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# Research and Innovative Approaches to Obtain Olive Oil with a Higher Level of Bioactive Constituents

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## Introduction

Virgin olive oil is the main component of the Mediterranean diet due to its sensory and nutritional qualities. The benefits of consuming olive oil have been known since antiquity. The ancient Greeks believed that the goddess Athena had created the olive tree. King David hired guards to protect Israel's olive groves and warehouses. Ancient peoples used olive oil not only for consumption and cooking, but also as perfume, ointment for the dead, soap, and in oil lamps. In ancient Greece, the athletes ritually rubbed it all over their bodies. It has been considered symbol of great wealth and power, therefore, it has anointed the noblest heads throughout history. Olive oil was used to produce both medicine and cosmetics; Hippocrates called it "great healer" and Homer "liquid gold," and Galen praised it for its positive effects on health (Clodoveo et al., 2014a). It is now well established that most of these effects can be attributed to the phenolic fraction of olive oil (Boskou, 2006 and 2011).

Olive fruit contains appreciable concentration, 1–3% of fresh pulp weight, of hydrophilic (phenolic acids, phenolic alcohols, flavonoids, and secoiridoids) and lipophilic phenolic compounds that are known to possess multiple biological properties such as antioxidant, anticarcinogenic, anti-inflammatory, antimicrobial, antihypertensive, antidyslipidemic, cardiotonic, laxative, and antiplatelet (Ghanbari et al., 2012). For some activities of olive oil phenolic compounds, the evidence is already strong enough to enable the legal use of health claims on foods (Martín-Peláez et al., 2013). A health claim is defined as any claim that states, suggests, or implies that a relationship exists between a food category, a food, or one of its constituents and health. A health claim on "olive oil polyphenols," even if not accurate in the choice of terminology and the subject of some controversies (Mastralexi et al., 2014a and 2014b; Romero and Brenes, 2014), was made only very recently after many years of discussion (EC No. 432/2012 of 16 May 2012; Servili, 2014). The EU la-

beling regulation 432/2012 allows the following health claim on extra virgin olive oil labels: "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress." The claim may be used only for olive oil that contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of extra virgin olive oil.

Wide ranges (50–1000 mg/kg) have been reported for the levels of total polar phenols in olive oils. Usual values range between 100 and 300 mg/kg (Boskou et al., 2006). The polar phenolic compounds of virgin olive oil belong to different classes: phenolic acids, phenyl ethyl alcohols, hydroxy-isochromans, flavonoids, lignans, and secoiridoids. This latter family of compounds is characteristic of *Oleaceae* plants; secoiridoids are the main compounds of the phenolic fraction. Many agronomical and technological factors can affect the presence of phenols in virgin olive oil. Its shelf life is higher than other vegetable oils, mainly due to the presence of phenolic molecules with a catechol group, such as hydroxytyrosol and its secoiridoid derivatives (Bendini et al., 2007). Several studies demostrated the relationships between the content of phenol compounds and the oxidative stability of virgin olive oil (Aparicio et al., 1999; Cinquanta et al., 2001; Gutierrez et al., 2001; Salvador et al., 2001a; Velasco and Dobarganes, 2002, Servili et al., 2004), as well as the influence of these substances on sensory properties of the final product. Typical sensory gustative properties of virgin olive oil, such as bitterness and pungency, have been attributed to secoiridoid molecules (Taticchi et al., 2013).

In the olive production, several factors have been modified in the last decades, particularly in the evolution of the mechanical extraction plants and the processing technologies that allow obtention of virgin olive oil with the desirable level of biophenols and other bioactive constituents.

## Virgin Olive Oil Production: Technological Aspects and Minor Components

Virgin olive oil is exclusively extracted from fruits by means of mechanical techniques that include crushing, malaxation, and extraction. Each of these technological operations—in addition to the olive fruit characteristics (cultivar, maturity stage, etc.), the preprocessing (fruit harvesting and storage) and the postprocessing (oil storage, filtering, and bottling) procedures—affects the nutritional and sensory properties of the product, in particular the quantity and the types of phenol compounds (Clodoveo, 2012; Clodoveo et al., 2014b). The fruit polar phenol content differs from the oil phenol content because, during the extraction process, phenolic substances undergo chemical and biochemical changes that modify their structure and influence their partition between the aqueous and oily phases.

High-quality virgin olive oil can be produced only from healthy, fresh fruits at the right ripening grade. The main purpose of the virgin olive oil elaboration process should be to extract the triacylglycerols and minor compounds with antioxidant activity and biological properties in the most possible intact form. At the same time, the synthesis of volatile compounds, which are not present in the fruit but are enzymatically generated after the crushing, should be favored (Angerosa et al., 2004; Clodoveo et al., 2014b, 2014c; Kalua et al., 2007; Vichi et al., 2003). If every single step of the mechanical process is not rationally conducted, it can lead to a dramatic reduction of antioxidants, particularly phenols, which are molecules susceptible to chemical and biochemical oxidation reactions, and consequently reducing the keepability of the final product. Enzymatic (Clodoveo et al., 2014b; De Leonardis et al., 2013) and nonenzymatic oxidative reactions (Frankel, 2010) are the main cause of phenol destruction during the fruit storage, the extraction process, and the storage of virgin olive oil. The phenol degradation kinetics depend on the availability of oxygen and is promoted by light, heat, metals, and enzymes; the presence of other antioxidants (tocopherols, carotenoids, etc.) may decrease the oxidation rates. Each single stage of the process leaves an oxidative imprint in the product. The quality of the oil present in the fruit cells can only be preserved during the elaboration process, but no technological solution is available that can create a quality product from poor-quality olives (Amirante et al., 2009). The final quality of virgin olive oil, and its content of bioactive compounds, arises, first of all, inside the orchard.

