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Abstract

Olive mill wastewater (OMW) is a pollutant by-product from the virgin olive oil production. Its high content in phenolic compounds makes them play an important role for their use in foods, for their high antioxidant significance. The present paper gives an overview on the techniques for OMW valuable ingredient separation, focusing on the most effective ones for their use in food products as functional ingredients. We report on effective methods to recover OMW phenolics, and give several examples on the usethese extracts in foods. When added into vegetable oils, their effect on retarding lipid oxidation improves the oxidative status of the product, whilst several challenges need to be faced. OMW phenolic extracts were also used in food emulsions, milk products or other model systems, showing promising results and little or no negative impact on the sensory characteristics or other properties. Their possible use as antimicrobial agents is also another promising approach, as positive results were obtained when applied in meat products.

Other examples of using natural phenolic extracts from other sources are suggested also for OMW extracts, to expand their use and thus to improve the nutritional and technological quality of foods.

Keywords

olive biophenols; functional foods; antioxidant activity; “green” chemistry; food industry by-products; natural phenolic extracts

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1. Introduction

Virgin olive oil (VOO) is the product of the olive fruit, obtained using only physical means and avoiding any solvent extraction or use of chemicals. High amounts of by-products, both as solids and liquids, are produced to obtain VOO, and this represents a major environmental issue. In Mediterranean countries, which is the biggest olive producing area worldwide, huge amounts of agro-industrial wastewaters are generated from the olive processing industry (Hanifi & El Hadrami, 2009; Jarboui, Sellami, Kharroubi, Gharsallah, & Ammar, 2008).

The olive mill by-products have environmental, social and economic significance in the olive producing countries. The type and amount of by-products obtained from the olive mills strongly depend on the type of mill, as schematised in **Fig. 1**. In particular, the more traditional press-based mill produces a solid by-product possessing the lowest moisture compared to other systems. The liquid by-product of this system consists of the vegetation water, the water added during the malaxation and the fraction added during pressing and subsequent centrifugation. In the case of "3-phase" centrifugation system, the olive oil, water and solid by-products are the three fractions obtained. The "2-phase" system is very similar to the previous one, but no water is added before or during malaxation, while water can be added during horizontal centrifugation. Thus, the first two mills separate the liquid from the solid by-products, while the latter produces one type of residue, i.e. slurry often named "*alperujo.*" This point is very important, as the 2-phase system gives a semi-solid residue of approximately 80% of the olive weight, and the usually reduced amount of water added during the process, while the 3-phase one can produce up to 1.2-1.5 times the weight of the treated olives. The solid by-product obtained from the 3-phase system and the traditional press system can be easily treated, for example by composting and using in the field or for energy production, due to their relatively low moisture content. The liquid residue of the 3-phase system is properly named “olive mill wastewater” (OMW). Around 6×10^6 m^3 OMW are produced yearly worldwide, of which 98% is produced in the Mediterranean basin. For each litre of olive oil produced, about 2.5 L of waste are released into the environment, with a total estimated amount of 1.4 million m^3 of OMW produced every year just in Italy and over 30 million m^3 produced in the Mediterranean area (Casa et al., 2003; Rinaldi, Rana, & Introna, 2003).

OMW has a high load of Biological Oxygen Demand (BOD) in the range of 35--110 g/L and Chemical Oxygen Demand (COD) 40--220 g/L (Niaounakis, and Halvadakis, 2006). Due to lack of strict regulation in some producing countries, the OMW are usually directly spread into sewer systems, lakes, valleys etc., and contaminating underground water (Hanifi & El Hadrami, 2009). Some European regulations allow spreading OMW onto agricultural fields but this solution seems more appropriate for Norther African countries and less suitable for other regions where the wet conditions following the harvest period do not require irrigation. Moreover, this practice is only allowed when there is no slope of the field for obvious reasons of surface runoff. It has been pointed out that each country might have its own regulations about OMW treatment, and uncontrolled land spreading of solid or liquid olive mill by-products is not an uncommon practice (Inglezakis, Moreno and Doula, 2012). This is usually due to the fact that a unique method which is both effective and cost affordable does not exist yet, thus many olive mill enterprises preferring to illegally dispose liquid waste into close aqueous receptors. However, it is important that sewer systems must be avoided because of the negative effect of phenolic compounds on the microflora.

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OMW could be an economic and natural source of antioxidants due to its high content of phenolic compounds. Olive oil phenolic compounds have potential beneficial nutritional properties, for their antioxidants, anti-allergic, anti-inflammatory, anticancer and antihypertensive effects, for which they have been linked to the positive effects of the Mediterranean diet (Visioli, Poli, & Gall, 2002). Their role has been clearly recognised by the EFSA, which allows the following health claim: "*olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.*" It has also been reported that a great variability exists in terms of concentration of phenolic compounds in virgin olive oils found on the retail market (Nicola Caporaso et al., 2015). Several review papers report on phenolic compounds in olive oil and their health benefits, thus the reader can refer to them for further details on the nutritional properties of these compounds (Martín-Peláez, Covas, Fitó, Kušar, & Pravst, 2013; M. Servili et al., 2009).

Generally, phenolic compounds have been described to inhibit the induction of oxidation by metals, scavenge free radicals, act as reductants and even preserving the flavour of foods, *e.g.* virgin olive oil (Genovese, Caporaso, Villani, Paduano, & Sacchi, 2015). In addition, olive oil biophenols have been shown to act as antimicrobial agents (Serra et al., 2008), which suggests their use as a natural mean to increase the shelf life of foods.

Therefore, the recovery of phenolic compounds provides not only an economic opportunity but also lowers the environmental charge of the wastewater. Natural polyphenols can be recovered to make food products with added value, or be used for other applications. These liquid by-products include 2-8% sugar and 1.8-2% salts of the total residue, including potassium and sodium, phosphates and carbonates. OMW also possess soluble dietary fibres and especially

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pectin with excellent gelling ability (Galanakis, Tornberg, & Gekas, 2010a). OMW can be used for energy purposes, as their bioenergy content is relatively high because of its high COD: 100--200 g/L (Khoufi, Aloui, & Sayadi, 2008) that can be converted to biogas rich of methane through a process of anaerobic digestion of the OMW or the “alperujo” (Khoufi, Louhichi, & Sayadi, 2015). There are important differences between OMW and alperujo in terms of physico-chemical properties, appearance and potential for biogas production and generally energy production.

Due to the high concentration of phenolics, the olive mill by-products could be conveniently converted into a valuable source of antioxidant compounds, which can be added to a variety of foods to preserve their quality and develop new functionality, e.g. improve the nutritional properties or better resistance to lipid oxidation. Previous reviews papers focused on the purification and quantification of phenolic compounds or non-conventional methods for their recovery from olive oil by-products (Araújo, Pimentel, Alves, & Oliveira, 2015). However, no review paper has been found in the literature reporting on OMW phenolic extraction for their possible use for the design of new food formulations, e.g. functional foods. Therefore, the present paper firstly gives an overview on the most promising methods currently available for the recovery of phenolics from OMW, and then deepens into their possible uses as food ingredients, by reporting on the current research carried out over the last few years and new potential applications in several food products, also including examples of other olive-derivative by-products to further stimulate into the application of OMW phenolic extracts as food ingredients.

