

OLIVE OIL HUILE D'OLIVE

The phenolic compounds: a commercial argument in the economic war to come on the quality of olive oil?

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Abstract – The quality of extra virgin olive oil (EVOO) is deeply related to the amount of its minor compounds, chiefly volatile and phenolic compounds, which confer the sensory note and the remarkable nutritional and biological properties of this traditional Mediterranean fruit juice. Several agronomic aspects and technological factors affect the qualitative and quantitative composition of these compounds in EVOO. The most abundant natural antioxidants of EVOO are tocopherols, carotenoids and hydrophilic phenols. The EVOO phenols represent a group of secondary plant metabolites not often present in other oils and fats. The class of the hydrophilic phenols includes phenolic alcohols and acids, flavonoids, lignans and secoiridoids. The latter group is exclusively found in the Oleaceae family plants of which the olive is the only edible fruit and it is considered as the most important fraction from a biological point of view. In particular, the secoiridoids are the most relevant phenols associated to health and biological properties and, at the same time, they are responsible for the bitter and pungency sensory notes of EVOO. The new approach to the EVOO extraction technologies is oriented towards the improvement of the virgin olive oil healthy and sensory properties by optimizing the oil mechanical extraction process conditions.

Keywords: Extra virgin olive oil / phenols / antioxidant activity / healthy and sensorial properties / agronomic factors and mechanical extraction process

Résumé – **Les composés phénoliques : un argument commercial dans la guerre économique à venir sur la qualité de l'huile d'olive ?** La qualité d'huiles d'olive vierges extra (HOVE) est profondément liée à la quantité de ses composés mineurs, principalement composés volatils et phénoliques, qui caractérisent la note sensorielle et les propriétés nutritionnelles et biologiques remarquables de ce jus de fruit de tradition méditerranéenne. Plusieurs aspects agronomiques et facteurs technologiques affectent la composition qualitative et quantitative de ces composés dans HOVE. Les antioxydants naturels les plus abondants des HOVE sont les tocophérols, les caroténoïdes et les phénols hydrophiles. Les phénols des HOVE représentent un groupe de métabolites secondaires des plantes pas souvent présents dans d'autres huiles et graisses. La classe des phénols hydrophiles comprend : les alcools et les acides phénoliques, les flavonoïdes, les lignanes et les sécoïridoïdes. Le dernier groupe est exclusivement présent dans les plantes de la famille Oléacées dont l'olive est le seul fruit comestible et il est considéré comme la fraction la plus importante d'un point de vue biologique. En particulier, les sécoïridoïdes sont les phénols les plus pertinents dans le domaine de la santé de par leurs propriétés biologiques et, en même temps, ils sont responsables des notes sensorielles d'amertume et de piquant dans les HOVE. La nouvelle approche en termes de technologies d'extraction de l'huile est orientée vers l'amélioration des propriétés sensorielles et biologiques du produit final grâce à l'optimisation des conditions d'extraction mécaniques d'obtention des huiles.

Mots clés : Huile d'olive extra vierge / phénols / activité antioxydant / propriétés saines et sensorielles / les facteurs agronomiques et le processus d'extraction mécanique