## Agricultural Practices, Harvesting System, and Preprocessing Conditions

Olive phenolic content depends both quantitatively and qualitatively on its genetic makeup (Conde et al., 2008). Several studies have revealed differences in the phenolic content of olive fruit from different olive cultivars (Aguilera et al., 2005; Hajimahmoodi et al., 2008; Luna, 2002; Morelló et al., 2005; Vinha et al., 2005). Studies related to the changes in fruit and their influence on the properties of extracted oils have indicated that during olive ripening, the concentration of phenols progressively increases to a maximum level at the "half pigmentation" stage, decreasing sharply as ripening progresses (Rotondi et al., 2004). However, there may be exceptions to this rule (see Chapter 5). Environmental factors and agronomic practices, such as fertilization and irrigation, have also been shown to affect the phenolic composition of virgin olive oil (see Chapter 4).

Fernández-Escobar et al. (2006) studied the effect of fertilization practices on oil quality. They found that virgin olive oil quality decreases with nitrogen overfertilization. Nitrogen in excess was accumulated in fruit and, consequently, phenol content significantly decreased in virgin olive oil as nitrogen increased in fruit. The

decrease in phenols induced a significant decrease in the oxidative stability of the oil and its bitterness. α-Tocopherol content, on the contrary, increased with nitrogen application. These findings are confirmed by Morales-Sillero et al. (2007) who studied the influence of fertilization in "Manzanilla de Sevilla" olive oil quality. They found that polar phenol content, $K_{225}$ (bitterness index), and oxidative stability were lower in the oils from trees receiving greater fertilizer doses.

Phenolic compounds of virgin olive oil are also influenced by irrigation management (Berenguer et al., 2006; Gómez-Rico et al., 2007; Grattan et al., 2006; Motilva et al., 2000; Romero et al., 2002; Tovar et al., 2001) during the growing season: thrifty watering increases the phenol level due to their involvement in defense against oxidative stress. The phenolic compound levels show an inverse relationship with the amount of water applied to the olive trees; bitterness and oxidative stability have been observed to decrease with the increase in applied water (Berenguer et al., 2006).

Because phenol levels naturally change as the olive fruit ripens (Baccouri et al., 2008; Beltrán et al., 2005; Dag et al., 2011; Salvador et al., 2001b), harvest time becomes very important. Early harvest results in oils with higher polyphenol values. After choosing the best harvesting time for each cultivar in each particular geographical area, the other two main factors that are crucial for establishing the final quality of virgin olive oil in terms of phenolic concentration should be considered: the harvesting methods and the postharvesting storage.

There are two main techniques for harvesting olives: the traditional harvest by hand picking or the newest mechanical methods. Mechanical harvesting systems can be categorized in two groups: systems based on vibration (manual aid branch shakers or trunk shakers) or contact canopy shakers (individual comb shakers and canopy shakers) (Ferguson et al., 2010). Harvesting by hand, although slow, guarantees the best quality of the olive fruit, creating better quality of the final product but also a higher price tag due to extra manual labor. Mechanical harvesting allows a higher working capacity. As an example, using continuous harvesters, one hectare of a high-density olive orchard is harvested within just 2–3 hours; this allows for harvest of a cultivar in a very short period with low costs (Godini et al., 2011). On the other hand, mechanical harvesting systems may sometimes reduce fruit quality due to damage and bruises, resulting in a bad quality of the final product.

Olive processing is often not well synchronized with crop harvests. So, the fruits are often piled into large heaps and stored at ambient temperature for up to several days prior to processing for oil extraction. If this happens, the greatest deterioration takes place. Pressure within the olive pile during storage may cause secretion of fluid from the olives, thereby providing an optimum medium for the growth of fungi and bacteria. Pseudomonas and other soil bacteria are able to metabolize a wide variety of flavor compounds, such as phenol and its derivatives. Moreover, breakdown of the cells may favor contact of the phenolic substances with the oxidative enzymes. Olives contain oxidoreductases,

such as polyphenoloxidase (PPO) and peroxidase (POD), that may oxidize polyphenols and impair the health-related qualities and sensory characteristics of olive oil (Servili et al., 2003; Servili et al., 2012). Under these conditions, anaerobiosis can also occur in the inner part of the pile, while aerobic losses can occur in the outer part. Furthermore, heat production from respiratory activity may accelerate the deterioration of the fruit and eventually cause the breakdown of cell structure. The oil extracted from these damaged olives can be high in acidity, low in stability (Amodio et al., 2005; Clodoveo et al., 2007), and poor in polyphenols and might develop off-flavors. When the amount of damaged fruit is high, the extraction of oil should be made promptly, avoiding fruit storage at ambient temperatures (Clodoveo et al., 2014b).

