolic compounds with strong antioxidant properties. An antioxidant is a substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents the oxidation of that substrate [5]. For health, antioxidants are needed to prevent the formation and oppose the actions of reactive oxygen and nitrogen species, which are generated *in vivo* and cause damage to DNA, lipids, proteins, and other biomolecules [6].

Olive mill wastewater is characterized by intensive violet-dark brown up to black color, strong specific olive oil smell, high degree of organic pollution (chemical oxygen demand; 40-220g/L and biochemical oxygen demand; 35-110g/L), pH between 3 and 6, total organic compound of 25-45g/L, high electrical conductivity, high content of polyphenols (0.5–24g/L), reducing sugars up to 60% of the dry substance, high content of solid matter, and potassium as the predominant inorganic material (~4g/L) [2]. The powerful pollutants prohibit OMW to be directly discharged into water or onto land. Several treatment procedures including physical, chemical, biological or combined technologies have been tested to reduce undesirable properties of OMW prior to disposal. On the other hand, the biophenolic fraction of olive oil comprises only 1-2% of the total phenolic content of the olive fruits, with remaining 53% and 45% being lost in OMW and the olive cake, respectively; depending on the extraction system used [2]. Olive biophenols are biologically active compounds and contain numerous simple and complex substances that are

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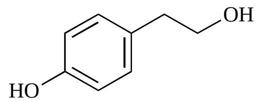
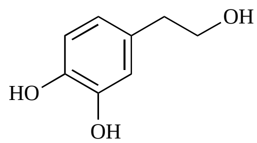
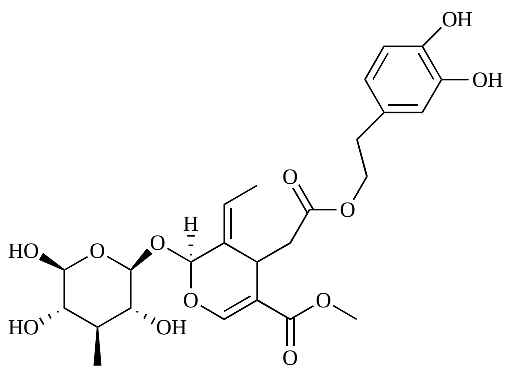
1874-4788/09 $100.00+.00

Table 1. The Main Biophenols Occurring in Olive Mill Wastewater

Biophenol	Chemical Structure	Properties
Tyrosol		IUPAC name: 4-(2-Hydroxyethyl)phenol Synonyms: 4-(2-Hydroxyethyl)phenol *p*-Hydroxyphenethyl alcohol 4-Hydroxyphenylethanol Molecular formula:$C_8H_{10}O_2$ Molecular weigth:138.164 CAS No: 501-94-0
Hydroxytyrosol		IUPAC name: 4-(2-Hydroxyethyl)-1,2-benzenediol Synonyms: 3-Hydroxytyrosol, 3,4-dihydroxyphenylethanol Molecular formula: $C_8H_{10}O_3$ Molecular weigth:154.16 CAS No: 10597-60-1
Oleuropein		IUPAC name: 4S,5E,6S)-4-[2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl]- 5-ethylidene-6-[[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)- 2-tetrahydropyranyl]oxy]-4H-pyran-3-carboxylic acid, methyl ester Molecular formula:$C_{25}H_{32}O_{13}$ Molecular weigth:540.514 CAS No: 32619-42-4

characterized by multifunctional moieties. The simple biophenols such as vanillic acid, gallic acid, cumaric acid, caffeic acid, tyrosol and hydroxytyrosol possess alkene, alcoholic, and carboxylic groups whereas more complex biophenols such as secoiridoids (oleuropein and ligstroside) possess glycosidic and monoterpenoid units [7]. Consequently, with its more than 30 phenolic compounds, OMW is now regarded as a potent source of natural antioxidants. During recent years, a number of reports have appeared in the literature following such benefit.