2. By-products obtained from the olive mills

Virgin olive oil extraction involves three major steps, i.e. crushing, malaxation and separation. This last step can be carried out using a traditional one pressing system or continuous systems based on centrifugation of the olive pulp. Centrifugation systems are classified into two-phase or three-phase extraction, with a dramatic importance in terms of amount and composition of by-products obtained, both liquid and solid by-products (**Fig. 1**). More detailed information on the effect of olive oil extraction system on its composition and the type of by-products obtained can be found elsewhere (N Caporaso, 2016). As previously mentioned, two-phase centrifugation systems generate a semisolid waste called two-phase wet pomace or "alperujo", rich in phenolic compounds and containing 98--99% of the phenols initially found in the olive fruit. The high moisture level of alperujo, which is between 65 and 70%, hinders its use in the olive oil industry for a second oil extraction. Moreover, olive mill liquid by-products have a high polluting organic load due to the presence of organic compounds, including sugars, tannins, polyphenols, poly-alcohols, pectins and lipids (Roig, Cayuela, & Sánchez-Monedero, 2006).

OMWs are composed of the olive washing waters (about 5% of the weight of the processed olives), the olive pulp water (40--50% of the initial weight of olives), the water added to olive paste in the centrifugation step and the water coming from washing of equipment (5--10% of the weight of olives processed) (Belaqziz, El-Abbassi, Lakhal, Agrafioti, & Galanakis, 2016). Thus, for one kg of processed olives, the liquid by-product obtained range from 550 to 750 g water, which corresponds to about 550-750 litre of water per ton of olive fruits. In general, the amount of water added during this process is not standardised or regulated and it is a technological decision made by the olive mill manager or operator depending on several factors such as the olive variety, ripening degree and olive moisture content.

OMW appears as a dark liquid, with a pH between 3 and 6, and has considerable amounts of sugars, tannins, phenolic compounds, polyalcohols, minerals, pectins and lipids. Compared to other organic wastes, it has a high concentration of potassium and considerable levels of nitrogen, phosphorus, calcium, magnesium and iron (Peri, 2014). Despite the high concentration of phenolic compounds in the olive fruit, only 2% of the initial concentration is found into VOO, while the remaining fraction is found in the OMW (approximately 53%) and in the pomace (approximately 45%). This is due to the necessary contact of the water phase and oil during the extraction steps, and the high hydrophilic nature of phenolics (Rodis, Karathanos, & Mantzavinou, 2002). The concentration of total phenolic compounds in OMW is up to 10 g/L (Mantzavinos & Kalogerakis, 2005), and its composition includes above 50 phenolic molecules, depending on the type of olive mill and the equipment used (Bianco et al., 2003). It was reported that approximately 90% of the phenols in olives are transferred to the water phase, i.e. OMW, during the pressing of the drupes, thus the biophenol concentration in OMW is around 1-1.8% w/v (Soni, Burdock, Christian, Bitler, & Crea, 2006).

Oleuropein is chemically a phenylethanoid and is the most abundant phenolic compound in intact olive fruit and olive leaves with high concentrations also found in the olive pomace. Oleuropein content in olive oil ranges between 0.005 and 0.12%, in pomace up to 0.9%, and in olive leaves between 1 and 14% (Savournin et al., 2001; Sanchez de Medina, Priego Capote & Luque De Castro, 2012). It is absent in OMW due to enzymatic hydrolysis during the olive oil extraction, inducing the formation of side products such as hydroxytyrosol and elenolic acid. Due to oleuropein degradation, hydroxytyrosol and tyrosol are the most abundant phenolics in OMW, with verbascoside being another potentially important compound (Cardinali et al., 2012). Other phenolic compounds reported in OMW are catechol, 4-methylcatechol, p-hydroxybenzoic acid, vanillic acid, syringic acid and gallic acid (De Marco, Savarese, Paduano, & Sacchi, 2007).

OMW phenolics are likely to possess great variability depending on the olive variety, location and maturity level, as well as technological factors applied for virgin olive oil extraction, thus the recovery process needs to be standardised to obtain a consistent product in terms of composition, when the aim its use as food ingredient.

3. Treatments and alternative uses of OMW

OMW treatments aim to lower its pollution charge and/or extract useful compounds for further applications. The methods for olive mill by-product treatment can be divided into physico-chemical, biological or combined methods, as summarised in **Fig. 2**. The physico-chemical methods are based on OMW treatment with flocculant and coagulant, electrocoagulation (Adhoum & Monser, 2004), oxidation cryogenesis, membrane filtration and reverse osmosis (Paraskeva, Papadakis, Tsarouchi, Kanellopoulou, & Koutsoukos, 2007a), spreading on agricultural soil as an organic fertilizer or simple evaporation in open tanks (Belaqziz et al., 2016). The majority of these methods are expensive and they do not completely solve the issue, as they usually produce a sludge or residue which needs to be eventually disposed or undergo further purification treatments. Moreover, when focusing at the objective of using OMW phenolic compounds as ingredients in food products, not all of these methods can be applied and a proper selection must be made.

Biological methods are based on the production of proteins and exopolysaccharides, energy production through anaerobic digestion, aerobic digestion and composting (Hachicha et al., 2009). The advantages related to these methods are the energy and chemicals saving and are related to the limited production of sludge, especially when wastewater of high COD needs to be treated. The solid and liquid by-products from olive oil industry have been proposed as an

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organic fertilizer (Araújo et al., 2015), being this solution the most rapid and easy one to dispose OMW and even *alperujo*. Furthermore, phenolic compounds from OMW may act against specific plant pathogens, and some experiments have been carried out to use them as natural pesticides (Cayuela, Millner, Meyer, & Roig, 2008). However, the main issue is that OMW production is concentrated in a few months and not spread throughout the year, i.e. mainly October-December in the Northern hemisphere. During this time of the year, there is no need to fertilise or to irrigate the soil in many of the producing countries. It should be also noted that spreading the OMW directly in the field is only applicable where the soil morphological conditions are satisfied, i.e. flat fields. Other strategies to use OMW include their application as natural supplement in feeds of small ruminants and fish, whereas it has been reported that the presence of phenolics shows a negative impact on nutrient digestibility and intestine flora (Araújo et al., 2015). For these reasons, a valid approach to reduce the pollution charge of OMW could be to recover the phenolic compounds in a food-grade manner and use them as ingredients in food products. Several studies reported on the recovery of phenolic compounds from OMW, as briefly described in the following sections, to give an overview on the most appropriate for their use in the food industry.

Solvent extraction methods include liquid-liquid extraction or solid-liquid extraction and solid phase extraction (SPE) (El-Abbassi, Khayet, Kiai, Hafidi, & García-Payo, 2013; Galanakis et al., 2010). This technique is the most frequently used approach to obtain crude extracts of antioxidants from plant matrices, usually applying solvents including water, ethanol, methanol, ethyl acetate and acetone. Suárez, Romero, Ramo, Macià, & Motilva (2009) evaluated several procedures to optimize the phenolic extraction from the two olive cake constituents, namely vegetation water and the solid residue. They proposed an accelerated solvent extraction from the *alperujo*. The method is a relatively simple and rapid procedure, alternative to solid-liquid extraction with a solution of methanol/water (80:20 v/v) at atmospheric pressure. A prior ultrasonic or thermal treatment is usually required to facilitate the phenols solubilisation (Suárez et al., 2009). However, the methanol concentration in food products is limited by European Regulation 2009/32, to a maximum concentration of 10 mg/kg (EC, 2009). Alternatively, for its lower toxicity in some research papers, ethanol was used instead. For example, Lafka, Lazou, Sinanoglou, & Lazos, (2011) recovered phenolic compounds from OMW using liquid extraction using several solvents, including supercritical CO_2 extraction. The results showed that ethanol was the most appropriate solvent for the extraction of phenolic compounds from OMW. Supercritical CO_2 was confirmed to be an efficient solvent for recovering phenolic compounds with relatively high antioxidant activity from OMW (Lafka et al., 2011). Bouaziz, Feki, Ayadi, Jemai, & Sayadi (2010) successfully used a mixture of ethanol and water (70:30 v/v) to extract polyphenols from olive leaves. These results are therefore promising in terms of using food-grade solvents and then use OMW phenols as food ingredients. Ethyl acetate was used by several authors, e.g. Kalogerakis et al. (Kalogerakis, Politi, Foteinis, Chatzisymeon, & Mantzavinos, 2013) described a pilot plant for the recovery of antioxidants compounds from OMW using a liquid-liquid solvent extraction.