The fruit deterioration can be reduced by controlling the storage temperature. It is known that low temperatures are able to reduce both microbial and endogenous enzyme activity. Many years ago, several studies tested the combined use of controlled atmosphere and cold storage of olive fruits with the aim to control microbial development (Amodio et al., 2005; Castellano et al., 1993; Clodoveo et al., 2007; García et al., 1996; Gutierrez et al., 1992; Kader et al., 1989; Kiritsakis et al., 1998; Özden and Bayindirli, 2002). Despite some promising results, this technology has not been widespread due to its high cost, which is not supported by the commercial value of the product (Amirante and Catalano, 2000). A rational approach requires coordination of the harvesting and milling operations to avoid fruit storage.

## Virgin Olive Oil Production: Process Elaboration

Virgin olive oils are obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil and that have not undergone any treatment other than washing, decantation, centrifugation, and filtration (International Olive Council, 2012; Hachicha Hbaieb et al., 2015).

### *Washing and Leaf Removal*

Most millers choose to pass the olives over a vibrating screen with a blower that removes leaves and other debris to protect the extraction plant from damages caused by stones and to avoid off-flavors deriving from the presence of leaves or other foreign bodies. Recently, Malheiro et al. (2013) studied the effect of crushing ripe olives with olive leaves (from 1% to 10% of the total weight of processed olives). Olive leaves are considered an excellent source of compounds with biological properties (see Chapter 11), but the resulting oils showed higher free fatty acids, peroxide value, and $K_{232}$. The authors assumed that the negative effect of addition of olive leaves on legally established parameters could be due to the presence of lipolytic enzymes in the olive leaves. This research revealed that it is important to include in the mill design a grading machine coupled with leaf removal equipment in order to improve virgin olive oil quality.

After the grading step, the olives can be also washed, especially if they have been picked from the soil or contain spray residues. A critical point of the washing step is the extra moisture of the resulting olive paste. Despite the absence of specific studies, several millers believe that oils made from washed olives are usually less desirable, with a reduction in bitterness and pungency probably due to the increasing humidity of olive paste, which can affect the partition phenomena of phenols between the aqueous and oily phases.

### *Crushing*

The washed olives are then crushed. The main purpose of crushing is the size reduction of olive fruit tissues and the breakdown of vegetal cells in order to facilitate the release of the oil from the elaioplasts.

#### *Crushing Equipment and Olive Paste Behavior*

This step can be done using a traditional stone mill (batch machine) or by means of a hammer or disk crusher (continuous machines) (Amirante et al., 2010a). A relatively new technology in virgin olive oil production is olive depitting (Amirante et al., 2006; Dugo et al., 2007). This ensures that the paste consists solely of the fleshy part of the olive (mesocarp), without the stone or pit (endocarp) that holds the seed. Each machine has some advantages and disadvantages, depending on the initial fruit characteristics and the type of oil that should be produced. The main objectives of cell disruption are:

- The release of the maximum amount of oil present in the cell
- The release of the bioactive compounds and their subsequent dissolution in the oily phase
- The preservation of the product limiting the detrimental effects of crushing in the subsequent processing steps (e.g., emulsion formation, enzyme inactivation, reduction of the oily drop diameter, excessive heating of olive paste)

The choice of crushing system depends on the raw material features (cultivar, ripening stage, water content) and the desired characteristics of the final product (phenol and volatile content, color, bitterness, and pungency). Inarejos-García et al. (2009; 2011) observed that the crushing conditions could affect both the concentration of bioactive compounds and oil yield due to:

- The temperature reached by the crushed olive drupes, which influences the viscosity of the olive paste and modifies the release of the oil from the fruit cells
- The different particle size of olive tissues
- The action of the several enzymes involved in the generation and transformation of polar phenols

### *Temperature of the Olive Paste after Crushing: Conversion of Mechanical to Thermal Energy*

At maturity, an olive usually consists of about 76% soft mesocarp, about 20% hard endocarp, and about 4% seed. The effects of the mechanical action of crusher equipment can differ depending on the different type of fruit tissues, which may undergo compressive, tensile, and shear stress. The kernel, compared with the pulp, is characterized by a high rigidity and requires more energy for the breakdown of tissues. The energy dissipated in grinding the pits is largely converted into heat. The increment in temperature of olive paste can be expressed by the following equation:

$$\Delta T = \frac{E}{C} \tag{1}$$

where $c$ (J/°C kg) is the average specific heat of the olive paste into the crusher and $E$ is the energy required to crush a kilogram of pits (J/kg). Moreover, the thermal power, $W_F$ (W), developed within the system is equal to:

$$W_F = \frac{M_p}{M_F} \cdot E \cdot G_F \tag{2}$$

where $M_P$ (kg) and $M_F$ (kg) represent the average mass of a pit and an olive fruit (pulp flesh and pit), respectively, and $G_F$ (kg/s) is the average total olive fruit mass flow rate through the crusher.

The increase in enthalpy per unit of time of the olive paste ($\dot{\Delta} I_O$[W]) is equal to:

$$\dot{\Delta} I_O = M_O \cdot c \cdot \frac{\Delta T}{\Delta t} \tag{3}$$

where $M_O$ (kg) is the mass of olives on average present in the crusher.

