JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Literature Review on Production Process To Obtain Extra Virgin Olive Oil Enriched in Bioactive Compounds. Potential Use of Byproducts as Alternative Sources of Polyphenols

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ABSTRACT: This review describes the olive oil production process to obtain extra virgin olive oil (EVOO) enriched in polyphenol and byproducts generated as sources of antioxidants. EVOO is obtained exclusively by mechanical and physical processes including collecting, washing, and crushing of olives, malaxation of olive paste, centrifugation, storage, and filtration. The effect of each step is discussed to minimize losses of polyphenols from large quantities of wastes. Phenolic compounds including phenolic acids, alcohols, secoiridoids, lignans, and flavonoids are characterized in olive oil mill wastewater, olive pomace, storage byproducts, and filter cake. Different industrial pilot plant processes are developed to recover phenolic compounds from olive oil byproducts with antioxidant and bioactive properties. The technological information compiled in this review will help olive oil producers to improve EVOO quality and establish new processes to obtain valuable extracts enriched in polyphenols from byproducts with food ingredient applications.

KEYWORDS: *EVOO, byproducts, polyphenol, production process, antioxidants, bioactive*

INTRODUCTION

The traditional Mediterranean diet is characterized by preferential consumption of vegetables, legumes, fruit, nuts, and cereals, as well as olive oil being the main fat in the diet.[1−3] Extra virgin olive oil (EVOO), extracted from fresh and healthy olive fruits (*Olea europaea* L.), properly processed and stored at low temperatures, is characterized by increased oxidative stability and unique aroma highly appreciated by consumers.[4−7]

EVOO is a natural juice obtained exclusively by mechanical and physical processes, in contrast to other edible oils, namely, sunflower and soybean oils, which must be refined before consumption, thus changing their original composition during this process.[8] The consumption of EVOO is associated with a low incidence of cardiovascular diseases, neurological disorders, and breast cancer.[9−11] Recently, several minor components have been related to the olive oil healthy properties, mainly polyphenols. These compounds are also associated with the oxidative stability and flavor characteristics of virgin olive oil.[12−14] However, the phenolic composition of EVOO is influenced by complex multivariate interactions from genotype, agronomical, environmental, and technological factors.[15] The qualitative and quantitative phenolic composition of EVOO is widely affected by many variables related to production processes, from the ripening stage of olive fruits to storage conditions.[16] The steps of the production process include collecting, washing, and crushing of olives, malaxation of olive paste, centrifugation, storage, and filtration. Most quality attributes of EVOO are determined by the chemical composition and biochemical status of the olive fruit.[17] Milling and malaxation are considered as the most critical steps during olive processing and oil extraction as the most important changes in EVOO phenolic composition.[15] However, qualitative and quantitative changes take place in olive oil polyphenols during storage and filtration.[18−20] Consequently, rigorous controls of all olive oil processes are recommended to produce olive oil of high phenolic quality.[21] Unfortunately, the production of EVOO is associated with the generation of large quantities of wastes,[22,23] and it is associated with the loss of olive polyphenols in olive oil byproducts. The well-known bioactivity of olive polyphenols[24] has stimulated qualitative and quantitative characterization of the phenolic profile in these wastes.

The aim of this review is focused on (a) the effect of different production processing steps taking into account the latest technological innovations to establish the best flow diagram for EVOO enriched in polyphenols, (b) phenolic composition and its bioactivity of some byproducts generated during the EVOO production process, and (c) development of pilot plant and industrial processes to recover polyphenols from olive oil byproducts.

Received: October 5, 2012
Revised: May 8, 2013
Accepted: May 9, 2013
Published: May 9, 2013