The phenolic compounds of virgin olive oil are classified by Bendini *et al.* [8] as follows: benzoic and derivatives acids (3-hydroxybenzoic acid, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, gentisic acid, vanillic acid, gallic acid, syringic acid), cinnamic acid and derivatives (*o*-coumaric acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapinic acid), phenyl ethyl alcohols (tyrosol (*p*-hydroxyphenylethanol) or p-HPEA, hydroxytyrosol ((3,4-dihydroxyphenylethanol) or 3,4-DHPEA), other phenolic acids and derivatives (*p*-hydroxyphenylacetic acid, *p*-hydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid, 3-(3,4-Dihydroxyphenyl)propanoic acid), dialdehydic forms of secoiridoids (decarboxymethyloleuropein aglycon (3,4-DHPEA-EDA), decarboxymethyl ligstroside aglycon (p-HPEA-EDA)), secoiridoid aglycons (oleuropein aglycon or 3,4-DHPEA-EA, ligstroside aglycon or *p*-HPEA-EA, aldehydic form of oleuropein aglycon, aldehydic form ligstroside aglycon), flavonols ((+)-taxifolin), flavones (apigenin, luteolin), lignans ((+)-pinoresinol, (+)-1-acetoxypinoresinol, (+)-1-hydroxypinoresinol) and hydroxyisochromans (1-phenyl-6,7-dihydroxyisochroman, 1-(3'-methoxy-4'-hydroxy)phenyl- 6,7-dihydroxyisochroman).

Excellent biological properties such as antioxidant, free radical scavenging, antimicrobial and anticarcinogenic activities of the biophenols of OMW shown by several studies are recently reviewed by Obied *et al.* [9]. The main biophenols occurring in OMW are given in Table **1**. Antioxidant property of *ortho*-diphenolic compounds of OMW, particularly of hydroxytyrosol attracts considerable attention to recover these compounds from OMW by developing an effective process. Oleuropein, which is an ester of hydroxytyrosol (3,4-DHPEA) and elenolic acid (EA) glucoside, also gains interest as a natural food antioxidant. On the other hand, intensive studies on phenolic composition of OMW have introduced novel antioxidants to olive oil sector. For instance, Obied *et al.* [10] recently identified two new biophenolic compounds in OMW extracts, namely hydroxytyrosyl acyclodihydroelenolate and p-coumaroyl-6'-secologanoside (comselogoside). The authors reported that antioxidant scavenging activity of these compounds was higher than hydroxytyrosol and oleuropein. Therefore, discovering unknown compounds still present in OMW will encourage studies that develop new processes to recover them from OMW to be used as health food supplements and natural food antioxidant.

Although hydroxytyrosol is a valuable antioxidant, it is not commercially available in high amounts since its production through chemical and enzymatic pathways is usually slow and expensive [11]. Consequently, recovery of hydroxytyrosol and other biophenols from olive oil processing by-products becomes more important to benefit from them as natural antioxidant. In addition to methods for their synthesis, several patents have been developed to recover biophenolic compounds from OMW. In this article, recent patents and published patent applications for the recovery of phenolic antioxidants from OMW are reviewed.

RECOVERY OF PHENOLIC ANTIOXIDANTS

Several techniques are used individually or in a combined form to recover the phenolic compounds from olive mill wastewater. These techniques largely comprise extraction, membrane separation, centrifugation, and chromatographic procedures. The patent publications in this field have mostly focused on the recovery of hydroxytyrosol, tyrosol, oleuropein and phenolic acids that possess more powerful antioxidant, free radical scavenging, antimicrobial and anticarcinogenic properties compared with other phenolic compounds in OMW. The patent publications on the recovery of antioxidants from OMW have been markedly appeared in the literature for the last decade; however, its number has not showed a considerable increase during this period. This can be addressed to the complex and varying characteristics of OMW as well as the requirement of sequential sub-processes differ in operation. In the following, patent publications between the years 2005 and 2009 on the recovery of antioxidants from OMW are reviewed after being classified according to the fundamental techniques employed.

SOLVENT EXTRACTION

Solvent extraction is the most widely used technique to recover phenolic compounds from olive mill wastewater in spite of its high cost resulting from the requirement of large amounts of organic solvents. In recent studies, the disadvantages of organic solvents such as toxicity and flammability are avoided by the use of supercritical fluids (SCFs), particularly supercritical CO_2. However, the extraction with SCFs has the disadvantage of the requirement of expensive high pressure equipment. Solvent extraction is used individually or preferably employed prior to membrane processes for the recovery of polyphenols from olive oil by-products.