A liquid-liquid extraction method applied after aerobic or anaerobic digestion can allow a recovery above 90% phenolic compounds using ethyl acetate (Khoufi et al. (2008). The aerobic and anaerobic pre-treatments resulted in the removal of the major phenolic compounds, i.e. a lowering of 78.7% and 80% of the soluble COD, respectively. Ethyl acetate was described as the

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most efficient solvent for the recovery of phenols from OMW obtained using a three-phase mill (Allouche, Fki, & Sayadi (2004). A continuous three-staged counter-current liquid-liquid extraction unit allowed a good recovery of hydroxytyrosol (85.46%) and the purification was done using a chromatographic system that could be adapted to a large scale. Using this system, the hydroxytyrosol extracted was 1.225 g per litre of OMW.

Solvent extraction (liquid-liquid extraction) is used individually or preferably employed prior the membrane filtration to recover the polyphenols from olive oil by-products (Lafka et al., 2011; Takaç & Karakaya, 2009). Despite its widespread application in OMW phenolics recovery, some challenges still exist, related to the large amounts of solvent required and its negative impact for the health and safety, environmental pollution and long extraction times required. The solvent extraction is still required for the *alperujo*, being this a semi-solid product, while several other techniques are possible for OMW, which is liquid.

Pressurized liquid extraction has been applied for the recovery of phenolic compounds from OMW (Lozano-Sánchez et al., 2014; Suárez et al., 2009). It is based on the use of organic solvents at a high temperature and pressure, with possible combinations of static and dynamic modes. Solvents such as acetonitrile, methanol, ethanol, ethyl acetate or water are used, while for OMW phenolic extraction mixtures of methanol/water and ethanol/water are the most efficient solvents (Lozano-Sánchez et al., 2014; Suárez et al., 2009). Generally, this method is faster than the traditional extraction techniques and uses a reduced volume of solvents.

Solid phase extraction (SPE) is a relatively new procedure applied with excellent results for the extraction of natural polyphenols (Bendini et al., 2003; Bertin, Ferri, Scoma, Marchetti, & Fava, 2011). An integrated physicochemical-biotechnological approach was described for the recovery

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of polyphenols from OMW with a procedure of extraction in solid phase, recovering more than 60% of polyphenols from the OMW (Scoma et al., 2011). SPE provides cleaner extracts, simpler protocol, shorter processing time, easier automation and low-cost procedures over conventional technologies, while it might be expensive for large scale applications. De Marco et al. (2007) applied a similar type of extraction to recovery biophenols from OMW, reporting that reversed phase solid phase extraction (RP-SPE) allowed the recovery of approximately one gram of purified hydroxytyrosol per litre of OMW.

While these methods are generally effective for analytical purposes, they are quite expensive on large scale, e.g. for applicative industrial uses, and one of the disadvantages is that traces of solvent can be sometimes found in the extract.

Supercritical fluid extraction is an interesting approach to avoid the use of flammable and toxic solvents, particularly supercritical CO_2. Supercritical fluids are used at temperatures and pressures above the critical point of the solvent in the absence of light and air, thus significantly reducing oxidation phenomena than other extraction methods (Al-Otoom et al., 2014; Dai & Mumper, 2010). Several supercritical fluids can be used, e.g. ethylene, methane, nitrogen, xenon or fluorocarbons, while CO_2 is the most common one used in the food industry. In addition, CO_2 is reputed an interesting alternative to organic solvents as non-explosive, non-toxic, and is generally recognised as safe (Al-Otoom et al., 2014). However, since this technique involves a high pressure, the instrumentation requires high investments for an industrial scale that exceed the technical benefits procured the use of CO_2 (Dai & Mumper, 2010).

Ultrasound-assisted extraction (UAE) is an advantageous technique that does not require complex instrumentation and can be carried out with relatively low costs. It is thus interesting for small and medium scale industries (Dai & Mumper, 2010). Jerman Klen & Mozetič Vodopivec (2011) compared five methods of phenolic extraction from OMW, including filtration, SPE, liquid-liquid and ultrasonic-assisted extraction of liquid and solid (freeze-drying). The results showed that UAE provides higher yield at both levels of individual and total phenol yields, thus it was suggested as a good alternative to conventional solvent extraction techniques.

Fungal enzymes are environmentally sustainable and a variety of them can be applied for OMW treatment, including cellulase, pectinase and hemicellulose to hydrolyse the structural components of the cell wall, and consequently increase their permeability, achieving high extraction yields (Ntougias et al., 2015). Fungal treatment is excellent from the point of view of yield, time and process sustainability. Nevertheless, the scale of industrial application is limited by the high cost of the enzymes and their effectiveness depends on the environment in which they are found, with factors such as amount of dissolved oxygen, temperature and availability of nutrients (García García et al., 2000).

Membrane extraction can be described as the most promising approach for the recovery of biophenols from OMW at the industrial scale. The application of the membrane separation process for the recovery, purification and concentration of polyphenols and other target molecules from OMW is getting more interest over time (Paraskeva, Papadakis, Tsarouchi, Kanellopoulou, & Koutsoukos, 2007b), due to their advantages over conventional methods. Membrane separation technologies require lower use of energy without the need of additives, they are characterised by high efficiency, mild operating conditions, separation efficiency and easy scaling up (Cassano, Conidi, Giorno, & Drioli, 2013). These include micro-filtration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), while a membrane distillation

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process (MD) has been also proposed (Khayet & Matsuura, 2011). Some studies have adopted osmotic distillation (OD) or membrane distillation (MD) for the recovery and concentration of OMW polyphenols, however some limitations were highlighted (El-Abbassi, Hafidi, García-Payo, & Khayet, 2009; Garcia-Castello, Cassano, Criscuoli, Conidi, & Drioli, 2010).

Membrane filtration offers the advantage of separating the phenolic compounds according to their molecular mass, which is not achievable through other methods. In fact, OMW comprises phenolic compounds with a high range of molecular masses, from benzoic acid and derivatives (MW up to 198) to secoiridoid aglycons (MW up to 378) and lignans (MW up to 416) (Takaç & Karakaya, 2009), and it might be of interest to separate these classes.

Several examples of successful application of membrane filtration for the separation and recovery of OMW phenolics can be found. Maurizio Servili et al. (2011) applied an industrial plant based on an initial enzymatic pre-treatment, followed by a three-phase membrane system for the recovery of OMW polyphenols and obtained a strong lowering of OMW pollution charge. Hamza & Sayadi (2015) evaluated the efficiency of an environment-friendly process on a pilot scale, which was based on a combination of enzymatic pre-treatment (with *Aspergillus niger* β-glucosidase) followed by membrane filtration, with a combination of MF and UF. Overall, this process was effective for the recovery of natural hydroxytyrosol on a large scale and free of chemicals. Microfiltration eliminates 72.12% of COD and the concentration of UF permeation which increases the hydroxytyrosol concentration up to 7.2 g/L.