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1 Introduction

The marketable quality of virgin olive oil (VOO) is related to the classification of the oil according to European legislation (EC), the international olive council (IOC) and also to the *Codex Alimentarius*, which have established three different categories of oil obtained from the olive, the “extra virgin olive oil”, “virgin olive oil” and “lampante olive oil”. The EVOO represents the highest commercial category according to well established production techniques, analytical parameters and sensory evaluation. Nowadays, it is well-known that chemical compounds such as natural antioxidants, oleic acid and squalene are responsible for the biological and sensory properties of oil. However, although the analysis of minor compounds (mainly phenols and volatiles) in VOOs is not recognized or normalized by neither European legislation nor the IOC, their concentrations in the oil determine its quality. Traditionally, the nutritional value of EVOO has been related to its composition, with particular emphasis to the amount of oleic acid. This monounsaturated fatty acid (MUFA) promotes the decrease of LDL cholesterol and it is related to the reduction and/or the prevention of cardiovascular diseases (Téres *et al.*, 2010). However, in the last ten years a significant variability has been observed in the oleic acid content of EVOO, which accounts for about 55–83% of the total fatty acids content (Tab. 1). This large range of variability is due to expansion of olive growing to several new cultivation areas in which the produced EVOOs are characterized by a low oleic acid content (in some cases below 50%). Clearly, this value involves an impact on the health and nutritional properties of EVOO. The EVOO biological and sensory properties have also been mainly associated to natural antioxidants. These compounds include vitamins, such as tocopherols and β -carotene (which together with chlorophylls is responsible for the oil colour), phytosterols, pigments, terpenic acids, flavonoids such as luteolin and quercetin, squalene, and hydrophilic phenolic compounds. Moreover, lipophilic phenols (especially tocopherols and tocotrienols) can also be found in other vegetable oils. Over 90% of tocopherols in EVOO is made by α -tocopherol, the concentration of which is also characterized by a strong variation depending on pedoclimatic factors and agronomic practices, such as the area of origin, the cultivar and the stage of fruit ripening (Inglese *et al.*, 2011). The data obtained assessing 430 samples of EVOO showed a range of variability between 23 and 751.1 mg/kg.

The phenolic fraction is the most representative one of EVOO (Servili *et al.*, 2014). These compounds have the characteristic to act as reducing agents, and several studies confirmed the correlation between the antioxidant power of EVOO and its content in derivatives of secoiridoids, with an increase of its oxidative stability. The antioxidant activity of 3,4-DHPEA (hydroxytyrosol) and its derivatives turns out to be higher than that of the *p*-HPEA (tyrosol) and α -tocopherol (Baldioli *et al.*, 1996; Servili *et al.*, 2009a, 2014). In food containing such compounds, therefore, they are able to reduce the formation, during storage and cooking, of potentially toxic radical species.

Phenolic compounds are responsible for the relative high resistance of EVOO against oxidative spoilage and play an important role in the health benefits which are commonly associ-

Table 1. Fatty acid composition of EVOO.

Fatty acids	(%)
Myristic (C14:0)	0.0–0.1
Palmitic (C16:0)	7.0–20.0
Palmitoleic (C16:1)	0.3–3.5
Heptadecanoic (C17:0)	0.0–0.4
Heptadecenoic (C17:1)	0.0–0.4
Stearic (C18:0)	1.0–4.0
Oleic (C18:1)	49.0–84.0
Linoleic (C18:2)	3.0–21.0
Linolenic (C18:3)	0.2–1.5
Arachidic (C20:0)	0.1–0.7
Eicosenoic (C20:1)	0.1–0.1
Behenic (C22:0)	0.0–0.3
Lignoceric (C24:0)	0.0–0.4

ated with EVOO in the “Mediterranean diet” concept. Regarding to this latter aspect, epidemiological investigations have demonstrated preventive properties of EVOO related to some chronic degenerative events based on inflammatory processes and chronic-degenerative diseases, such as cardiovascular-cerebral ones and cancer (Casaburi *et al.*, 2013; Covas *et al.*, 2006; López-Miranda *et al.*, 2010; Martín-Peláez *et al.*, 2013; Obied *et al.*, 2012; Servili *et al.*, 2009a, 2014).

Recently, both observational epidemiology and intervention studies have confirmed that the above mentioned healthy properties seem to be mediated by the presence of some phenolic compounds as the secoiridoids, which are found exclusively in EVOO. In general, the studies carried out have shown that the phenolic compounds could interfere in those chemical reactions deeply involved in both atherosclerosis and cancerogenic processes. At this regard, in Table 2 the pharmacological properties of EVOO biophenols are summarized (Obied *et al.*, 2012).