By applying the principle of conservation of energy, equaling the thermal power of Eq. 2 ($W_F$ [W] developed into the system) with the increase in enthalpy per unit of time of the olive paste of Eq. 3 ($\dot{\Delta} I_O$[W]), the following equation is obtained

$$\frac{\Delta T}{\Delta t} = \frac{M_p}{M_F} \cdot \frac{E \cdot G_F}{M_O \cdot c} \tag{4}$$

Considering the amount of heat $Q$ (J) dissipated through the crusher walls, characterized by a surface $S$ ($m^2$) and a conductance coefficient $K$ (W/°C $m^2$), we can write:

$$Q = KS\Delta T \Delta t \tag{5}$$

By combining Eq. 5 and Eq. 4, we obtain the following equation:

$$\Delta T = \frac{M_P}{M_F} \cdot \frac{E \cdot G_F}{M_{O \cdot c}} \cdot \frac{Q}{KS\Delta T} \tag{6}$$

Considering that the heat dissipated through the crusher walls $Q$ (J) is connected to the temperature variation $\Delta T$ by the following relation:

$$Q = M_F c \Delta T \tag{7}$$

the temperature of olive paste after crushing $T$ (°C) can be calculated after a simple equation rearrangement:

$$T = T_e + \frac{M_P}{M_O} \cdot \frac{E \cdot G}{K \cdot S} \tag{8}$$

where $T_e$ (°C) is the external temperature. Considering that the olives are usually stored at ambient temperature for many hours, it coincides with the olive temperature before crushing.

This last equation shows a direct proportionality of the thermal gradient ($T - T_e$) in respect to the total mass flow rate $G_F$. Eq. 8 also demonstrates that it is possible to reduce the thermal gradient, maintaining the mass flow rate constant and preserving the olive paste from overheating by means of the increment of the crusher surface $S$, which is necessary for the heat dispersion. Moreover, this equation explains the differences in olive paste temperature obtained if a hammer crusher is used instead of the stone mill. Even if the energy required for crushing the kernels expressed in mass units assumes the same value for both systems, the temperature increase is much greater in the hammer crusher than the stone mill due to the great difference of the surface of heat exchange.

Caponio et al. (2003) evaluated the quality of virgin olive oils obtained when either a hammer crusher or a disk crusher was used for the preparation of olive paste. They found that a hammer crusher caused a substantially higher rise in the output paste temperature than the disk crusher due to the energy needed to obtain a smaller fragment size. The more intensive crushing action of the hammer crusher on the olive pits inevitably has a major impact on the quality and shelf life of the resulting virgin olive oil. In fact, oxidative degradation in the oils obtained from hammer-crushed olives was found to be significantly higher than in those obtained from disk-crushed olives; this was shown by the higher levels of oxidized triacylglycerols and the results of the oven storage stability test.

As a consequence of the crushed olive paste temperature increment, the following phenomena can occur:

- A higher rate of oxidative reactions
- Inactivation of useful thermolabile enzymes such as lipoxygenases

### *Particle Size of Olive Fruit Fragments and the Polar Phenols Transfer into the Oily Phase*

The different crushing systems produce particles with different sizes, and this has various effects on virgin olive oil quality (Figure 7.1). Crushing disrupts the cell structure

and liberates the oily and aqueous phases containing a complex mixture of triacylglycerols, minor compounds, and various enzymes that were previously trapped within the cell compartments. All these enzymes can catalyze both desirable and undesirable reactions (Clodoveo, 2012; Clodoveo et al., 2014b), modifying the final quality of the product. The olive fruit mesocarp accumulates a wide range of secondary metabolites. The main category of secondary metabolites is represented by secoiridoids: oleuropein, demethyloleuropein, oleuroside, ligstroside, and nüzhenide. Glycosidic forms are more solubile in water, and after the process of mechanical extraction, only a small portion is recovered in the oil. During the crushing, malaxing, and extraction of olive fruits to obtain virgin olive oil, the glycosides oleuropein, demethyloleuropein, and ligstroside are hydrolyzed by endogenous β-glycosidases to form aldehydic aglycones. The aglycones become soluble in the oil phase because of the change in their polarity.

Theoretically, a finer particle size means a greater amount of material released from the ruptured cells and a greater hydrolyzation, favoring the dissolution of the aglycones in the oil. On the other hand, β-glycosidase is not the unique enzyme freed by the crushing. Polyphenoloxidases (PPO) and peroxidases (POD) catalyze, in the presence of oxygen and water, the oxidation of phenolic compounds to corresponding quinones (Zanoni, 2014). These phenomena cause a degradation of phenolic compounds and can bring about changes in both intensity of sensory descriptors, such as bitterness and pungency, and antioxidant power, resulting in decreased shelf life and nutritional value of the oil. However, alternative technological procedures can be implemented to obtain a selective inhibition of this class of enzymes (destoning, reducing the oxygen concentration into the crusher, etc.) (Clodoveo et al., 2014b).