Cassano et al. (2013) applied a membrane integrated system for the recovery of low molecular weight phenolic compounds from OMW. The process included a sequence of two UF steps followed by a NF and the authors claimed the possibility to re-use the obtained water in the olive

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mill. Similarly, D'Antuono et al. (2014) applied membrane filtration to recover OMW phenolic compounds from two Italian and three Greek olive cultivars. Three fractions were obtained by MF, UF and NF. Each fraction contained a different degree of purity and a different content of phenolic compounds. The phenolic concentration for the different fractions was 2.5-5.3 g/L for MF; 1.4-3.1 g/L for UF and 0.4-1.6 g/L for NF. Interestingly, significant quantitative differences were observed not only due to the filtration process, but also to the different olive cultivars.

Zagklis, Vavouraki, Kornaros, & Paraskeva (2015) characterised the fractions obtained by a membrane process, specifically the reverse osmosis concentrate, after using NF. These phenolic compounds were further treated with XAD4, XAD16, and XAD7HP adsorption/desorption resins. Finally, the recovered phenolic compounds were concentrated by vacuum evaporation, obtaining a final product with phenolics concentration of 378 g/L in gallic acid equivalents, from an initial concentration in the raw OMW of 2.64 g/L. The use of membrane filtration to treat OMW was also reported by others, describing that a two-step treatment using MF and NF produced a retentate with phenolic concentration above 3.7 times its initial concentration, i.e. 1500 mg/kg. Also, the potential pollutant charge (COD) was lowered by 97% (di Lecce et al., 2014).

Membrane systems have been also used to treat the wastewater obtained from table olives processing, which has a similar composition to OMW, but with a higher level of sodium chloride and sodium hydroxide (used for lowering the bitter taste of the olives) and lower concentration of organic matter and polyphenols (Kiai, García-Payo, Hafidi, & Khayet, 2014). UF was shown to decrease the initial phenolic content by 40%, while reverse osmosis concentration was below 1% in the initial content and the final OMW toxicity was decreased significantly (Comandini, Lerma-García, Massanova, Simó-Alfonso, & Gallina Toschi, 2015). Other examples and details on this aspect can be found in a review paper recently published by others (Castro-Munoz, Yanez-Fernandez, & Fila, 2016)

4. Use of OMW and olive-derivative phenolic extracts in foods

The exploitation of by-products of fruit and vegetable processing industry as a source of functional compounds for their application in foods is a promising field requiring interdisciplinary research of food technologists, food chemists, nutritionists and toxicologists. The use of OMW extract in foods is a new trend in the food sector to formulate new products with positive effect on consumers' health. In addition, the recovery of natural phenolic compounds is of great interest for their importance in terms of antioxidant effect to better preserve the quality and shelf life of food (Galanakis, Yucetepe, Kasapoglu & Ozcelik, 2017). Consequently, the production of functional foods from OMW extracts constitutes a viable alternative to transform this agro-industrial waste stream into a useful and relevant ingredient (Hassan K. Obied, Bedgood, Mailer, Prenzler, & Robards, 2008; Galanakis, Tornberg, & Gekas, 2010). A general overview on the possible uses of phenolic compounds, extracted from olive by-products, with some recent food applications is summarised in **Fig. 3**. The majority of literature dealing with this issue is applied on olive-derivative phenolic extracts in vegetable oils, with the aim of extending their shelf life by adding "extra" antioxidant activity. Several authors extracted phenolic compounds from other olive sources, such as leaves, to be applied to food product; this choice is driven by the more straightforward extraction required and higher yield obtained. An alternative approach to fortify food products with olive-derivative phenolics includes the direct addition of pure phenolic compounds such as hydroxytyrosol of oleuropein. However this approach does not solve the issue of the olive industry by-products and it is hardly applicable at the industrial level, and its major application is at the laboratory scale. Several other food products have been functionalised, as described in the following sections.

4.1 Use to fortify vegetable oils and functionalise other food products

Polyphenols extracted from the olive fruit and OMW have been applied as food ingredients in order to fortify edible oils both to preserve from lipid oxidation and increase their antioxidant activity. In this context, the addition of individual phenolic compounds (hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid) and OMW extract to refine olive and husk oils was compared to BHA and BHT (Fki, Allouche, & Sayadi, 2005). 3,4-Dihydroxyphenyl acetic acid had the highest protective effect against oil oxidation, as it led to oils with the lowest peroxide value. The addition of OMW extract at 500 ppm resulted in lower PV values than BHA, p-hydroxyphenylacetic acid and tyrosol.

Sánchez De Medina, Priego-Capote, & Luque De Castro (2012) enriched refined vegetable oils, i.e. maize, soya, high-oleic sunflower, sunflower, olive and rapeseed oils, with two levels of polyphenols isolated from pomace and olive leaves. The functionalised oils were shown to possess a similar composition to extra virgin olive oil in terms of amount of biophenols. A similar work was carried out by Paiva-Martins, Correia, Félix, Ferreira, & Gordon (2007) to increase the level of phenolic compounds in refined olive oils by adding polyphenols extracted from olive leaves. The authors evaluated the stability of the enriched oil and reported that 1 kg of leaves extract was sufficient to fortify 50-320 litres of refined oil, obtaining a chemical stability to oxidation similar to extra virgin olive oils.

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Phenolics recovered from the *alperujo* using microwave assisted extraction were used to fortify vegetable oils, as reported by Girón, Ruiz-Jiménez, & De Castro (2009). The authors studied the phenol mass-transfer in the oil depending on the product fatty acid composition, showing that the more the unsaturation of the oil, the easiest is the inclusion of phenolic compounds. Other researchers added the phenolic compounds recovered from OMW in virgin and extra virgin olive oils in order to enhance their concentration. The crude phenolic extract was obtained by membrane filtration followed by a solvent extraction by ethyl acetate and ethanol (Maurizio Servili et al., 2011). The authors also used the crude phenolic concentrate during virgin olive oil extraction, in particular before the malaxation step, with the aim to improve its phenolic content. The addition of 5 or 10% of this phenolic concentrate led to significantly higher content of total phenolic compounds, and particularly 3,4-DHPEA and 3,4-DHPEA-EDA compared to the control olive oil. This effect, however, was shown to also depend on the olive variety used (Servili et al, 2011). The authors suggested this approach as one of the possible solutions for the utilisation of OMW and their exploitation to enhance the health properties of virgin olive oils.

Even olive leaves were used to extract phenolic compounds, which were hydrolysed and added to refined olive oil and olive pomace oil to improve their oxidation resistance. The performance of the leaves extract was similar to the one obtained using oleuropein or α-tocopherol at a concentration of 400 μg/kg oil. The oleuropein hydrolysate had even better performance than the native oleuropein and α-tocopherol (Bouaziz et al., 2010).

Phenolics extracted from OMW using ethyl acetate were added to lard in concentrations ranging from 50 and 350 mg kg^{-1}. Effective protection was demonstrated against lipid oxidation in a dose-dependent manner. In addition, the cytotoxic effect was tested to assess whether high phenolic content inhibit cell growth: it was reported that concentrations of 10 mM had toxic effects, but concentrations of 100-200 mg^{-1} of phenolics did not inhibit cell growth (De Leonardis et al., 2007).