dx.doi.org/10.1021/jf400806z | J. Agric. Food Chem. 2013, 61, 5179−5188

EXTRA-VIRGIN OLIVE OIL PRODUCTION PROCESS: TECHNOLOGICAL ALTERNATIVE TO OBTAIN OLIVE OIL ENRICHED IN PHENOLIC COMPOUNDS

Harvest Period. The olive ripening stages that include harvest time and maturity index are the most important factors associated with the quality evaluation of olive oil. Indeed, during the ripening, several metabolic processes take place in olives followed by variations in the phenolic composition due to different biosyntheses and biotransformation pathways of phenolic compounds. Different anabolic and catabolic pathways in olive fruits were established from Arbequina and Hojiblanca cultivars,[17] related to the oleuropein and its derivatives and to the activity of β-glucosidase during the growth and ripening of olive fruits. The main phenolic compounds and derivatives, including hydroxytyrosol, ligstroside aglycon, oleuropein aglycon, acetoxy-pinoresinol, and elenolic acid, showed an increase in EVOO at the early stages of olive harvest, followed by a reduction of their concentrations at more advanced stages of maturity.[25] Consequently, early harvested fruit produces olive oil with high polyphenol content and high oxidative stability. It is well-known and widely accepted that both phenolic amount and oxidative stability are linked to the antioxidant capacity of EVOO polyphenols. However, these compounds have also been associated with the flavor characteristics, and harvesting too early produces olive oils that have occasionally unacceptable sensory quality due to excessive polyphenol concentrations. It was suggested that the majority of olive oil produced does not have the best commercial quality because the fruit has not been picked at the optimal harvest time.[26] There is therefore a need to determine the most appropriate maturation stage of each olive cultivar before processing the olive fruit. According to literature reports, it is possible to establish that the best harvest time is carried out early, when the fruit reaches optimum ripeness, when the "*envero*" or the change in color of olives starts taking place. From the point of view of phenolic composition, oxidative stability, and organoleptic properties, the best olive oil was shown to be obtained with maturity index values between 2.5 and 3.5 in Nostrana di Brisighella cultivar cultivated in Jaen, Spain.[27]

Crushing. Crushing of olives is a physical process used to break the fruits' tissues and release the oil drops contained in the vegetable cell vacuoles. The olive paste preparation is currently performed in industrial oil mills either with the traditional discontinuous stone-mill or with the continuous hammer crusher. The latter is mainly used in the olive oil industry where the oil extraction is usually performed by centrifugation.[28]

Olive crushing is one of the most important steps that affect the phenolic profile of EVOO produced.[29] Indeed, after olive crushing, several enzymes that can be activated are involved in the generation and transformation of phenolic compounds. Secoiridoid aglycons such as the elenolic acid linked to hydroxytyrosol and the decarboxymethylated form of elenolic acid linked to hydroxytyrosol and to tyrosol are produced during crushing, by hydrolysis and loss of carboxymethyl groups of oleuropein, dimethyl-oleuropein, and ligstroside. These changes take place when the reaction is catalyzed by the endogenous β-glucosidases followed by other chemical reactions.[30]

The systems used and crushing conditions have an influence on these reactions and the partitioning behavior of polyphenols. A comparative study carried out between both systems described above, traditional discontinuous stone-mill and continuous hammer crusher, showed that phenolic compounds were better preserved in olive oil obtained with the continuous system.[31] These results were associated with the homogeneous and smaller sizes of the solid fragments obtained by the continuous hammer crusher, favoring the substance exchange process between the oily phase and the aqueous phase of the olive paste. The crushing conditions, which have been evaluated by several authors, include the use of a hammer crusher, the grid hole size, and rotation speed. Stronger conditions using smaller grid holes and higher rotation speed increase the final phenolic content of EVOO, this effect being higher for the hydroxytyrosol than for tyrosol.[29]

Malaxation. Malaxation of the olive paste is carried out with a stainless steel device made of a semicylindrical vat with a horizontal shaft, rotating arms, and blades of different shapes and sizes. This vat is equipped with a heating jacket, circulating hot water to warm the olive paste.[32] The efficiency of malaxation depends on the rheological characteristics of the olive paste and the technological parameters of the operation, such as temperature and time.[33]

Regarding the phenolic composition, temperature, time, and the activity of several enzymes are involved in the evolution of these compounds during the malaxation step. On one hand, it has been described that increasing malaxation temperature from 15 to 30 to 37 to 42 °C and times from 20 to 45 to 60 min improved the phenol contents and oxidative stability of EVOO. On the other hand, a longer malaxation time more than 60 min apparently affected the phenol contents negatively.[34] However, in another study the secoiridoid group showed a quasi-linear increment of concentrations with increasing temperature up to 30 °C, followed by a corresponding marked decrease with the highest malaxation temperatures (33 and 36 °C).[35] Furthermore, increasing the temperature during the olive paste malaxation process increases the activity of oxidoreductase enzymes such as polyphenol oxidase present in olive fruit which is rather high at 35 °C. The lipoxygenases that catalyze the formation of hydroperoxides could also be responsible for an indirect oxidation of secoiridoids. Another active enzyme, β-glucosidase, could play a role in the production of phenol-aglycons (secoiridoids) by hydrolysis of the oleuropein and dimethyloleuropein.[32] These enzymatic activities explain also the lineal increase of hydroytyrosol and tyrosol obtained by degradation of complex phenolic compounds during malaxation.[36]

The most important losses of different phenolic groups present in olive paste occur in the solid phase (wet pomace) and aqueous phase by the low lipophilic behavior of the phenolic structures that led to a low concentration in EVOO.[37] However, in some EVOOs, a low phenolic concentration may improve their sensory quality. Cornicabra EVOO obtained under malaxation conditions of temperature below 28 °C and a time longer than 60 min improved its bitterness by reducing phenolic content, and the expected decrease in oxidative stability would not affect its shelf life.[38]