Emmons and Guttersen [12] described a process for collecting oleuropein aglycon from olive oil vegetation water comprising oleuropein, oleuropein aglycon and conversion enzymes. The method involves addition of citric acid to the raw material, subsequent heating in order to precipitate the solids, and extraction of oleuropein aglycon from water immiscible constituent with a non-polar organic solvent mixture, preferably mixture of 50/50 v:v hexane and acetone. The solvent is then evaporated under vacuum and/or heat.

In a process for producing triacetylhydroxytyrosol, De Martino *et al.* [13] used the organic extract of olive mill wastewater rich in hydroxytyrosol as a raw material. A possible industrial production of triacetylhydroxytyrosol, which is a stabilized form of hydroxytyrosol, is expected to find convenient applications, mainly for the exploitation of triacetylhydroxytyrosol as additive in nutritional, cosmetic and pharmaceutical preparations. The inventors described the reaction conditions of organic extract of OMW with an acylating mixture consisting of perchloric acid adsorbed on silica gel as a catalyst and an acetyl donor compound preferably acetic anhydride, followed by chromatographic purification of the reaction product. The procedure, consists of three steps and starts by using OMW, provides an overall yield of triacetylhydroxytyrosol at least 35%. In the first step of the process, OMW is treated with a polar organic solvent to obtain an organic extract containing hydroxytyrosol by means of a Soxhlet, continuous countercurrent or batch extraction system. The organic solvent suggested for the process is ethyl acetate, methyl isobutyl ketone, methyl ethyl ketone, diethyl ether, methanol or *n*-butanol. In the process, the pH of the OMW can be native pH (4.5-5.5) or adjusted in the range from pH 2 to 8. The most convenient extraction procedure is declared to use OMW at its native pH in a Soxhlet apparatus. In a preferred embodiment of the process, the pH of the OMW is the native pH, the polar organic solvent is ethyl acetate and the ratio solvent/OMW is between 1:1 and 4:1.

Tornberg and Galanakis [14] disclosed a method for isolating dietary fibers, such as pectins, and valuable polyphenols from olive mill wastewater, as well as the use of isolated products as additive in other food items and as an antioxidant foodstuff, respectively. In the process, firstly, OMW is defatted by centrifugation and concentrated by removing the water content. Afterwards, it is extracted by using ethanol up to 7% (v/v) and an organic acid in the range of 0.5% to 3% by weight of the extraction solution. The process uses one of the following organic acids; citric, tartaric, malonic, maleic, malic, oxalic, adipic, or fumaric. The polyphenols remaining within the dietary fibers are then extracted by at least 85% (v/v) ethanol and separated by filtration. After dilution with 15-40% (v/v) ethanol, the liquid phase containing polyphenols is clarified by filtration. In a preferred embodiment of the invention, the polyphenol-rich liquid is used to produce a once-a-day administrable dose of antioxidant polyphenols. Finally, the soluble dietary fibers are further separated from the insoluble dietary fibers by centrifugation and concentrated by evaporation.

MEMBRANE SEPARATIONS

Application of membrane technologies to recovery of antioxidants from olive mill wastewater is of interest due to their several advantages mainly low energy consumption, no additive requirements and no phase change. Although conventional filtration membranes still find a considerable application in the treatment of OMW, microfiltration (MF), ultrafiltration (UF), nanofiltation (NF) and reverse osmosis (RO) membranes, mostly in a sequential form, successfully meet the requirement for the recovery, purification and concentration of antioxidants with regard to their specific molecular weight cut-off values. In recent applications, membrane filtration is preferred to be applied in crossflow mode. In crosslow or tangentinal flow filtration, the feed is pumped into the membrane module where it is separated into two streams namely the filtrate (or permeate) and the retentate in which the retained species has been concentrated. Crossflow ultrafiltration differs from conventional dead-end filtration in that the retentate flows parallel to and across the membrane surface rather than perpendicular to and towards it [15]. The tangentinal movement of the fluid targets to remove most of the rejected material from the membrane surface, and consequently to minimize the accumulation at the membrane surface. Olive mill wastewater comprises phenolic compounds of different molecular masses ranging from low molecular weight phenolics such as benzoic acid and derivatives (MW up to 198) to high molecular weight phenolics such as secoiridoid aglycons (MW up to 378) and lignans (MW up to 416) [8]. The wide range of molecular weights of OMW compounds complicates their recovery with high purities, which can be overcome by using membrane technologies. Microfiltration membranes are capable of retaining microparticles in the range of 0.1-10 µm where UF membranes retain 1-100 nm macromolecules. The molecules in the size of 0.5-5 nm can be separated by NF membranes and the molecules smaller than 1nm are retained on RO membranes [16]. Accordingly, several methods employing membrane technologies for the recovery of natural antioxidants from OMW have been studied and patented.