Esposto et al., (2015) recently used an OMW phenolic extract to verify its effectiveness in retarding lipid oxidation during the frying procedure. The extract was obtained by enzymatic treatment followed by a membrane filtration and a drying process in a rotary evaporator; it was added to refined olive oil at concentrations ranging from 100 to 1200 mg/kg. OMW extract was effective in protecting α-tocopherol content and avoid excessive production of off-flavour volatiles. The authors suggested that a minimum concentration of 400 mg/kg should be used to have a significant effect.

It should be mentioned that no other ingredients can be added to virgin or extra-virgin olive oil in any case, nor to influence the sensory properties or its shelf-life, but the approach of adding natural antioxidants from OMW by-products is feasible and valid for vegetable oils, where the legislation allows its use. However, there are commercial examples even of extra virgin olive oils added with other ingredients, e.g. vitamin D, so that the application of fortified olive oils using phenolic extract is likely not to represent a great drawback for the industry, especially for vegetable oils for which other antioxidants are allowed by the current legislation. Regarding this latest aspect, natural antioxidant are generally more accepted by consumers compared to synthetic ones, so there would be an advantage in commercialising such products, regardless the effectiveness of natural phenolic extract compared to the synthetic antioxidants.

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As OMW phenolic compounds have strong hydrophilic nature, the moisture content of the food and the thermal treatments required for its production should be carefully evaluated, when designing applications of phenolic extract, due their easy degradation under high temperatures. Oil-in-water (O/W) emulsions are examples of food products with promising characteristics in terms of OMW phenolics addition, whereas some technological challenges still need to be solved.

Caporaso, Genovese, Burke, Barry-Ryan, & Sacchi, (2016a; 2016b) used OMW phenolic extracts obtained from membrane separation process followed with spray-drying, added to model olive O/W emulsions, in combination with stabilising agents such as whey protein isolate and xanthan gum. The aim was to assess the effect of OMW phenolic extracts on the physical properties and stability of the emulsions and the oxidation status over accelerated ageing conditions. OMW spray-dried extract did not result in a dramatic improvement of the primary and secondary oxidation status of the emulsions over accelerated storage. Moreover, higher production of secondary oxidation products was observed at a very high concentration of phenolic powder. This result should be taken into consideration when formulating food systems with these types of extracts. It was suggested that further research is needed to understand the effect of the coating material used in spray-drying, as this can influence the reactivity of the phenolic compounds and their interaction with other ingredients at the interface. Similar applications of olive phenolic compounds have been reported by Di Mattia, Sacchetti, & Pittia (2011), by using pure tyrosol and oleuropein in O/W emulsions to assess their interfacial behaviour. The presence of these phenolic compounds improved the primary and secondary oxidation, especially for oleuropein also with respect to pure tyrosol. However, important

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differences exist between pure laboratory phenolic compounds and natural extracts, in terms of purity, reactivity and effectiveness.

The use of natural phenolic extracts to improve the nutritional value of table olives was described by Lalas et al. (2011). The authors tested 1%, 5% and 10% of leave water extract, which was used for the treatment of table olives de-bittering. The use of 10% extract had the highest antioxidant activity and polyphenol content, when the extraction was performed at room temperature for 24 h. The treated olives showed an increase of 457% of oleuropein, 109% of hydroxytyrosol and the bitterness perception. However, the treated table olives showed equal overall acceptability and overall preference compared to the untreated ones (Lalas et al., 2011).

As previously mentioned, the use of polyphenols extracted from olive leaves has already been applied in order to increase olive oil shelf life (Paiva-Martins & Gordon, 2001; Paiva-Martins, Barbosa, Pinheiro, Mourão, & Outor-Monteiro, 2009; Paiva-Martins, Correia, Felix, Ferreira, & Gordon, 2007). A similar approach can be also proposed for OMW phenolic extracts, which is likely to open new opportunities for the food industry. Indeed, one of the biggest differences in terms of nutritional value between virgin olive oils and refined olive oil is the absence of phenolic compounds, and the fortification of refined olive oils is likely to improve both its nutritional profile and shelf life.

Phenolic extracts from OMW have been applied in a milk system to study their effect in modulating the Maillard reaction when milk is treated by ultrahigh-temperature. The phenolic powder extract was added at a concentration of 0.1-0.5% (w/v) and the products of the Maillard reaction were monitored. The authors reported that the phenolic extracts trapped the reactive carbonyl species which led to off-flavour development. OMW phenolic also inhibited the formation of reactive carbonyl species, i.e. Amadori products (Troise et al., 2014). Other research papers reported on the use of OMW phenolic compounds in milk beverages to enhance their nutritional properties, showing that the concentration of the majority of phenolic compounds decreased over storage, while the level of hydroxytyrosol increased as a result of the hydrolysis of more complex compounds (Servili et al., 2011).

Moreover, it should be noted that several interational projects, mostly in the public sector, have been carried out over the past years to solve the issue of OMW and their possible use in foods. In particular, in the European Union, some LIFE projects fostered the collaboration between research bodies and the food industry, e.g. a project named “RE-WASTE - Recovery, recycling, resource. Valorisation of olive mill effluents by recovering high added value bio-products” led by an Italian olive oil industry in collaboration with several public-private partners, carried out over the period 2009-2012. Another example of LIFE project is “Olèico+ -European awareness raising campaign for an environmentally sustainable olive mill waste management”, which had the objective to revise the technologies currently available for the treatment of olive mill waste for their disposal, and mainly worked in Italy, Spain, Portugal and Greece. Also, the project aimed to raise awareness to olive mill owners on the negative effects on improper disposal of olive mill by-products.

4.2 Use as antimicrobial agents

The use of OMW phenolic compounds in food products is mainly justified by their antioxidant activity and the possibility of retarding lipid oxidation. In addition, some new applications include their use as antimicrobial agents. Olive-derivative phenolic extracts have been described as powerful antimicrobial agents against some pathogens, e.g. olive leaves extract

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was effective against several Gram positive, Gram negative bacteria and fungi even at low concentration (Pereira et al., 2007). Their antimicrobial activity has been exploited for the elaboration of green table olives, as olive leaf extract showed the power to partially inhibit the growth of several bacteria strains, including *E. coli*, *Salmonella typhimurium* and *S. aureus*. Thus, its use was proposed to be added as a starter in the fermentation of table olives to better control unwanted microbes (Shaide et al., 2016). Olive leaves extracts, however, are abundant in oleuropein, representing approximately 70% of total phenolic compounds (Pereira et al., 2007).

Olive oil lees extract has a more similar profile to OMW phenolics. This extract was tested against *L. monocytogenes*, *S. aureus*, *E. coli* and *S. enteritidis*, and it was reported that a concentration of 40 µl ml^{-1} reduced the pathogens number of 2.5-3.2 log10 CFU ml^{-1}, over 7 days of storage at 10°C, while it was not effective when the storage temperature was 37°C (Janakat, Al-Nabulsi, Allehdan, Olaimat, & Holley, 2015).

In general, OMW possesses an antimicrobial potential against pathogenic bacteria and fungi, as well as molluscicidal activity (Carraro et al., 2014; H. K. Obied, Bedgood, Prenzler, & Robards, 2007; Yangui, Sayadi, Rhouma, & Dhouib, 2010). Olive mill by-products were shown to possess antimicrobial activity against several microbial species, including *Escherichia coli*, *Salmonella poona*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Candida albicans* (Serra et al., 2008). In this work the natural OMW extract had the strongest antimicrobial activity compared to the three antioxidants used alone, i.e. quercetin, hydroxytyrosol and oleuropein, which suggests a useful application in the future as natural antimicrobial agents for the food industry. In addition, the results highlighted the potential of OMW extracts against some food-borne bacteria, especially against Gram-positive species. Nevertheless, its potential antagonist

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effect on lactic acid bacteria which plays a key role in the processing and preservation of fermented products that needs to be taken into consideration.