Centrifugation. Centrifugation is usually applied for a primary separation of the olive oil fraction from the vegetable solid material and vegetation water. This step may be carried out using the combination of two different systems: horizontal centrifugation (three- and two-phase decanter) and vertical centrifugation. Horizontal centrifugation using three-phase decanter requires the addition of warm water to dilute the

Table 1. Best Process Conditions To Produce EVOO with High Phenolic Content

steps	control parameters	conditions	systems	refs
ripening	maturity index or harvesting time	early harvest time with low value of maturity index		17, 25
crushing	techniques, grid hole diameter, and rotation speed	small grid holes and high rotation speed	hammer crusher	29, 31
malaxation	temperature and time of malaxation	temperature lower than 30 °C and time shorter than 60 min		34−36
horizontal centrifugation	two- and three-phase decanter	without addition of warm water	two-phase decanter	40, 42
vertical centrifugation	water	small water amounts added		44
storage	time, temperature, oxygen, and light	short time, room temperature, darkness, and absence of oxygen		49
filtration	filter aids	nitrogen gas flow	filtration using inert gas	20

olive paste to facilitate the separation described above[39] while the two-phase decanter consists of "no-water" centrifugation plants for separating the oily phase from malaxed pastes without requiring adding warm water. It should be considered that the two-phase decanter requires a minimal moisture value on the olive paste (50%) to facilitate the separation process. When this value is not reached, a low amount of water is loaded into the decanter.[40,41] This decanter has the advantage of recovering more complex hydrophilic phenolic compounds and preserving them more efficiently in EVOO than by the three-phase method.[34,39,42] Concerning vertical centrifugation, this system is used to separate the oily must obtained from horizontal centrifugation. Phenolic composition of the wash water added during this step has also been characterized. Hydroxytyrosol, tyrosol, and the dialdehydic form of elenolic acid linked to hydroxytyrosol were the most representative phenolic compounds identified.[43] However, another study showed a slight variation in the concentrations of phenolic compounds when the comparison between olive oil composition before and after vertical centrifugation process has been carried out. It could be attributed to the small water amounts added in the experimental conditions developed by the research.[44] Therefore, the best way to reduce the loss of phenolic compounds during horizontal centrifugation (two and three phases) and vertical centrifugation is established by the equilibrium between the volume of water added and by a good separation of phases.

Storage. In the Mediterranean area, olive oil is generally produced from September to February and stored in the mill until filtration and commercialization. Several studies have focused on the possible hydrolytic and oxidative degradation of phenolic compounds present in EVOO over the shelf life in commercial containers. As expected, after storage for 9 months the peroxide values increased and the total phenol content and oxidative stability of olive oil decreased.[45] With the aim of understanding chemical changes produced in EVOO polyphenols, different stress conditions have been applied. In this way, EVOO samples were kept in the dark at 60 °C for up to 7 weeks and removed every week from the oven to carry out the analysis. The results showed that secoiridoids were apparently oxidized.[46] In a more recent study, EVOO samples were stored in different commercial containers (glass, polyethylene terephthalate, and Tetra-Brik1) at room temperature (20 °C) and refrigerated temperature (4 °C). After 9 months of storage, the smallest decrease in phenolic content was in EVOOs stored in Tetra-Brik, due to a minor degradation process by preventing the passage of light and oxygen.[47]

The oxidation and storage conditions applied in these studies do not exactly reflect the real storage conditions of EVOO in the mill companies until the oil is sold. The phenolic patterns discerned could depend on storage conditions including time, temperature, oxygen availability, and industrial or commercial containers.[48] The formation of oxidized and hydrolyzed products and changes in the phenolic patterns of EVOO after storage for 10 months in industrial tanks without headspace at room temperature in the dark have also been evaluated. Degradation pathways were proposed based on the half-life, elimination, and appearance rate of the complex phenolic compounds, and their oxidized and hydrolyzed products.[49] In fact, when the correlation for the pair oleuropein aglycon or decarboxymethyl oleuropein aglycon and their hydrolyzed derivatives was evaluated, the determination coefficients of the mathematical function proved higher than 0.950.

From all studies carried out, the higher phenolic contents and oxidative stability were obtained when the EVOO was stored for shorter times under the best conditions of temperature, light, and oxygen.

Filtration. Filtration is a special important final step to remove suspended solids and moisture to produce a brilliant olive oil for consumer acceptance. Different filtration systems have been applied in the pilot plant and olive oil industry, including conventional filtration systems (filter tanks and filter presses), cross-flow filtration (tangential flow filtration), inert gas flow filtration systems, and filter bags. Controversial results were published by different authors on how filtration affects the phenolic composition of EVOO. A laboratory scale study[50] showed that similar amounts of phenolic compounds were found with almost all cultivars in unfiltered as in filtered EVOO. Another laboratory scale study[18] showed that the hydroxytyrosol concentration decreased after filtration with cotton compared to the unfiltered olive oils. However, an apparent increase in hydroxytyrosol was produced in EVOOs after filtration with paper plus anhydrous sodium sulfate. A pilot plant scale study[20] using inert gas flow filtration systems and filter bags showed that the concentration of the most phenolic compounds seemed to increase after filtration. Among these, mainly secoiridoids were responsible for the apparent increase in the total phenolic content. Regarding the oxidative stability, it was reduced after filtration by the effects of water content on the polyphenolic antioxidant capacity. However, filtration of olive oil in the presence of inert gases did not decrease the main positive sensory attributes.