Castanas *et al.* [17] disclosed a system of filters and a method in which the proposed system of filters is used for the treatment of olive mill wastewater. The inventors reported that the system of filters is composed of sub-stratums of natural products selected from the group consisting of turf, sand, and sawdust, and optionally one or more filters of resins selected from the group consisting of cationic, anionic, mixed type or polyvinylpolypyrrolidone resins. Olive mill wastewater is passed through different combinations of filters to obtain an effluent. A flocculant such as aluminum or other adsorbent salts is added to the effluent obtained in order to eliminate its color. The effluent can then be used for agricultural irrigation. A method is also proposed for recovery of highly valuable products, such as antioxidant biophenols, present in OMW, by use of the system of filters where polyphenols are retained on the filters of resins. After separating the filters of resins from other filters and washing them by passing a solvent, a highly valuable product is recovered. The whole or part of the system of filters can be regenerated by passing an organic solvent, preferably methanol, through the system. The system offers some advantages such as being made of inexpensive filters and possessing a considerably degree of flexibility as it is made up of a discrete number of filters providing a change in the number of each kind of filter in the system easily.

Pizzichini and Russo [18] revealed a process for totally recovering the chemical components of olive mill wastewater using membrane technologies. The patent application concerns a selective fractionating process of the components present in OMW, enabling recovery of the polyphenolic compounds contained therein, reusing the concentrate residues for the production of fertilizers and biogas, and obtaining a highly purified aqueous product which can also be used as a basic component for beverages. In the invention, membrane operations are used after adjusting the pH of the fresh OMW to within an acidic range (3-4.5) to prevent oxidation of polyphenols, to favor the transformation of oleuropein to hydroxytyrosol, and to create optimal conditions for the subsequent enzymatic treatment. According to the proposed process, the acidified wastewater is subjected to enzymatic hydrolysis by a pectinase enzyme complex to remove the cellulose, hemicellulose and pectin microdispersed components from OMW. The liquid fraction separated from the degradation products is then used in a membrane system including tangential MF, tangential NF and RO units, in sequence. The ultimate retentate of the RO system is rich in polyphenols. The retentate of MF unit can also be used for extracting polyphenolic compounds therefrom. The utilization of a diafiltration unit, following the MF unit, is notified to be useful for further recovery of polyphenols in the retentate of the MF unit. The sum of the permeate and of the diafiltrate is then subjected to NF. An additional tangential ultrafiltration unit on the permeate line of MF provides a substrate for compost production or for fermentation processes. The properties of the membranes used in the process are reported as follows: for tangential MF, ceramic membranes of molecular size ranging between 0.10 and 1.4µm, preferably made up of a ceramic block with 23 filtration channels in a daisy-like or sunflower-like arrangement with a filtering surface of $0.35m^2$ per ceramic block; for tangential UF, polymeric membranes of molecular size ranging between 1 and 20kDa, and spiral-shaped, made of polysulphone, polyethersulphone, polyamide or regenerated cellulose acetate; for tangential NF, polymeric membranes of molecular size ranging between 150 and 250Da, preferably of about 200Da, spiral-shaped and made of composite polyamide or nylon; and for RO, high saline rejection spiral-shaped polymeric membranes made of composite polyamide. The proposed process conditions are the following: for MF operation, entry flow pressure 2.5bar, exit flow pressure 2.3bar, pressure on permeated side 0.7bar, and temperature 25-30^{o}C; for UF operation, entry flow pressure 3-4bar, exit flow pressure 2.8-3.9bar, pressure on permeated side zero, and temperature 15-25°C; for NF operation, entry flow pressure 12bar, exit flow pressure 10bar, and temperature 20-25^{o}C; and for RO, entry flow pressure 30bar, exit flow pressure 25bar, pressure on the permeate side zero, and temperature 15-25^{o}C.