The mechanical thrust exerted by the hammer crusher is stronger than that of the disk crusher; therefore, the size of the stone fragments produced by the first is

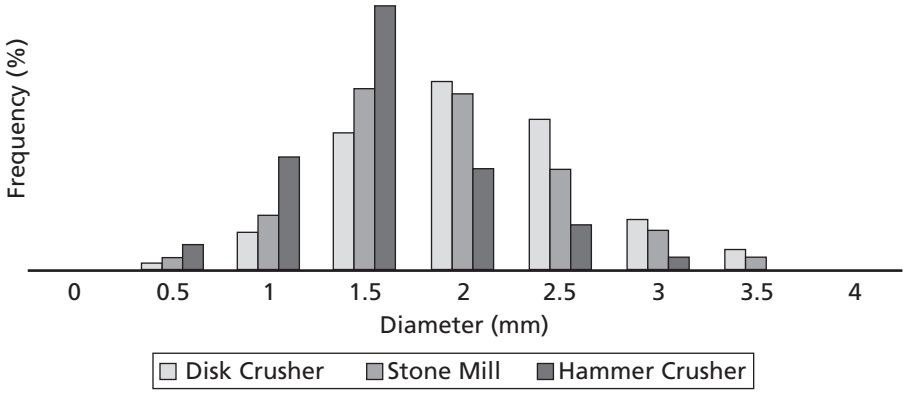


**Figure 7.1** Distribution of particle size of olive fruit fragments in three different crushing system. Data from an unpublished study.

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smaller than those produced by the latter. Amirante et al. (2010a), after many years of experimentation in various industrial olive mills, concluded that the oils extracted via the stone mill were characterized by the lowest content in total polyphenols, due to the long time required and the high exposition of the olive paste to air. The oils extracted using the disk crusher were characterized by a medium value, and the highest value of total polyphenols was obtained using the hammer crusher.

Similar conclusions are reported by Inarejos-García et al. (2011). Olives from two different cultivars, Arbequina and Cornicabra, were processed using hammer mills with various breakage forces applied and various grid hole diameters. The researchers observed that the stronger crushing conditions (i.e., smaller grid holes and higher rotation speed) resulted in a higher phenolic content in the olive paste. Figure 7.2 shows the effect of rotation speed (rpm) and hole diameter of the hammer crusher on the phenolic compound concentration (mg/kg) in the Cornicabra olive paste. When the higher rotation speed of the hammers is combined with the smallest hole, the olive paste has the highest concentration of oleuropein and the lowest concentration of hydroxytyrosol (Figure 7.2). This phenomenon indicates a reduced activity of the β-glucosidase enzymes, probably due to the higher olive paste temperature. However, the oil obtained from this olive paste was the richest in hydroxytyrosol and tyrosol probably due to the small size and large surface area of olive fruit fragments that favor the dissolution of nonpolar phenol into the oil. Analyzing the graphical representation, it is clear that the diameter of the grid holes, which determines the average particle size, was the main source of variation in the phenolic compositions in the olive paste (Figure 7.2) and virgin olive oil (Figure 7.3).

The olive fruit is very rich in phenolic compounds, but only 2% of the total phenolic content of the olive fruit passes in the oil phase, while the remaining amount is lost in the olive mill wastewater (~53%) and in the pomace (~45%) (Rodis et al., 2002; Alesci et al., 2014). It is, therefore, important to have an insight into the mechanisms involved in the dissolution of phenols.

The dissolution of phenols in virgin olive oil starts during the crushing and continues during the malaxation. The basic factors involved in the phenol transfer from the crushed fruit fragments (solid) to the oily phase include the processes of diffusion and solubility, chemical and biochemical reactivity, and hydrodynamic behavior. This phenomenon is based on the affinity of these substances (solute) (e.g., secoiridoid aglycones or other classes of polyphenols) toward a component of the solution (oily phase). Starting from the Fick's law principle, it is possible to make a balance of the aglycon phenol concentration throughout contact time