Recently, the Panel NDA of EFSA (European Food Safety Authority) has granted a healthy claim related to capability of olive fruits and EVOO phenols to reduce cardiovascular diseases (Reg. EU 432/2012). Several scientific investigations have demonstrated that the daily consumption in the ratio of 5 mg/day of EVOO phenolic compounds (hydroxytyrosol and its derivatives, in particular) would have a positive impact in the prevention of cardiovascular disease by reducing the peroxidation of blood lipids. In this regard, the disclosure of EFSA highlights that the daily intake of phenolic substances has to be in the ratio of 20 g of oil/day, a value compatible with a moderate daily intake of recommended fatty substances for an adult (EFSA, 2011). According to the Panel, 5 mg of hydroxytyrosol and/or its derivatives should be ingested daily with oil, but it must be considered that some olive oils have such a low concentration of polyphenols that they do not guarantee the proper amount for a balanced diet. This concentration corresponds to a minimum content of the total phenolic compounds in EVOO no less than 250–300 mg/kg. However, oils belonging to the marketable class of EVOO show a large range of variability in the phenolic compounds concentration between 40 mg/kg and 1000 mg/kg on 713 analyzed samples (Fig. 1).

Table 2. Olive biophenols (OBP) properties (Obied *et al.*, 2012).

1. Antioxidant effect	“OBP have RONS scavenging, reducing power, and metal chelating activities, induce endogenous antioxidant enzymes such as catalase, superoxide dismutase, quinone reductase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, and g-glutamylcysteine synthetase”.
2. Anti-inflammatory effect	OBP act against cardiovascular diseases (CVD) and some types of cancer by inhibition of proinflammatory enzymes, phosphoinositide 3 kinase, tyrosine kinases, and downregulation of various proinflammatory cytokines, tumor necrosis factor alpha, interleukins including and monocyte chemotactic protein-1.
3. Cardiovascular effects	3.1. blood pressure-antihypertensive activities; 3.2. platelet and endothelial function; 3.3. platelet and endothelial function; 3.4. atherosclerosis; 3.5. other cardioprotective properties.
4. Immunomodulatory effects	OBP have been shown to modulate immune function, particularly inflammatory processes associated with the immune system.
5. Gastrointestinal effects	5.1. gastroprotective effects; 5.2. modulation of digestive enzymes.
6. Endocrine effects	6.1. antidiabetic effects; 6.2. osteoprotective effects; 6.3. other endocrine effects.
7. Respiratory effects	OBP antioxidant and anti-inflammatory properties against lung diseases.
8. Autonomic effects	8.1. cholinergic effects; 8.2. adrenergic effects.
9. Central nervous system effects	9.1. neuroprotective effects; 9.2. analgesic and antinociceptive effects; 9.3. behavioral effects.
10. Antimicrobial and chemotherapeutic effects	10.1. antibacterial properties; 10.2. antifungal properties; 10.3. antiviral properties; 10.4. antiprotozoal and antiparasitic activities.
11. Anticancer and chemopreventive effects	Biophenols can directly control cell growth at different stages of carcinogenesis <i>via</i> inducing apoptosis or inhibiting proliferation by diverse mechanisms.

In the olive fruit a large amount of phenolic compounds can be found, with concentration ranging between 1% and 3% of the weight of the fresh pulp. The phenolic acids, phenolic alcohols, hydroxy-isochromans, flavonoids, lignans and secoiridoids are mainly included into this class of chemical compounds. Secoiridoids that in combination with lignans are the main hydrophilic phenols of EVOO include the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA or p-HPEA-EDA), an isomer

of the oleuropein aglycon (3,4-DHPEA-EA) and the listroside aglycon (p-HPEA-EA) (Montedoro *et al.*, 1993). Lignans include (+)-1-acetoxypinoresinol and (+)-1-pinoresinol (Owen *et al.*, 2000) (Fig. 2).

The hydrophilic phenols content found in EVOO is affected by the agronomic conditions and the technological factors adopted in EVOO production. These substances (aglycon derivatives of secoiridoid glucosides contained in the olive fruit) are released in the EVOO during the