De Magalhaes *et al.* [19] described a process for obtaining a concentrate rich in hydroxytyrosol from olive tree residues and sub-products using supercritical fluid extraction, nanofiltration and reverse osmosis techniques individually or in an integrated mode. In the patent application, a recovery of natural extract of hydroxytyrosol between 15 and 98% mass fraction is achieved, and its utilization in the form of solid particles, as an aqueous solution, as an emulsion or as lipidic based nanoparticles in the food, pharmaceutical and cosmetics industries is proposed. It is suggested that vegetation water or the extracts of solid or semi-solid residues resulting from olive oil production and other by-products from the olive tree, such as olive stones and olive leaves can be used as feedstock in the process. The inventors report that hydroxytyrosol and other bioactive compounds are recovered by supplying a flow stream from the feedstock either to a supercritical fluid extraction column to recover the compounds in the extract stream, or to a nanofiltration unit to recover the compounds in the permeate stream. Following one of these steps, the extract or permeate stream is fed to a RO system to obtain hydroxytyrosol and other bioactive compounds in the retentate stream. It is claimed that bioactive compounds supplied to the flow stream and in the retentate of the RO system comprise at least hydroxytyrosol, luteolin, and hydroxycinnamic acids, preferably caffeic and *p*-coumaric acids. In the supercritical extraction system, CO_2 is used as supercritical solvent in a packed bed column where the liquid feedstock is continuously fed at the top of the column. The supercritical fluid, which is fed at the bottom of the column, leaves the column with extracted solutes by means of an outlet at the top. The supercritical extraction step is accomplished at a pressure between 6-40MPa, and at a temperature between 30-200^{o}C. In the NF unit, the utilization of not only the extracts of solid or semi-solid residues and other by-products of olive tree and olive oil process, but also the extracts produced by supercritical fluid extraction is claimed. The operation of the NF unit is accomplished at an absolute pressure difference between the feed retentate compartment and the permeate compartment in the range of 0.5MPa and 3.0MPa, and the operation of RO unit is accomplished at an absolute pressure difference in the range of 3MPa and 8MPa. For the selective fractionation of bioactive compounds present in the feed stream, NF membranes with the molecular weight cut-off lower than 1,000Da, preferably lower than 400Da, most preferably below 300Da, are proposed. The NF and the RO membranes are reported to be homogeneous or composites, being polymeric or inorganic or comprising both materials; and to have a flat geometry or a tubular geometry, being arranged in a plate-and-frame module, in a spiral-wound module, in a hollow fiber module, in a capillary module or in a tubular module. The utilization of one or more modules arranged in series or in parallel where at least one membrane module is submerged in one or more feedstock vessels, or is placed externally to the at least one feedstock vessel is also proposed. At least one feedstock vessel is claimed to be temperature, pressure and pH controlled and fed at least one nanofiltration membrane module in a continuous, semi-continuous or batch mode. Finally, it is reported that the natural hydroxytyrosol-rich concentrate, recovered from olive tree residues and by-products using clean technologies, exhibits antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic activities superior to those observed for pure hydroxytyrosol, in equivalent concentration.