Filtration is therefore recommended because moisture reduction improves the quality of EVOO. Furthermore, the higher polar phase content in unfiltered olive oils may augment

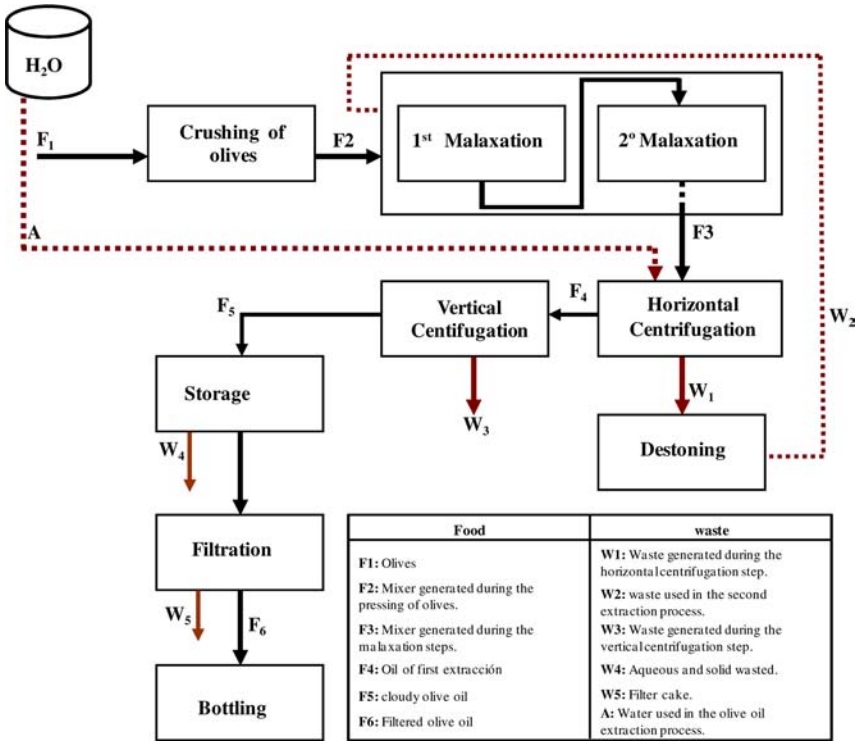

Figure 1. Flow diagram of EVOO production industrial process.

the degradation process and reduce the shelf life. From the phenolic composition point of view, the effects of this step are controversial. Indeed, in polar matrix such as unfiltered EVOO, the affinity of phenols to solvent extraction is lower than in a filtered EVOO, and the majority of phenolic compounds located around water droplets remain in unfiltered olive oil. Future investigations are warranted to develop a new analytical methodology taking into account the different water content in unfiltered and filtered EVOO and its effect on the extraction process used to qualitative and quantitative characterization of these compounds.

Table 1 shows the best process conditions to produce EVOO with high phenolic content.

Olive Oil Byproducts. A huge quantity of olive byproducts produced from olive processing of different kinds have been described in the literature according to the extraction, filtration, and storage systems. Traditionally, large volumes of water are used in the three-phase mill to aid the separation of olive oil and generate two byproducts. The first byproduct of liquid waste is known as olive mill wastewater, vegetation water, or *alpechin*. The second byproduct is a solid waste called *pomace* or *orujo*. The use of a modern two-phase processing technique, in which no water is added, generates a new byproduct called *alperujo* or *pomace* and includes a combination of liquid and solid waste.[51] Other olive oil byproducts generated by storage and filtration of EVOO are composed of solid and liquid storage wastes and cakes used for EVOO filtration.[52,53] Figure 1 shows the many processing steps of olive oil producing food and waste byproducts, including crushing, malaxations, two centrifugations, destoning, storage, filtration, and bottling.