$$V_L \frac{dC_{PL}}{dt} = kA_s \left(C^*_{PS} - C_{PL}\right) \tag{9}$$

$$t = 0, C_{PL} = C_{PL,0} \tag{10}$$

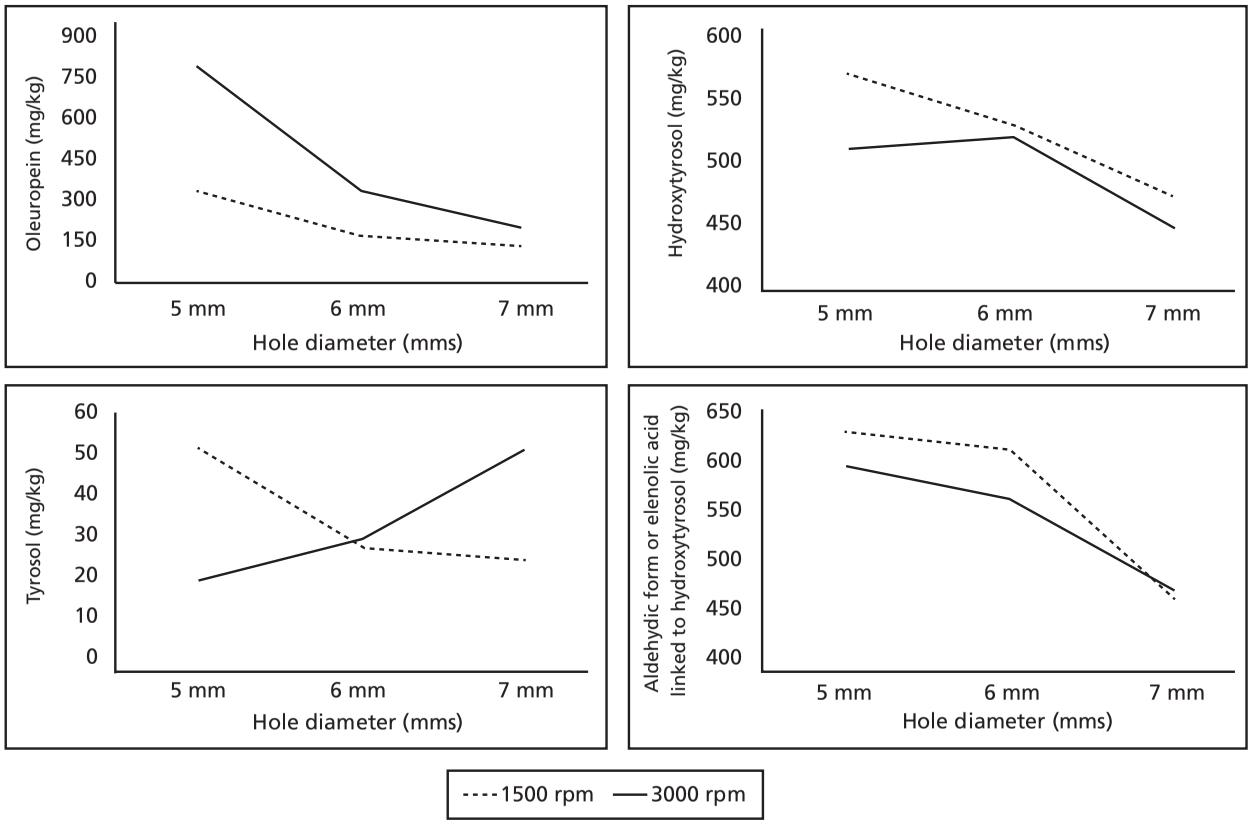


**Figure 7.2** Effects of rotation speed (rpm) and hole diameter of the hammer crusher on the phenolic compounds (mg/kg) in the Cornicabra olive paste. Data from Inarejos-García et al. (2011).

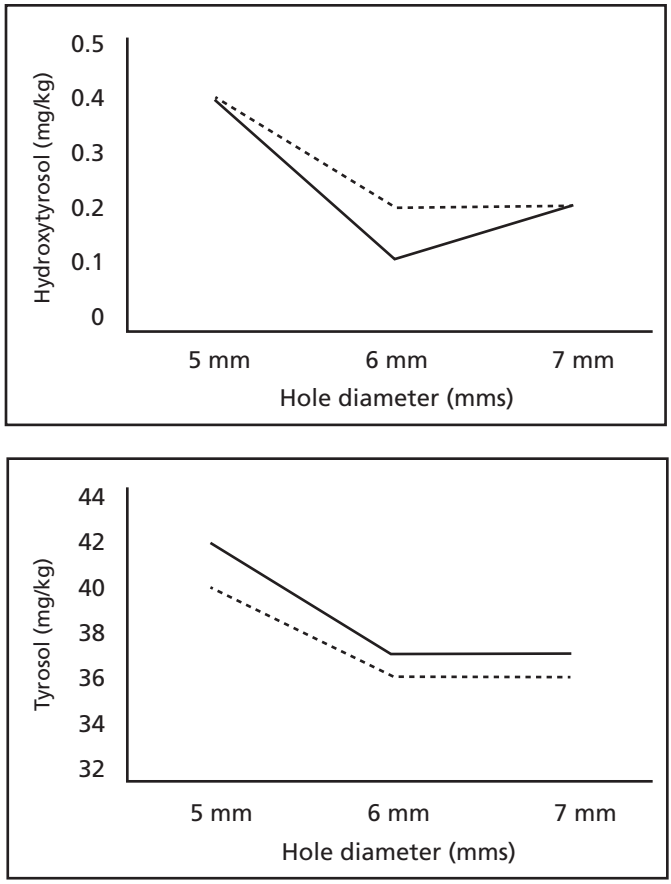


**Figure 7.3** Effects of rotation speed (rpm) and hole diameter of the hammer crusher on the phenolic compounds (mg/kg) in the Cornicabra virgin olive oil. Data from Inarejos-García et al. (2011).

where $V_L$ is the volume of the oily phase, $C_{PL}$ is the concentration of each class of phenols in the oily phase, $t$ is time, $k$ is the mass transfer coefficient in the oily phase, $A_s$ is the area of the solid phase, $C^*_{PS}$ is the solubility of each class of phenols in the oily phase, and $C_{PL,0}$ is the initial value of $C_{PL}$. Defining $a$ as the specific area of the olive fruit fragment, calculated as

$$a = \frac{A_s}{V_s} \tag{11}$$

and defining $R$ as the volume ratio of the solid ($V_s$) to the oily phase ($V_L$), namely

$$R = \frac{V_s}{V_L} \tag{12}$$

then Eq. 9 can be rewritten as

$$\frac{dC_{PL}}{dt} = kaR\left(C^*_{PS} - C_{PL}\right) \tag{13}$$

Assuming that there is an excess of solute in the solid phase, then $C^*_{PS}$ can be taken as essentially constant; consequently, integration of Eq. 13 leads finally to

$$C_{PL} = C^*_{PS}\left(1 - e^{-kaRt}\right) \tag{14}$$

The concentration of each class of phenols in the oily phase ($C_{PL}$) is directly proportional to the solubility of the considered class of phenols in the oily phase ($C^*_{PS}$), and it increases if the volume ratio ($R$) of the solid ($V_s$) to the oily phase ($V_L$), the specific area of the olive fruit fragment ($a$), and the contact time increase. So, according to this relation, by modifying the size of the particles, it is possible to modify the dissolution rate of each class of phenols in the oily phase.