Villanova *et al.* [20] described a process for the recovery of tyrosol and hydroxytyrosol from olive mill wastewater and a method for the catalytic oxidation of tyrosol to hydroxytyrosol. The process suggested for the recovery of tyrosol and hydroxytyrosol from polyphenolic fractions or their purified components of OMW comprised rough filtration (RF), microfiltration, ultrafiltration, nanofiltration and reverse osmosis units followed by column chromatography. The sequential stages of RF, MF, UF, NF and RO with different modules of different molecular weight cut-offs provide the recovery of at least 1g/L hydroxytyrosol and 0.6g/L tyrosol. The concentration ratio of feed/extract in the stages of MF, UF, NF and RO is reported to be preferably higher than 8. In the chromatographic separation, a preparatory column filled with a macroporous co-polymer of divinylbenzene and N-vinylpyrrolidone is used to isolate tyrosol, hydroxytyrosol, trihydroxybenzoic acid, catechol, etc., available in the extract of RO, and in particular to prepare hydroxytyrosol with a high level of purity and free from catechol, which is toxic for humans. The chromatographic separation affords tyrosol with a yield higher than 84% (purity > 80%), and hydroxytyrosol with a yield higher than 80% (purity > 80%). Hydroxytyrosol and tyrosol are then brought to purity higher than 98% by a semi-preparative reverse phase HPLC column. In the proposed process for the catalytic oxidation of tyrosol to hydroxytyrosol, methyl rhenium trioxide (MTO) is used as catalyst in both homogeneous and heterogeneous forms with hydrogen peroxide in a protic solvent as oxidant. The highest yields in the homogenous MTO oxidation of tyrosol to hydroxytyrosol are obtained in the presence of ethanol at 20^{o}C, and of water at 45^{o}C with 100% and 75% yields, respectively. Two types of heterogeneous MTO catalysts, poly (4-viniylpyridine) 2% MTO and microencapsulated MTO, are also successfully used in the oxidation of tyrosol providing hydroxytyrosol yields of 43% in water and 40% in ethanol, respectively. Polyphenolic fractions or their purified components obtained by described process are formulated to be used as antioxidant agents, free radical scavengers, varnish additives, composites, natural fibers, and plastic materials.

CHROMATOGRAPHIC SEPARATIONS

Fernandez-Bolanos *et al.* [21] suggested a method for obtaining hydroxytyrosol from products and by-products of olive tree by two-step chromatographic treatment. In the first column, the polystyrene based non-activated ion exchange resins, in the form of a gel or macroreticular, is used to provide partial purification of hydroxytyrosol. After elution with water, the solution containing hydroxytyrosol from the previous stage is fed to the second column involving non-ionic, polystyrene based XAD resins to enable hydroxytyrosol to be completely purified after eluting with 30-33% methanol-water or ethanol-water mixture. In the first column, at least 85% of tyrosol present in the feed, and in the second column, at least 75% of hydroxytyrosol present in the olive oil by-product with the minimum purity of 95% are claimed to be recovered. The method used in the invention is reported to be also applied to two-phase pomaces, three-phase pomaces and stone if they are subjected to a steam explosion process for hydroxytyrosol solubilization.

Liu and Wang [22] disclosed a method for the recovery of hydroxytyrosol from an olive extraction liquid. After dilution with water, the liquid is passed through a styrene resin chromatographic column. The transparent elute obtained by washing the column with distilled water is subjected to extraction with ethyl acetate and to distillation at low temperature for the recovery of hydroxytyrosol.

INTEGRAL PROCESSES

Ibarra and Sniderman Zagiary [23] claimed a process for producing liquid and powdered olive polyphenols concentrate from by-products of two-phase and three-phase olive oil extraction processes. The by-products used as a source of polyphenols were semi-solid (orujo) or liquid (alpechin) materials obtained in three-phase process, semi-solid material (alperujo) obtained in two-phase process, and liquid extracts from either these semi-solids or particulate material formed from orujo or alperujo. The process starts by mixing one of the by-products defined above with a polar solvent to give a by-product/solvent mixture so as to that polyphenols present go into solution. Water, ethanol or a mixture of water and ethanol are preferred solvents for the first step of the process. In the second step, the by-product/solvent mixture is subjected to an extraction procedure where the solvent may be water, ethanol or a mixture of water and ethanol with the weight ratio of the by-product to solvent is in the range 1/3 to 1/30. In this step, olive polyphenols solution and extracted solids are obtained. The olive polyphenols solution separated from solids in a decanter and/or by means of a filtration step is then clarified in a disk centrifuge or by using microfiltration membranes. Metallic, ceramic or polymeric membranes are suggested for use in MF unit. The loss of polyphenols in the extraction step was claimed to be less than 15wt% based on the dry extract in the starting material. Partial or complete fat removal was also claimed by means of solvent extraction, decanting or cryogenic separation. Although vacuum evaporation or chromatographic methods can be used to concentrate the polyphenols solution obtained, membrane separation techniques are preferred. Ultrafiltration membranes made of ceramic, cellulosics, polysulfone and polyvinylidene fluoride in the forms of tubular, hollow fiber, plate and spiral are suggested to pass almost all the polyphenol through the membrane. The loss of polyphenols in the retentate of UF is reported to be less than 10wt% based on the total dissolved solids of the olive polyphenol extract. The permeate of the UF unit is preferred to pass through NF membranes made of thin film composites and cellulosic materials to remove the dissolved minerals from the solution. In one embodiment of the invention, UF permeate, NF permeate or a mixture of them is subjected to vacuum concentration. In the last step, the olive polyphenol concentrate may be dried by using a pan vacuum drier or a spry-drier to obtain a powder. The process yields a concentrate wherein the concentration of polyphenols present at least 10wt%. In different steps of the process, some enzymatic treatments are also carried out. Deactivation or inhibition of polyphenol oxidase that is responsible for the undesirable polymerization of poly-phenols can be employed after the first step of the process. Hydrolysis of oleuropein and/or demethyoleuropein present in the by-products mainly into hydroxytyrosol, tyrosol, eleanolic acid and glucose as derivatives by means of β-glucosidase or esterase is another enzymatic treatment suggested before or after the second step of the process. The olive polyphenol concentrate obtained is directed to be used as ingredients in many foods to impart a strong bitter profile to food products, to add antioxidant activity and anti-microbial properties to the foods, and consequently, to increase the food's shelf life.