Olive Mill Wastewater. The olive mill wastewater (OMWW) is a liquid of violet to dark brown color with a strong smell of olive oil.[54] This byproduct is composed of vegetable water from the fruit and the water used in different stages of oil extraction that contain olive pulp, mucilage, pectin, oil, and other suspended components in a relatively stable emulsion.[55] The chemical composition of OMWW is variable depending on olive cultivars, growing techniques, harvesting period, and especially the technology used for oil extraction.[56,57] Olive wastewater is characterized by diverse specific components and a high degree of organic pollution [chemical oxygen demand (COD) and biological oxygen demand (BOD)], acidic pH, high electrical conductivity, and phenolic content. Many published physical−chemical characteristics of OMWW show wide variations in pH, electrical conductivity, chemical and biological oxygen demand, and total phenolic compounds.[58−63] The composition and amounts of the OMWW are serious environmental problems in the Mediterranean areas, and the discharge of large quantities of these pollutants in the sewage system is not possible without any treatment. Different biological and chemical/physical methods have been proposed to reduce the organic matter, polyphenols, and tannins present in OMWW to detoxify their effects on the environment.[64−66]

On the other hand, phenolic extracts from OMWW can be used as natural alternatives to commercial synthetic antioxidants with applications in food as well as the development of nutraceutical and medical products.[67−69] According to the extraction process, the partitioning behavior of polyphenols and their distribution between the oil and waste fractions are affected by the processing temperature and the quantity of water used for extraction. Although the partitioning of polyphenols into the oil is increased at higher temperatures, more amphiphilic polyphenols are lost in the wastewater if more water is added.[70]

Different analytical extraction methods have been used to recover the phenolic components of OMWW which include oleuropein aglycon derivatives, elenolic acid, luteolin 7-glucoside, quercetin, and phenolic alcohols.[71] Moreover, 20 phenolic compounds have been identified and 16 were

quantified in the olive wastewater of Canino olives using high performance liquid chromatography (HPLC) coupled to mass spectrometry (MS).[72] Phenolic compounds were also recovered by liquid−liquid extraction from centrifuged OMWW and characterized by gas chromatography coupled to MS.[73] The phenolic compounds identified in samples include hydroxytyrosol as the major component (66.5%), together with tyrosol, cafeic acid, *p*-coumaric acid, homovanillic acid, protocatechuic acid, 3,4-dihydroxymandelic acid, vanillic acid, and ferulic acid. Furthermore, the phenolic compounds were identified and quantitated in two different OMWW samples; hydroxytyrosol was the most abundant compound and represented about 70% and 55% of the total phenolic concentration of both OMWW extracts.[74] Other phenolic compounds which have also been characterized in OMWW include verbascoside, isoverbascoside, *β*-hydroxyverbascoside, and various oxidized phenolic compounds.[75,76]

Olive Pomace. Olive pomace consists of olive pulp, skin, stone, and water. Different terms may be given depending on factors such as composition and oil content (crude or extracted olive pomace), stones, or moisture (fresh or dry olive pomace).[77] The different olive oil extraction procedures and resulting byproducts have recently been documented.[51] The olive pomace obtained from the two-phase extraction procedure may be differentiated by the higher moisture and the lower oil content than from the three-phase centrifugation procedure, resulting in a more efficient and environmentally friendly centrifugation process, compared to the traditional three-phase system.

Table 2 shows the composition of olive pomace produced by three- and two-phase decanter.[78] Thus, olive pomace is an inexpensive biomass that is generated in large quantities in Mediterranean countries that also represents serious environmental problems.[79] Many studies have been aimed at reducing the environmental impact of olive pomace and/or harnessing its potential economic value. Olive pomace has been used as fuel, fertilizer, or animal feed.[80,81] Nevertheless, the profitability of olive pomace treatment plants is still in doubt because these activities only represent a very small percentage of the olive pomace produced.

Table 2. Quantity and Characteristics of Virgin Pomace Obtained with Different Extraction Systems for Olive Oil

measurements	3-phase decanter	2-phase decanter
quantity (kg/t olives)	450−550	800−850
moisture (%)	45−55	65−75
oil (% on fresh pomace)	3.5−4.5	3−4
pulp (%)	15−25	10−15
stones (%)	20−28	12−18
ash (%)	2−4	3−4
nitrogen (mg/100 g)	200−300	250−350
phosphorus (mg/100 g)	30−40	40−50
potassium (mg/100 g)	100−150	150−250
total phenolic compounds (mg/100 g)	200−300	400−600

Evidently, there is a demand for alternative benefits from olive pomace, which is characterized by high contents of polyphenols.[78] The potential antioxidative activity of polyphenols in olive pomace would provide a cheap source of natural antioxidants in concentrations up to 100 times higher than in EVOO.[82] In fact, many scientific studies have been published on the phenolic characterization of olive pomace.[83−86] Methanolic extracts of olive pomace (two-phase extraction) were analyzed by HPLC−MS. Phenolic compounds identified included phenolic alcohols, flavonoids, and secoiridoids, including 10-hydroxyoleuropein, identified for the first time. In the same study, the comparison between olive pulp and olive pomace showed a change in phenolic structure. Because some phenolic compounds were not degraded during olive oil extraction, the olive pomace from the two-phase system may be considered as a good source of these compounds as olive pulp.[87]

Different studies were carried out to use phenolic extracts from olive pomace to develop potential applications as food antioxidants. Edible oils and other foods were enriched with polyphenols extracted from olive pomace, and many phenolic compounds have been identified.[88,89] Studies of the optimization, characterization, and quantification of phenolic compounds in olive pomace showed that the highest yield of total phenolic compounds was achieved by extraction with methanol at 70 °C for 12 h.[85] The major bound phenolic compounds in full-fat olive pomace included syringic acid (22%), protocatechuic acid (21%), caffeic acid (14%), sinapic acid (13%), and rutin (12%). In defatted olive pomace the relative concentrations were 23%, 14%, 11%, 17%, and 8% respectively.