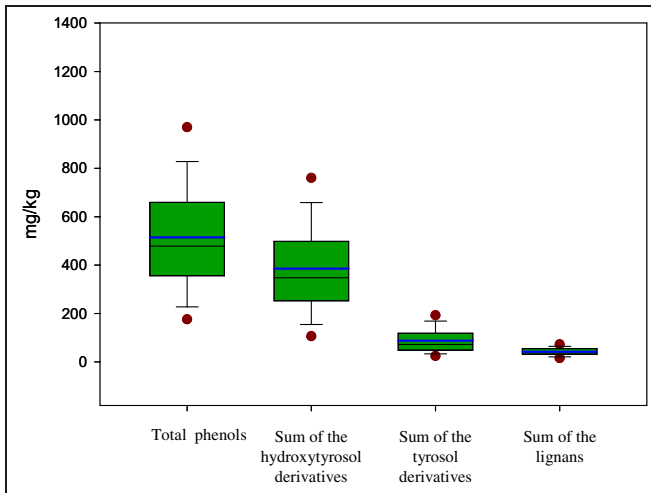


Fig. 1. Variability (mg/kg) of phenolic compounds evaluated on 713 Italian EVOO industrial plants' samples* (*Unpublished data*). Limits in percentile: box = lower 25%, upper 75%; respectively; whiskers = lower 10%, upper 90%, the red points = lower 5%, upper 95%; the blue and black lines in the box represent the median and the average, respectively.

mechanical extraction process, by means of the reactions of oleuropein, demethyloleuropein and ligstroside hydrolysis, catalysed by endogenous β -glucosidases. The hydrophilic phenolic compounds have been subject of several studies aimed at assessing their antioxidant effect. In fact, as primary antioxidants, these chemical compounds delay the EVOO autoxidation process, thus guaranteeing protection against rancidity and prolonging product shelf-life (Servili *et al.*, 2009a). Many authors have studied the relationship between the natural antioxidants content in EVOO and its oxidative stability (Servili *et al.*, 2004). The general conclusion of the researches is that the increase of the antioxidants content in oil enhances its oxidative stability. In particular, several investigations were focused on the antioxidant activities and health benefits of oleuropein derivatives (3,4-DHPEA and 3,4-DHPEA-EDA), 3,4-DHPEA-EA derivatives, ligstroside derivatives (*p*-HPEA and *p*-HPEA-EDA) and lignans. The results obtained have shown that the oxidation resistance of EVOO is mainly due to the oleuropein derivatives, such as the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and an isomer of oleuropein aglycone (3,4-DHPEA-EA), whereas other components, such as lignans and ligstroside derivatives,