Benavent [24] described an integral procedure for obtaining a concentrated liquid of organic material from olive mill wastewater, which contains a high concentration of polyphenols and is directed towards the food, cosmetic, pharmaceutical and farming industries due to its high antioxidant capacity. The procedure starts with a solid-liquid separation or centrifugation step to obtain the liquid fraction of the raw material, i.e. of alperujo or alphechin. The liquid fraction is subjected to centrifugation, biological treatment with pectolytic enzymes and amylases with the aim of increasing its filterability, alcohol fermentation of sugars and filtration procedures before being concentrated using a multiple step vacuum evaporator. The filtered alphechin is reported to contain 1.2-1.6g/L hydroxytyrosol, 0.2-0.4g/L tyrosol and 0.1-0.2g/L oleuropein, which can be used as natural antioxidants. Other polyphenols obtained are notified as caffeic, vanillic, ferulic and *p*-coumaric acids. These compounds can be recovered by using selective resins to obtain a compound rich in antioxidants for dietary uses. The product concentrated in an evaporator has the characteristics of being a colloidal suspension, dark grayish-brown, slightly acidic, and free of sedimentable solids. The ultimate product contains polyphenols with the following concentrations: hydroxytyrosol 12.0-16.0g/L; tyrosol 2-4g/L; and oleuropein 1.0-2.0g/L.

The patent application by Leuenberger and Ulm [25] is directed to a process to manufacture a powder comprising vegetation water rich in water-soluble polyphenols. The water-soluble polyphenols are consisted of hydroxytyrosol, oleuropein, tyrosol and their mixtures. The process fundamentally comprises spry-drying or granulating the water-soluble part of the vegetation water. A centrifugation technique to separate the water soluble part of vegetation water can be optionally used before spry-drying or granulating. The spry-dying step is performed, preferably in the presence of an adjuvant such as a monosaccharide, a disaccharide, an oligosaccharide and a polysaccharide, where drying air inlet and outlet temperatures are 120-200^{o}C and 75-90^{o}C, respectively. The granulating step is carried out by using fluid-bed granulation, extrusion granulation or mixer-granulation procedures. The obtained powder comprising vegetation water rich in polyphenols can optionally be washed with an organic solvent. The organic solvent used in the last step is suggested to be dichloromethane, *n*-hexane, acetone, toluene, *i*-propanol, ethyl acetate or their mixtures. The amount of the water-soluble polyphenols/hydroxy-tyrosol in the ultimate powder is targeted to be at least ≥25 wt% based on the total weight of the powder.