Olive Oil Byproducts Generated by Storage and Filtration of EVOO. The potential use of waste generated during the storage of EVOO as a natural source of phenolic antioxidant compounds has been evaluated[52] by solid−liquid and liquid−liquid extraction processes followed by rapid resolution liquid chromatography (RRLC) coupled to electrospray time-of-flight and ion trap mass spectrometry (TOF/IT-MS). Several degradation pathways of phenolic compounds were proposed based on hydrolysis, oxidation, hydration, and loss of the carboxylic group. These reactions occur during storage time, and the byproducts generated may be considered an important natural source of secoiridoid derivatives and flavones, mainly hydroxytyrosol, tyrosol, decarboxymethyl oleuropein aglycon, and luteolin. In solid waste, the dialdehyde form of decarboxymethyl-elenolic acid was the most abundant derivative, followed by hydroxytyrosol, luteolin, vanillin, and decarboxymethyl-oleuropein aglycon. Although the aqueous waste contained a small amount of phenolic compounds, it contained the highest amounts of phenolic alcohols.

Filtration may be carried out with various materials or filter aids in combination with filtration hardware to improve performance. Filter cake used during filtration could be used as a source of bioactive compounds. The hydrophilic phenolic compounds retained in different organic and inorganic filter aids included phenolic acids and alcohols, secoiridoids, lignans and flavones, vanillin, vanillic, ferulic, and *p*-coumaric acids, tyrosol, and hydroxytyrosol.[53] Although the healthy properties of the polyphenols have been identified in the wastes and in the byproducts and filter cake,[90−93] additional investigations are needed to evaluate their applications as food antioxidant and nutraceutical products.

Figure 2 shows the structure of the main phenolic compounds from each family identified in olive oil byproducts.

BIOLOGICAL ACTIVITIES AND POTENTIAL ANTIOXIDANTS OF OLIVE MILL WASTEWATER POLYPHENOLS

Several in vitro and in vivo studies showed that OMWW phenolic compounds exert potent biological activities including,