### *Differential Crushing: The Destoning Machine as a Tool to Modulate the Polyphenol Composition of Oil*

In the last decade, many researchers studied the distribution and the activity of the endogenous enzymes of the drupe (García-Rodríguez et al., 2011; Mazzuca et al., 2006; Ortega-García et al., 2008; Salas et al., 1999; Saraiva et al., 2007; Taticchi et al., 2013) in order to employ extraction machines in a way that permits a modification of enzyme action. The correct and strategic choice of each machine can activate or inhibit the beneficial and harmful enzymes by selecting the different parts of the drupe (whole fruit or only the mesocarp, disrupting or preserving the integrity of endocarp). An alternative crushing system is the destoner, also called a "depitting" machine. It crushes only the pulp tissues (Amirante and et al., 2006; Dugo et al., 2007; Rodríguez et al., 2008; Servili et al., 2007). The resulting virgin olive oil has higher phenol content than those obtained by other crushing systems (Amirante et al., 2006; Servili et al., 2007) due to the different distribution of endogenous enzymes and of phenolic compounds in the various parts of the olive fruit (pulp, stone, and seed).

Servili et al. (1999) examined the distribution of phenolic compounds in the various parts of the olive fruit; they found that oleuropein and dimethyl-oleuropein were present in all parts of the fruit, with the highest concentrations in the pulp, whereas luteolin-7-glucoside and rutin were present only in the peel and nuzhenide in the seed. With regard to the distribution of enzymes in the different parts of the

fruit, POD is highly concentrated in the olive seed (Amirante et al., 2006, 2010a; Servili et al., 2007). So, the exclusion of seed can reduce the enzymatic oxidation of the pulp phenols. García-Rodríguez et al. (2011) studied the role of seed POD in the pool of oxidative reactions that determine the final content of phenolics in the oil. The seed POD and PPO are able to oxidize both the main phenolic glucosides found in the olive fruit and phenolics arising during the industrial processing. The modulation of olive POD with the use of a destoner could have a great impact on nutritional and sensory qualities of virgin olive oil. Luaces et al. (2007) studied the role of the kernel and its effect on the phenolic composition by mixing increasing seed proportion to the destoned pulp before the oil extraction. The destoned fruit oils were characterized by a higher phenol content. This observation indicated a real role for the seed in the oxidation of phenolic compounds during the extraction processes due to the high levels of POD activity observed. The small loss of the oil yield (about 1.5 kg of oil per 100 kg of olives in comparison to the traditional process) is the main disadvantage of this system; however, it is insignificant compared to the advantages.

### *Challenges for the Development of Innovative Crushing Systems to Reduce Undesirable Oxidation Reactions*

The influence of the atmosphere composition in contact with the olive paste during crushing is a poorly studied aspect. Oxygen concentration inside the olive paste after crushing has an average value of 18% (very similar to the atmosphere composition) (Amirante et al., 2012). It is important to develop innovative crushing systems able to modulate atmosphere composition inside the crushing chamber to obtain a strategic control the oxygen concentration in the olive paste. In this way, undesirable oxidative reactions, mainly catalyzed by POD and PPO, can be prevented in the subsequent malaxing step in which the process parameters are favorable for enzymatic activities. An example of a plant that allows modulation of the atmosphere composition from the crusher to the malaxer has been described in a recent patent (Clodoveo, 2013a) (Figure 7.4).

The atmosphere upstream of the crusher is balanced by the hydrostatic pressure of a water vat, whereas downstream of the crusher is delimited by the hermetic closure of the malaxer. The atmosphere composition can be modulated, choosing the appropriate mix of gas, both in the crusher and in the malaxer by means of a valve implemented on the malaxer.

An American company that produces machinery for the olive oil industry has recently introduced in the market a new product named the "Apollo-Culitvar 500." It is a small-size extraction plant that operates under vacuum. The producer built this equipment in order to reduce the olive paste exposure to oxygen by operating under vacuum and obtaining a product richer in polyphenols. However, this new

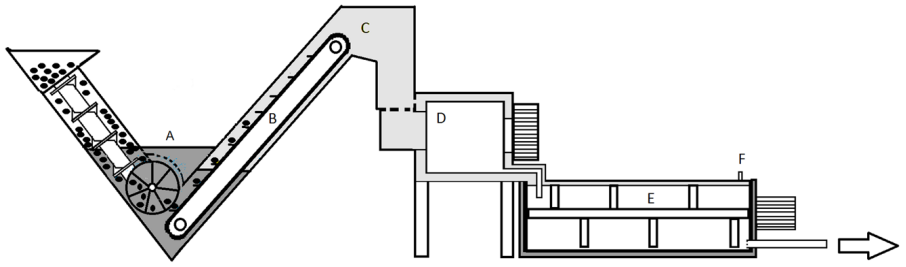


**Figure 7.4** Method and apparatus for thermal conditioning of olives or other oleaginous fruits combined with a crushing and kneading system of olives or other oleaginous fruits in controlled or modified atmosphere (Patent No WO2013076592 A1). In the vat (A) filled with water, a conveyor belt (B) is immersed to transfer the olives coming from the washing machine toward the crushing section. A transfer duct (C) is isolated from the outside environment and the crusher (D) is apt to operate in controlled or modified atmosphere. The crushed product is then transferred directly to a hermetic kneader (E) equipped with a one-way valve (F) that allows the outletting of air or gas during the loading step, but not allowing their inletting into the crushing/kneading environment.

equipment has not yet been validated by scientific research. A probable weakness of vacuum employment could be the loss of aromas due to their low vapor pressure (Clodoveo et al., 2014b).