Crea [26] claimed obtaining hydroxytyrosol from vegetation water produced from olives, preferably depitted olive meat. The vegetation water is incubated in the presence of an acid, preferably in the presence of citric acid, for a period until at least 75% of oleuropein originally present in the vegetation water has been converted to hydroxytyrosol. For purification, it is claimed to recover hydroxytyrosol-rich composition, including up to 99wt % hydroxytyrosol, by contacting vegetation water with a SCF. In one embodiment, porous membranes, configured as hollow fiber bundles or spiral wound sheets, is used for SCF extraction instead of contacting columns. Supercritical CO_2, flowing on one side of the membrane, extracts hydroxytyrosol from liquid passing through the membrane within a pressurized module. Hydroxytyrosol produced by this method is reported to be

Table 2. Comparison of Techniques Used for Recovery of Antioxidants from OMW

Technique	Additional Chemicals	High Pressure	High Temperature	Phase Change	Application
Solvent extraction	Organic solvent	-	-	-	Individually or prior to membrane separation
SCF extraction	SCF preferably SC CO_2	+	+	+	Individually or prior to membrane separation
Membrane separation	Membrane	+	-	-	After solvent extraction, usually in a sequential form
Column chromatography	Resin; regeneration solution; elution solvent	-	-	-	Individually or after solvent extraction or membrane separation, usually in a sequential form

useful as antibacterial, antiviral and fungicidal product for agricultural applications.

Lopez Mas *et al.* [27] used an extraction process to recover hydroxytyrosol from olives and/or from the solid residues of olives after carrying out an acid hydrolysis at 105-140^{o}C, pH 1.0 to 6.0 and pressure 10 to 20psi, followed by filtration and/or centrifugation to clarify the mixture. These steps are followed by purification in two columns containing anionic resins and non-ionic resins, in sequence; both columns being eluted with water to recover hydroxytyrosol. The acid-activated anion exchange resins are polyamine-type resins such as Diaion WA10, WA20, WA21, WA30; Amberlite IRA-35, IRA-67 (IRA-68), IRA-93ZU, IRA-94S, IRA-478; and WGR-2, where the non-ionic resins are styrene and divinylbenzene copolymers such as Amberlite.RTM. XAD-4, XAD-7, XAD-1180, XAD-1600; XUS-40323.00, XUS-40285.00, XUS-40283.00; SP-700, SP-825, SP-850; Diaion HP 10, HP 20, HP 30, HP 40 and HP 50. The liquid product is then concentrated by reverse osmosis and is brought to a solid form by freeze-drying, vacuum evaporation or spry-drying, with or without carriers such as maltodextrines. According to a preferred aspect of the invention, the solid product possess a hydroxytyrosol content of at least 90% (w/w), a purity of at least 90% and total phenols content of at least 92%.

CURRENT AND FUTURE DEVELOPMENTS

The survey and analysis of recent patent documents on the recovery of phenolic antioxidants from olive mill wastewater show that the companies have been seeking a simple, effective and non-expensive process that provides a concentrate rich in antioxidants, particularly in hydroxytyrosol, with a high yield and purity. However, despite considerable research efforts have been made, the number of patented system that is successful for its exploitation is limited. The system patented by Fernández-Bolaños *et al.* [21] was commercialized and the ultimate products are marketed under the trademark Hytolive®1 and Hytolive®2 [28]. Organic solvent extraction, filtration, chromatographic adsorption, centrifugation and steam explosion techniques were mostly employed to recover polyphenols from OMW in early studies. The recent patent applications, nevertheless, use supercritical fluids instead of organic solvents in extraction procedures, and employ tangential ultrafiltration and reverse osmosis systems instead of conventional filtration procedures in membrane processes. All processes developed usually employ pre-treatments such as steam explosion, acidic or enzymatic hydrolysis and centrifugation to remove impurities from OMW. It is doubtless that the employment of these methods has some complexities resulting not only from their cost and operational characteristics, but also from hard and varying chemical characteristics of OMW. Although these techniques minimize the wastes generated in the recovery process, the requirements of comparatively high pressure equipment or expensive membrane materials prevent them to become economically feasible processes for the recovery of antioxidants from OMW. A comparison of techniques used in antioxidant recovery from OMW is given in Table **2**. The interest on membrane technologies in processing olive oil by-products as represented in this paper indicates that the future direction of the processes for the recovery of antioxidants from OMW is presumably toward the utilization of membranes in a sequential design. The further developed processes will possibly involve some enzymatic and hydrothermal pre-treatments to provide higher purity in the antioxidant recovered, and will be most probably able to employ other by-products of olive processing industry in addition to OMW. The improvement of the antioxidant stability of the recovered product is another topic to be considered important for the advanced studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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