OMW phenolic extracts obtained by membrane separation process were applied to several spoilers, starters and food-borne bacteria to assess their bactericidal effect and propose them as natural ingredients to extend food shelf life. The authors tested antibacterial activity of phenolics by the minimum bactericidal concentration (MBC), against *Staphylococcus, Listeria, Escherichia, Salmonella, Pseudomonas, Lactobacillus and Pediococcus*. *Staphylococcus aureus* and *Listeria monocytogens* had the lowest level of resistance to phenolics. In contrast, the Gram negative strains (e.g. *S. Typhimurium* and *Pseudomonas spp*.) were in some cases unaffected by the tested doses, while starter cultures (e.g. *Staphylococcus xylosus*) growth was dramatically reduced (Fasolato et al., 2015).

Other authors added purified OMW phenolic compounds into hamburgers made of white meat, at a concentration of 750 mg/kg of meat or 1500 mg/kg, to assess their effect on the product shelf life over storage of 11 days at 4°C. The inhibition of total mesophilic count was effective especially at the highest concentration tested, whereas a statistical significance was not described (Veneziani, Novelli, Taticchi, & Servili, 2017). A similar work was carried out by Chavez-Lopez et al. (2015), where OMW phenolics were added to fermented sausages for the control of mould populations. The authors used a surface treatment and concluded that dipping the product in a 2.5% solution of phenolics was an effective mean for some fungi (Chaves-López et al., 2015). Thus, OMW phenolics have been proposed as an alternative to synthetic antifungal compounds to preserve from oxidation and undesired fungi. Despite the work carried out on these aspects, however, systematic data on the effect of OMW phenolic extracts at several

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concentrations is missing and a careful evaluation about the correct level of addition is a key parameter to be evaluated, to obtain the desired effect but not compromise the sensory impact of the product.

The addition of OMW phenolic compounds in milk beverages was assessed to verify their influence on beneficial bacteria in yogurt and similar products (Servili et al., 2011). As olive phenolic compounds possess antimicrobial activity, it is useful to understand their possible influence when added to milk during fermentation for yogurt production. It was shown that the addition of phenolic extract had little influence on *Lactobacillus* and *Streptococcus* bacteria concentration over fermentation. From a sensory point of view, no statistical difference was obtained between the control and the product with 100 mg/kg phenolic extract, while the assessors recognised the product with 200 mg/kg extract, which also had the highest score for preference. For this reason, the authors suggest that the addition of OMW extracts to milk beverages is an opportunity for the sector to extend olive phenolics health benefits (Servili et al., 2011). However, the concentration of the added OMW phenolic extract should be carefully assessed in order to obtain the desired effect and avoid unwanted consequences, both in term of product stability and negative sensory impact. Despite the fact that the bitter and pungent sensory note is desired for virgin olive oil -- even within certain limit dictated by consumer acceptability -- there is limited research on the undesirable effects of using an excessive level of olive-derivative phenolic compounds in food products. Thus, further studies are needed to verify this aspect and also to suggest possible limits in terms of food regulations.

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4.3 Use as feed and other applications

Alternative uses of OMW phenolic extracts are represented by their application as feed, to improve the quality of meat and animal products. For example, the extract was added at a concentration of 4% in piglet feed (Gerasopoulos, Stagos, Kokkas, et al., 2015) and in broiler chicken (Gerasopoulos, Stagos, Petrotos, et al., 2015), demonstrating its effectiveness to reduce the oxidative stress and obtaining higher antioxidant capacity of the plasma and tissues. The concentration used, however, might seem excessive for a wide application in the animal feeding industry. Other authors applied concentrations as little as 1.5% of crude phenolic extract, corresponding to approximately 300 mg/kg total phenolic compounds, for swine (F??tima Paiva-Martins, Barbosa, Pinheiro, Mour??o, & Outor-Monteiro, 2009).

The feed digestibility, growth performance of pigs and pork meat quality was also investigated modifying the diet of pigs with the incorporation of olive leaves in their diet, using concentrations from 2.5 to 10%. The results showed that even at lower levels, olives leaves increased tocopherol content of meat, with positive effects on primary and secondary oxidation indices of the fat, while it did not change meat fatty acid profile. However, the growth performances were negatively affected by the addition of olive leaves, which is negative for animal growth but it might be useful for the control of human weight (Fátima Paiva-Martins et al., 2014).

Other components extracted from OMW beyond the sole phenolic compounds can be conveniently extracted and used in food products. For example, Galanakis, Tornberg and Gekas (2010b) used suspensions of dietary fiber extracted from OMW as fat replacement in meatballs. The concentration of the residues used ranged from 0.02 to 0.07% of the total batter weight,

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showing that the two types of fiber present in the OMW lead to different water holding ability and oil uptake during frying.

Moreover, alternative uses of phenolics extracted from OMW might be found in different areas: i) in the cosmetic industry (Rodrigues, da Mota Nunez, & Pinto Olveira, 2016); ii) use as supplements by the pharmaceutical industry (Mirabella, Castellani, & Sala, 2014); iii) use in the packaging of food products to extend their shelf life and design an “active packaging” (Barbato et al., 2015). These aspects are not reported in details in the current review, as we focused on their direct uses in food products; however, research on alternative uses of OMW might be highly beneficial for the sector and for the general public, as it is likely to positively affect eventually the public health and the environment.

3. Examples of the use of by-products phenolics from other food industries

In a vision of “clean” or “green” labels for food products, the current trend toward more “natural” ingredient is likely to foster the food industry toward the use of natural extracts which give improved functionality to the final product, including antimicrobial activity and extended shelf life. Despite the fact that olive mill wastewater extracts might not be as effective as pure or synthetic compounds, their interest will be growing in future years. The literature is rich of examples on the use of high added-value components from other food industry by-products, where minor ingredients are recovered from by-products and incorporated into foods (Galanakis, 2012). We shortly describe a few case studies to give examples on applications of polyphenols extracted from the industry by-products.

Grape marc is an important by-product in several wine-producing countries, for its high polyphenol content. The possibility of extracting valuable compounds from grape marc and using them for specific application in the food industry has been reported by Bonilla, Mayen, Merida, & Medina (1999). The authors extracted polyphenols from red grape marc using ethyl acetate and water, adding this extract to refined olive oil to improve its oxidation stability. A concentration as little as 100 mg/kg phenolic extracts was used, in comparison to synthetic antioxidants such as BTA, BTH and propyl gallate. Whilst synthetic antioxidants had a better performance in the retarding lipid oxidation, the use of natural polyphenols from red grape marc as food additives was still suggested due to its advantages in terms of consumer acceptability of natural products. A similar experiment was reported by other authors using ethanol and methanol extracts from winery waste, i.e. grape skin and seeds, added to olive oil and sunflower oil to increase their oxidative stability. Oil shelf life was extended and lower PV values were obtained when the phenolic extracts were used, in a concentration dependent manner, from 50 to 150 mg kg^{-1} (Lafka, Sinanoglou, & Lazos, 2007). Other authors applied a different method for the purification of phenolic compounds from grape marc, testing several solvent composition and solid/solvent ratios when using UF and NF followed by the separation of polar vs non-polar compounds by resin separation. The resin absorption step helped to further increase the phenolic content of the retentate; the initial content was 0.44 g L^{-1} polyphenols expresses as gallic acid equivalents, while the obtained product had concentration of 190 g L^{-1} (Zagklis and Paraskeva, 2015).