dx.doi.org/10.1021/jf400806z | J. Agric. Food Chem. 2013, 61, 5179−5188

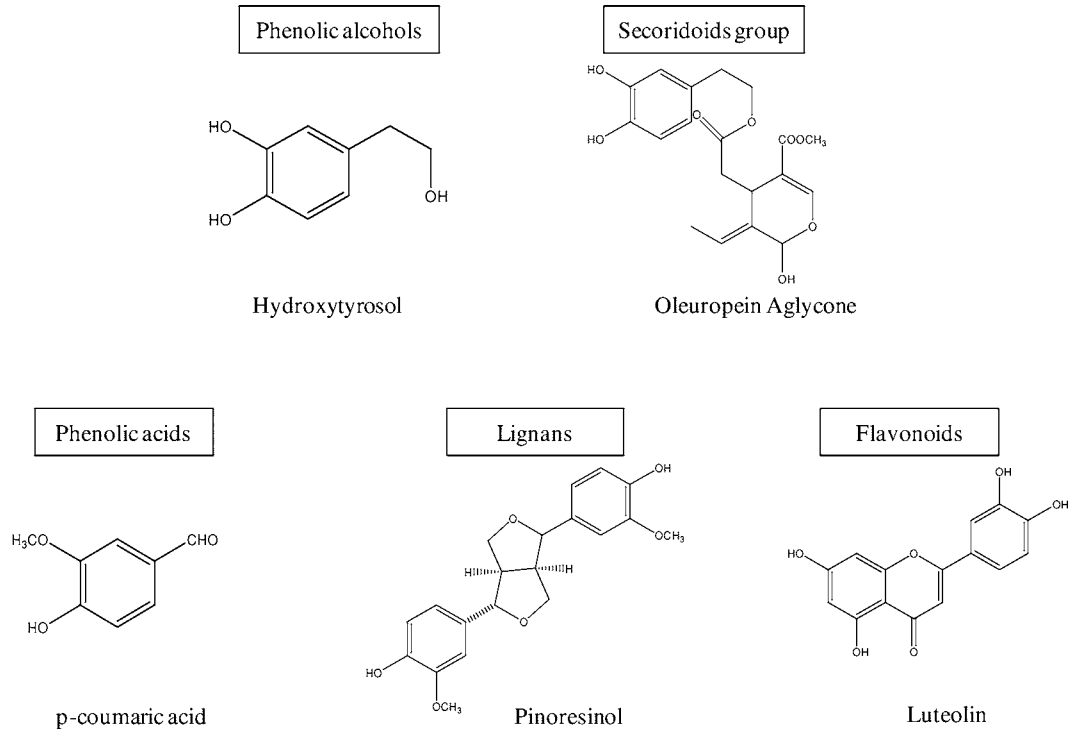

Figure 2. Structures of the main phenolic compounds from each family identified in olive oil byproducts.

but not limited to, antioxidant and free radical scavenging properties.[24,90,94] Hydroxytyrosol from OMWW is one of the most promising compounds as a potent inhibitor of copper and peroxyl radical-induced oxidation of low-density lipoprotein (LDL), representing one of the initial steps in the onset of atherosclerosis. Hydroxytyrosol may also scavenge free radicals and modulate several enzymatic activities linked to cardiovascular diseases. The superoxide anion scavenging activity of four OMWW phenolic extracts in cultured human promonocyte cells (THP-1) has also been described,[95] being attributed to hydroxytyrosol as the most active component responsible. Moreover, the administration of OMWW extract fractions and purified hydroxytyrosol to diabetic rats caused a decrease in the glucose level in plasma.[96−98]

On the other hand, the efficacy of a hydroxytyrosol-rich OMWW extract to attenuate Fe^{2+} and nitric oxide (NO) induced cytotoxicity in murine-dissociated brain cells was supported by ex vivo data providing the first evidence of neuroprotective effects of oral hydroxytyrosol intake.[99] Besides, to better understand the absorption potential for verbascoside and its derivatives recovered from OMWW, both in vitro digestion and Caco-2 human intestinal cell absorption studies were carried out to establish digestive stability and recovery (bioaccessibility) and efficiency of intestinal uptake/accumulation. During the experiment carried out, verbascoside was found to be moderately stable to in vitro digestive conditions with recovery of 53%, and its uptake by highly differentiated Caco-2 monolayers was rapid with peak accumulation occurring after 30 min. The total accumulation efficiency was 0.1% of the original amount of verbascosides present in a partially purified phenolic fraction of OMWW.[75] The verbascoside derivatives present in OMWW were also shown to provide a rationale in subsequent bioavailability and bioactivity studies. In another study, individual verbascoside from OMWW was active as a scavenger of reactive oxygen species and as a chemopreventive agent protecting LDL from oxidative damage.[76]

BIOLOGICAL ACTIVITIES AND POTENTIAL ANTIOXIDANT OF OLIVE POMACE

The antioxidant activity of olive pomace due to its phenolic content has been evaluated and demonstrated by several authors. A positive correlation was reported between olive pomace total phenolic content and the antioxidant activity; these results suggest that the phenolic compounds in olive pomace could be used at different concentrations as antioxidant foods over the shelf life.[85] It has recently been reported that the oxidative stability of EVOO and other edible oils was improved by using phenolic compounds extracted from olive pomace.[88,89] Finally, the analysis of rat tissues obtained after administration of a phenolic extract from olive pomace[83] showed a wide distribution of phenolic compounds and their metabolites, with a main detoxification route through the kidneys. The free forms of some phenolic compounds, such as oleuropein derivative, were quantitated in plasma and brain, luteolin in kidney, testicle, and heart, and hydroxytyrosol in plasma, kidney, and testicle.

PILOT PLANT AND INDUSTRIAL PROCESSES TO RECOVER PHENOLIC COMPOUNDS FROM OLIVE OIL BYPRODUCTS

Taking into account the phenolic composition of olive oil byproducts and their biological activity, these wastes may be used as valuable sources of components for nutraceuticals, food, and pharmaceutical preparations or in the cosmetics industry.[100] Although diverse synthetic procedures have been developed for the production of hydroxytyrosol and other phenolic compounds, the technological processes proposed so far are expensive and/or produce low yields.[101] Consequently, other types of natural compounds that could be used as

antioxidants are urgently needed. Several extraction and purification technologies have been reported to obtain polyphenol enriched extracts, mainly in hydroxytyrosol, from olive oil byproducts. The main systems proposed to recover the phenolic compounds from olive waste include resin chromatography, microfiltration, ultrafiltration, nanofiltration reverse osmosis, and solid−liquid or liquid−liquid solvent extractions.[102]