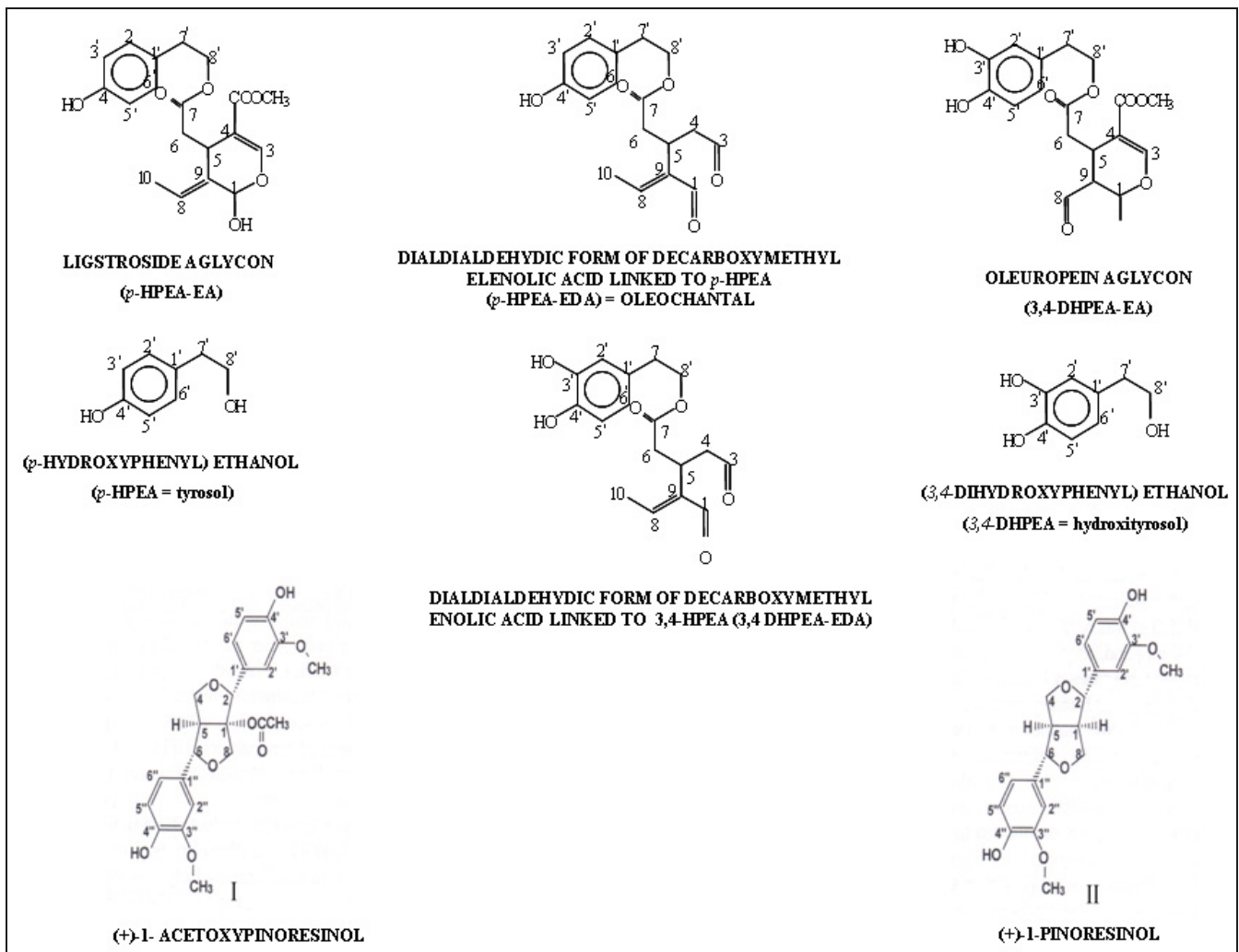


Fig. 2. Chemical structures of EVOO secoiridoids derivatives and phenyl alcohols (Servili *et al.*, 2004).

have a marginal role in EVOO oxidation stability (Carrasco-Pancorbo *et al.*, 2005; Obied *et al.*, 2008). Moreover, due to the concrete contribution of the phenolic compounds to the olive oil oxidative stability and to the human health, consumers are now increasing their consumption of oils with high bitterness intensity (Inarejos-Garcia *et al.*, 2009). In fact, in EVOO the intensity of bitterness and pungency is related to the phenolic compounds, with secoiridoid derivatives being the most representative ones (Servili *et al.*, 2014). In particular, several investigations focused on EVOO bitterness have hypothesized that their main contributors are compounds characterized by an aromatic ring in their chemical structure, as in the case of the secoiridoid derivatives of oleuropein and demethyloleuropein, 3,4-DHPEA-EDA and 3,4-DHPEA-EA respectively (Mateos *et al.*, 2004; Servili *et al.*, 2009a, 2014). Furthermore, in a study carried out by Gutiérrez Rosales *et al.* (2003) it has been confirmed that the main compounds responsible for the bitter taste of EVOO are the 3,4-DHPEA-EDA, 3,4-DHPEA-EA (Tovar *et al.*, 2001) and ligstroside derivatives such as *p*-HPEA-EDA (also called oleocanthal) which give the perception of the bitter and pungent sensory notes of EVOO. They also reported on a linear correlation between the bitter taste and derivatives of oleuropein and ligstroside aglycones concentrations. Concerning the pungency, a slight burning sensation has been attributed to 3,4-DHPEA-EDA, whereas in EVOO the deacetylglucoside aglycone (*p*-HPEA-EDA) is mainly responsible for the pungent sensation perceived mostly at the back of the tongue (Andrewes *et al.*, 2003; Beauchamp *et al.*, 2005; Peyrot des Gachons *et al.*, 2011; Taticchi *et al.*, 2014). As a result, bitterness evaluation is becoming an important area in olive oil research.