Apple pomace extracts were used as a source of fibre and phenolic compounds in baked products (Sudha, Baskaran, & Leelavathi, 2007). In this case, the extraction was not focused to recover polyphenols, instead apple pomace was directly mixed with flour for the formulation of a functional cake with improved fibre and phenolic content. The addition of apple pomace to flour

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increased the water absorption and mixing tolerance index, while it decreased dough stability, indicating weakening of the dough. Resistance to extension values significantly increased whereas extensibility values decreased. Despite the worse rheological characteristic of the cakes made using this flour, the product had good values for fibre and polyphenol content, and therefore apple pomace can serve as a source of the latter (Sudha et al., 2007).

Pomegranate by-products were utilised as ingredients in ice creams (Çam, Erdoğan, Aslan, & Dinç, 2013). Pomegranate seed oil and peel phenolic compounds were extracted from the fruit by-products. The products were separately dried and the oil was extracted by solvent, while polyphenols were obtained by microencapsulation. The concentration of phenolic extracts added to the ice cream was 0.1-0.4%. The authors reported that the functional properties of the ice creams were significantly improved, while it was also highlighted that pomegranate oil is highly susceptible to oxidation, with the consequent risk of adding rancid flavour to the product (Çam et al., 2013).

These examples can be used as stimuli or suggestions to researchers working in the olive oil sector to test OMW phenolic extracts in a wider range of food products, as this demonstrates that many other potential applications are possible and also expected in the near future. For example, still little research has been published so far on the use of OMW phenolics in bakery products, which might be an interesting application. Also, the good solubility of hydroxytyrosol especially in aqueous media and its high bioavailability enable its application in multi-component foods, and offers encouraging prospects in the marketing in functional food and natural cosmetics (Bouzid et al., 2005).

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4. Conclusions and future perspectives

Over 15 years ago, Schieber, Stintzing, & Carle (2001) reported that the exploitation of by-products of fruit and vegetable processing as a source of functional compounds and their application in food is a promising field which requires interdisciplinary research. Nowadays this is still true, as this topic is regarded as one of the priorities in the food sector.

Natural phenolic compounds -- especially those recovered from food industry by-products -- are interesting compounds to be used as antioxidants in some foods, as an alternative to synthetic antioxidants. The use of OMW as a valuable source of low-cost chemical compounds able to add functional value to food products is of great interest from an environmental point of view and a food technology perspective. The addition of OMW phenolic extract into foods will help in solving the problem of OMW high pollution charge and find an efficient use of the obtained extract.

Nowadays the olive oil industry is facing two parallel issues, beyond the economic sustainability: the first one is related to the pollution charge of by-products and to sustainable methods for their purification, as well as to recover valuable compounds; the second challenge is to get a sufficient demand of these extracts for practical applications in the food industry. Indeed, the use as feed additives does not worth the price needed to obtain the extracts and therefore this use might not be appealing for the industry, while the pharmaceutical industry might prefer other types or sources of similar phenolic extracts, e.g. direct extraction from olive leaves instead of OMW extraction, as their concern for the environmental impact of olive oil processing might be modest.

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Whilst the current technology allows treatment of OMW in an effective way, its efficiency and sustainability if far from being satisfactory, especially for small-medium industry, which is the typical situation of the olive oil industry in the majority of producing counties. This fact brings several considerations, which need to be addressed both at scientific level through the research of more efficient treatments, but also at political level by finding appropriate solutions that could bring together the olive mill industry for a co-operative organization of the by-product treatments.

From a technological point of view, it is important to address the question from a holistic point of view, i.e. from the field management to the final product disposal and use. In fact, the massive use of agrochemicals containing copper might influence the oxidation stability of the OMW due to their pro-oxidant nature. Similarly, the high salt content in table olive processing industry represents an agronomical and technological problem that needs to be further studied.

It appears that the current trend on the research on olive oil sector is strongly linked to the nutraceutical effects of olive-derivative phenolics, and further research is expected on the possible synergistic effects among different olive phenolic compounds, also from OMW. Moreover, the consumer awareness is of strong importance to foster the food industry into the research on the use of these extracts and consequently create potential economic benefits.

The design of functional foods requires investigations on the stability and interactions of the phenolic compounds with other food ingredients, because of their complex matrix and the composition of their bioactive compounds. Further research is also needed on the sensory impact of OMW by-products, as these are characterised by fermentative and “table olives” odours, as well as a dark colour which can influence the colour of the food systems where they are added.

In summary, the future trends of OMW phenolics that are expected to be addressed over the next years are listed as follows: i) to study new rapid, cheaper and efficient “green” methods for the recovery and separation of OMW biophenols; ii) to apply OMW extracts on a wider range of food products and assess their possible impact and interactions; iii) to study *in vitro* and *in vivo* these phenolic extracts to demonostrate their properties on the human health and their technological properties; iv) to perform sensory studies of these extracts in the final foods, and v) to development policies able to stimulate the consumer awareness on the environmental and health consequences of using food products fortified with OMW phenolics.

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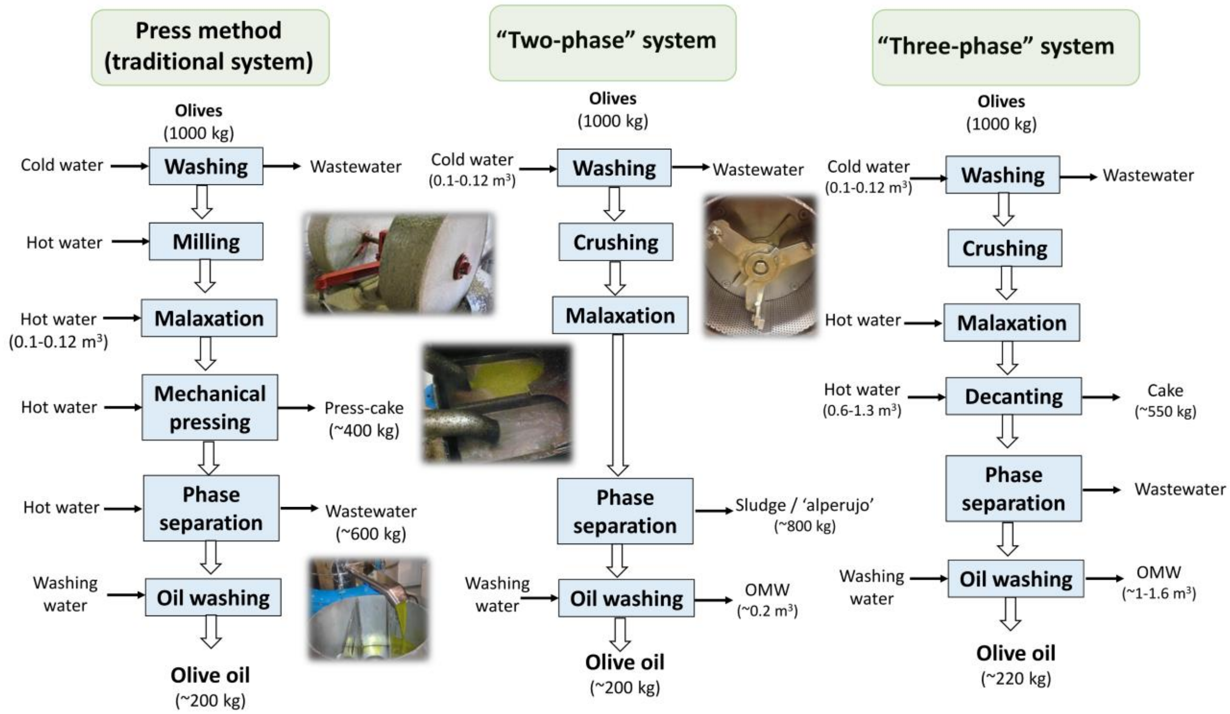

Figure 1. Extraction methods applied for virgin olive oil production, with regard to the by-products obtained from each processing step. Modified from Zbakh & El Abbassi (2012) and Albuquerque et al. (2004).