A patented system proposed to purify hydroxytyrosol from OMWW[103] includes passing the liquid source of hydroxytyrosol through an ion-exchange resin to trap the antioxidant and eluting with water, followed by adsorption through an XAD-type nonionic resin. This matrix is washed with mixtures of methanol or ethanol and water (30−33%), to produce a solution containing at least 75% of hydroxytyrosol, followed by removal of the polar organic solvent to produce a solid containing 95% by weight of hydroxytyrosol, plus significant fractions reaching up to 99% of purity. Another patented process is claimed[104] for totally recovering the polyphenolic compounds in OMWW to reuse the concentrate residues in the production of fertilizers, biogas, and highly purified aqueous products that may also be used as a basic component of beverages. The process includes adjusting the pH of the freshly produced wastewater to within an acidic range, and an enzymatic hydrolysis followed by separation of the permeate streams obtained, by means of centrifugation and subsequent treatments with membrane technologies, consisting of microfiltration, ultrafiltration, nanofiltration, and reverse osmosis.

Another pilot scale system for the treatment of OMWW was developed for the recovery of valuable polyphenols and reduction of environmental problems.[105] The treatment consists of four steps: (a) successive filtration stages to gradually reduce and decolorize water suspended solids, (b) passage of the filtered wastewater through adsorbent resins to deodorize and decolorize the wastewater and recover the polyphenol and lactone components, (c) thermal evaporation and recovery of the organic solvent mixtures used to regenerate the resin, and (d) separation of the polyphenols and other organic substances by fast centrifuge partition chromatography. This procedure is claimed to reduce 99.99% of the polyphenols and 98% of chemical oxygen demand (COD) and to produce an extract rich in polyphenols and lactones of high antioxidant activity and added value, and an extract containing the coloring substances of olive fruit, and pure hydroxytyrosol. The extracts and pure compounds obtained are claimed to be useful not only for the pharmaceutical and cosmetic industries but also to produce wastewater free of polyphenols.

In another study, the application of a novel process based on the hydrothermal treatment of olive oil waste (alperujo) led to a final liquid phase that contained a high concentration of simple phenolic compounds.[106] During thermal treatment, either 10 or 20 kg of alperujo was loaded into a 100 L reactor and for different heating times (15−90 min) was evaluated at 160 °C. The wet material was then centrifuged at 4700*g* to separate solids and liquids. After centrifugation, 10 L of the liquid phase from each treatment was concentrated to 1 L by rotary vacuum evaporation at 30 °C. A maximum concentration of phenolic extract (11 g/kg) was finally obtained after 75 min of thermal treatment.

A new filtration process of EVOO to produce a filter cake enriched in polyphenols, which may be used as ingredient in functional foods and nutraceuticals, has been recently developed.[107] The filtration systems consist of using native starch as filter aid with filter tanks and filter presses. The composition of the final byproduct is based on native starch enriched in polyphenols, mainly hydroxytyrosol yields about 30 times higher than that from EVOO.

The interests in the healthy benefits of EVOO polyphenols have been increased due to recent different studies supporting their biological properties in reducing oxidative stress, especially when they are derived from more concentrated olive oil sources than in EVOO. Therefore, researching for the best ways of concentrating phenolic compounds in EVOO and recovering them from its byproducts could be very promising. To achieve this goal, many studies about the effects of olive oil process steps on final olive oil phenolic composition have been summarized to establish the best conditions to obtain EVOO with higher phenolic content. Taking into account the literature used in this review, harvesting too early, crushing olives using hammer crusher equipped with small grid holes and high rotation speed, malaxation of paste at temperature lower than 30 °C and time shorter than 60 min, centrifugation of paste using two-phase decanter followed by vertical centrifugation with a minimum water added, storage of EVOO at short time and low temperature, and filtration using inert gases, contributed to obtain EVOO enriched in phenolic compounds and to conserve its positive sensory attributes.

The next aim of this review was to summarize the qualitative and quantitative characterization of the phenolic compounds in olive oil byproducts. Phenolic alcohols, consisting of hydroxytyrosol, phenolic acids, secoiridoids, lignans, and flavonoids, were found as the main phenolic families. Furthermore, the antioxidant and biological activity of phenolic extracts from OMWW and olive pomace showed interesting results in all works carried out and summarized in the review. However, phenolic extracts from waste generated during storage of olive oil and filter cake have not been evaluated, and future investigations are needed to evaluate their applications in food antioxidant and nutraceutical products. Finally, different pilot plant and industrial processes employed to recover phenolic compounds from olive oil byproducts have been widely reviewed.

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Funding

We are grateful to the Spanish Ministry of Education and Science for Projects AGL2011-29857-C03-02, to the Andalusian Regional Government Council of Innovation and Science for Projects P09-CTS-4564, P10-FQM-6563, P11-CTS-7625, and GREIB.PT.2011.18, and to the University of California, Davis, CA, USA, the University of Granada, Functional Food Research and Development Center, the University of Bologna, and the International Olive Oil Council (IOC).

Notes

The authors declare no competing financial interest.

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