2 Agronomical and technological factors affecting the phenolic compounds content in EVOO

In the field of EVOO the innovation process is oriented towards the qualitative improving of EVOO through the optimization of agronomical and technological parameters, which most affect the phenolic fraction concentration. In this section, the effects of agronomic and technological factors on the phenolic composition of EVOO are summarised.

The olive cultivar (variety), the degree of ripening of the fruit, the climatic conditions, soils and water management are the main agronomical factors determining the content and the profile of phenolic compounds of an EVOO (El Riachy *et al.*, 2011; Inglese *et al.*, 2011). In particular, the phenolic composition of olive fruit is strongly related to the cultivar (Servili *et al.*, 2004). A few papers have pointed out the cultivar effect on qualitative and quantitative characterization of the phenolic fractions of olive fruit. In particular, it was observed that the oleuropein is generally present in the drupes of all olive cultivars while demethyloleuropein and verbascoside are cultivar dependent, since they occur only in some olive varieties. The absolute concentration of the specific hydrophilic phenols of EVOO is affected by the cultivar, whereas the phenolic profile is almost the same.

During the ripening several changes take place in the fruit: these involve texture and colour changes, the regulation of enzymatic pathways and evolution in the phenolic and volatile profiles. All these changes significantly affect oil yield and quality and, therefore, the correct choice of the harvesting time is of paramount importance. In particular, during the olive fruit maturation the concentration of oleuropein decreases, while the one of the demethyloleuropein increases. However, the amount of both compounds strongly decreases at higher maturity stages. Moreover, climatic conditions modify the phenolic composition of EVOO with a negative impact of high temperatures during the olive ripening in the phenolic concentration of oils (Di Vaio *et al.*, 2006; Inglese *et al.*, 2011; Ripa *et al.*, 2008; Tura *et al.*, 2008). With respect to the relationships between EVOO quality and seasonal conditions of olive growing it was observed that the high rainfall reduces the EVOO phenolic content. Several results related to the relationships between water availability during olive growing and phenolic concentration of EVOO show that their concentration is strongly affected by the absolute disposability and distribution of water during the vegetative cycle of olive tree (Servili *et al.*, 2007a). A negative correlation between the water disposal and the phenolic concentration in EVOO has been confirmed by many authors (Caruso *et al.*, 2014; Inglese *et al.*, 2011). Secoiridoids are largely affected by the irrigation treatments that, on the contrary, show a low impact on lignans.

The importance of the different EVOO processing stages on the phenolic fraction found in the final product has been studied extensively. The technological operations most affecting the EVOO composition and quality during the extraction process are crushing and malaxation, because they give rise to changes in the phenolic fraction of EVOO (Servili *et al.*, 2012; Fregapane, Salvador, 2013; Taticchi *et al.*, 2013; Clodoveo *et al.*, 2014). Because of the fact that the occurrence of hydrophilic phenols in EVOO is strictly related to the activities of some endogenous enzymes of olive fruit, their concentration in the oil is strongly affected by the extraction conditions. The main endogenous enzymes involved in the determination of the final concentration of hydrophilic phenols in EVOO are polyphenoloxidase (PPO) and peroxidase (POD) and β -glucosidases. In fact, upon olive crushing, several enzymes involved in the generation and transformation of hydrophilic phenols and volatile compounds are activated. In particular, secoiridoid aglycons such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA and 3,4-DHPEA-EA are originated by the hydrolysis of oleuropein, demethyloleuropein and ligstroside, catalysed by the endogenous β -glucosidases. At the same time, endogenous oxidoreductases such PPO and POD have the effect of decreasing the concentration of phenolic compounds by catalyzing their oxidation in the paste and in the oil during the mechanical extraction process (Clodoveo *et al.*, 2013; Servili *et al.*, 2004, 2007b, 2008a; Taticchi *et al.*, 2013).