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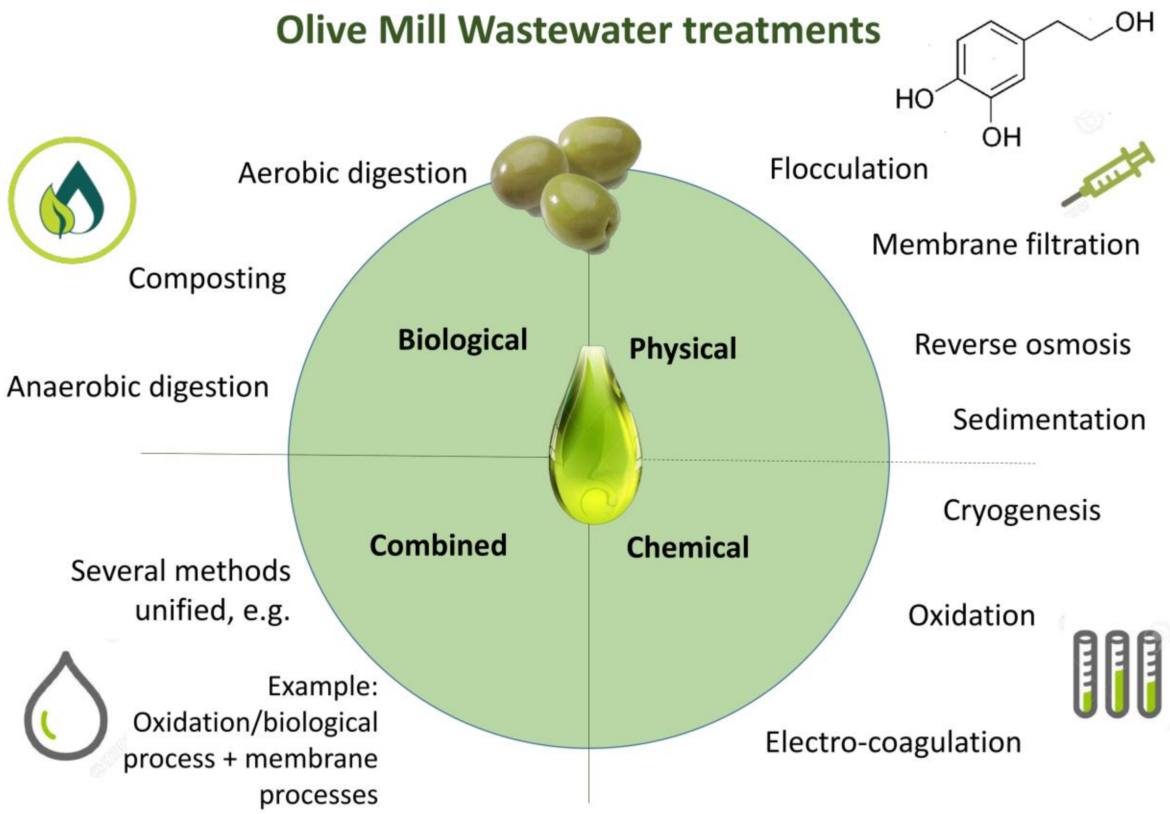

Figure 2. General scheme of different approaches applicable for olive mill wastewater treatment, to lower the pollution charge or to extract useful biomolecules for further applications.

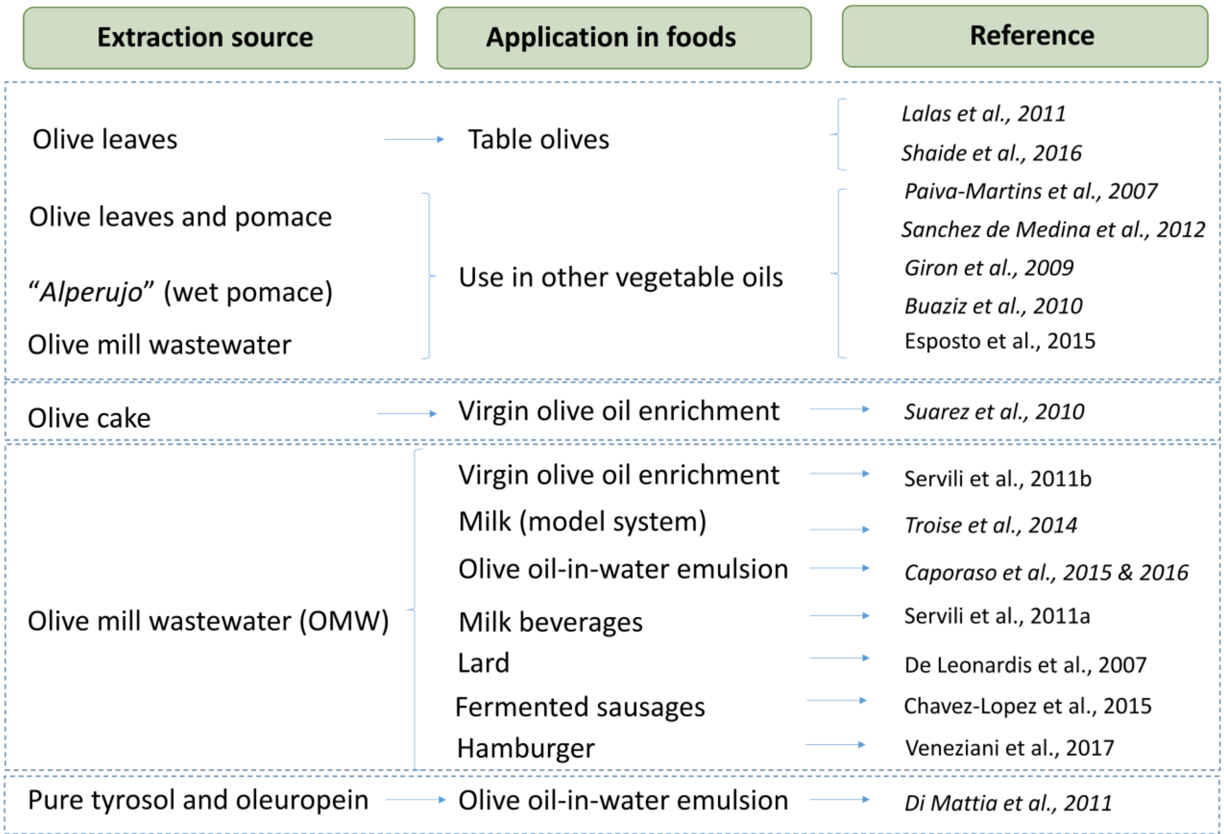

Figure 3. Use of phenolic compounds extracted from olive by-products: some recent examples of applications in food products.