Several studies were focused on the distribution of enzymes on the different parts of the fruit (pulp, stone and seed). They showed that the seed is particularly rich in POD activity, whereas the phenolic compounds are largely concentrated in the pulp. On the other hand, stone and seed contain small quantities of these substances. These investigations

allowed to introduce the technological basis for the new approach to EVOO mechanical extraction process, represented by the use of a hammer with a differentiated effect on the constitutive parts of the drupes (such as blade crusher, teeth crusher, pre-crusher or stoning crushing) that reduces the seed tissues degradation by limiting the release of POD in the pastes and improves the concentration of hydrophilic phenols in the EVOO by preventing their oxidation during malaxation (Servili *et al.*, 2007b; Taticchi *et al.*, 2013). Several researches have shown that the destoning process, by removing the olive seed before malaxation, partially reduces the peroxidase activity in the pastes and, consequently, can inhibit the enzymatic degradation of the hydrophilic phenols in the oils thus enriching their concentration and improving oil oxidative stability (Angerosa *et al.*, 1999; Lavelli and Bondesan, 2005; Mulinacci *et al.*, 2005).

Malaxation and related selective control of enzymes as PPO and POD are other critical factors of the mechanical extraction process of EVOO. At this regard, the role of the operative conditions applied during malaxation, strongly affecting EVOO quality, has been deeply investigated. In fact, by monitoring the main process parameters (oxygen availability in the malaxer head-space, temperature and time) it can be modulated the endogenous enzymatic activities. The introduction of technological innovations, such as covered malaxer, has allowed regulating the O₂ concentration in the malaxer head-space, giving rise to an increase of the amount of hydrophilic phenols in the olive pastes and in the corresponding EVOO, through a decrease of phenolic oxidation catalyzed by endogenous reductases.

Furthermore, in covered malaxer the O₂ concentration can be regulated by using inert gases or the CO₂ naturally produced by the olive pastes during the malaxation phase (Parenti *et al.*, 2006a, 2006b; Servili *et al.*, 2008a). In fact, the CO₂ saturating the head-space of the malaxer allows the reduction of oxidative phenomena without resorting to the expensive inert gases.

Temperature and time of malaxation significantly have the effect of modifying the phenolic profile of EVOO. Recently, the influence of the malaxation temperature on the concentration of phenolic compounds in EVOO has recently been object of new investigations (Boselli *et al.*, 2009; Gómez-Rico *et al.*, 2009; Taticchi *et al.*, 2013). The temperature effect on the phenolic concentration is also affected by the small amount of O₂ occurring in the covered malaxer. Low O₂ concentration in the malaxed pastes inhibits the activity of PPO and POD which carry out the phenolic oxidative degradation and, at the same time, the temperature increase enhance the solubility of phenols in the EVOO (Servili *et al.*, 2008a; Taticchi *et al.*, 2013). These results indicate that temperatures higher than 30 °C partially inactivate the PPO. On the other hand, these temperature values could increase the activity of depolymerizing enzymes which promote the release of hydrophilic phenols in the oil and vegetation waters by hydrolyzing the olive cell wall (Servili *et al.*, 2008a, 2008b; Vierhuis *et al.*, 2001). However, high temperatures of malaxation promote a fall of volatile compounds, such esters and the cis-3-hexen-1-ol, and an accumulation of hexan-1-ol and trans-2-hexen-1-ol, both considered by some authors as eliciting smell not

completely agreeable (Angerosa *et al.*, 2004; Servili *et al.*, 2009b). The sensory analysis of the related EVOOs points out a weakening of the typical “green” attributes with the increase of malaxation time and of all sensory notes when high temperatures are adopted during malaxation (Angerosa *et al.*, 2004; Servili *et al.*, 2009b). Preliminary studies on some Italian cultivars have been performed to define the best malaxation conditions, in terms of temperature and O₂ concentration. The results obtained suggest that a malaxation temperature included between than 25–30 °C, while the oxygen concentration should range between 50 and 30 KPa represent a good compromise in order to obtain a high-quality EVOO in the extraction process, in terms of phenols and volatile compounds (Servili, 2012).

With respect to the times of malaxation, in the confined malaxers there is not a direct relation between the time of malaxation and the loss of phenolic compounds. However, times of malaxation greater than 35–40 min do not involve an extraction yield increase and, therefore, even if a loss of the oil quality is not observed prolonged periods of malaxation are negative for a correct plant management.

Thus, during the malaxation the O₂ concentration, in combination with time and temperature can be managed to optimize the EVOO phenolic and volatile concentrations.

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