The restricted data available in the international literature on this topic demonstrates that there is an important scientific lacuna in the control of atmosphere composition inside the crusher. Future research may well clarify the relevance of the plant implementation, but in any case, the atmosphere control limited to the malaxer machine is ineffectual because of the high rate of enzymatic reactions during crushing, which makes useless any subsequent modulation attempts.

Very recently, an Italian research group (Zoani et al., 2014) proposed a new method employing solid-state carbon dioxide, commonly known as "dry ice" added to the olives before crushing. The water molecules in the fruit cells freeze, causing the collapse of the cell walls of the olives. The new method provides a significant increment of yield of oil compared to traditional methods. The oil contained more vitamin E and it was characterized by a greater resistance to oxidation because gaseous carbon dioxide protects olive paste inside the malaxer from oxidation. The high operating costs related to the fruit cooling through liquid $CO_2$, which are well covered in the enology industry by the high price of the best quality wine, should be properly evaluated for the olive oil industry because often the market price of olive oil does not cover the production costs (Clodoveo et al., 2014a).

### *Malaxation*

Malaxation prepares the olive paste for the subsequent separation of the oil. It is a slow mixing of the paste at a carefully monitored temperature (28–30 °C) for a period between 30 and 60 minutes, depending on the features of the raw material. Malaxation promotes the coalescence of the tiny oil drops in drops of greater sizes; these can be more easily separated in a centrifugal field and this reduces the olive paste viscosity to optimize the phase separation inside the decanter (oil/vegetal water/pomace). Malaxation of olive paste is more than a simple physical separation; it is a bioprocess related to the quality and composition of the final product. Malaxing conditions can modify the phenol contents in virgin olive oil and, as a consequence, its nutritional and sensory properties. A deeper knowledge of the working parameters (process duration, temperature, atmosphere in contact with the olive paste, addition of lukewarm water) is necessary to diversify the oil quality (Clodoveo et al., 2014b, 2014c).

#### *Duration of Process and Its Effect on Phenol Content*

Two main effects of the malaxation time on the phenol content of virgin olive oil should be taken into account:

1. The mechanisms that are involved in the dissolution of polyphenol classes in the oily phase (Eq. 14)
2. The activity of PPO and POD and the rate of phenol oxidation over time in the presence of oxygen and high water content

Usually, a long malaxing time in nonhermetic malaxers produces a decrease in oil phenol content and related parameters, such as oxidative stability and bitterness.

Evidence for the influence of air exposure time of olive pastes on the phenolic composition of virgin olive oil has been provided in the study conducted by Servili et al. (2003). The authors demonstrated that total phenols and the sum of orthodiphenols were negatively correlated with the time and the presence of oxygen during malaxation. Masella et al. (2012) tested a hermetically sealed malaxer and concluded that, under sealed conditions, large $CO_2$ emissions coupled with $O_2$ depletion occur. The oil samples produced under sealed conditions were less oxidized and had a higher concentration of antioxidant compounds (especially secoiridoids phenols) than the control (nonhermetic malaxer).

#### *Temperature: Evolution of Heat-Transfer Systems, Innovative Equipment, and Emerging Technologies*

Malaxing time and temperature are inversely correlated factors. Oil millers tend to increase malaxing temperature (Clodoveo, 2013c; Clodoveo and Hachicha Hbaieb, 2013) in order to favor the coalescence of the oil and hence a decrease in viscosity. The coalescence of oily drops inside the olive paste is due to hydrophobic interactions. The

hydrophobic effect is largely due to the special ability of water molecules to form hydrogen bonds with themselves. The attractiveness of the water molecules for each other causes the formation of larger drops. A high malaxing temperature also promotes the oil/water separation in the decanter centrifuge (Clodoveo et al., 2014b, 2014c).

The simultaneous enlargement of oil drop diameters and the reduction of olive paste viscosity facilitate the subsequent separation of the oily phase into a centrifugal field (decanter). These principles are well explained by the Stoke's law:

$$V_S = \frac{d^2\left(\rho_p - \rho_o\right)w^2 r}{18\eta} \tag{15}$$

where $V_S$ is velocity of oily droplet (m/s), $d$ is the oily droplet diameter (m), $r$ is the radius of the centrifuge, ω is the acceleration due to centrifugation (m/s$^2$), ρ is the density of the olive paste ($p$) and oily phase ($o$) (kg/m$^3$), and η is the viscosity of olive paste (kg m$^{-1}$ s$^{-1}$ ).

The temperature also affects the phenol content of the oil. Older literature data indicate an inverse relationship between the temperature and the phenolic content (Angerosa et al., 2001; Servili et al, 2003). More recent research shows an increase of the phenolic fraction with a temperature increase (Boselli et al., 2009; Kalua et al., 2006). These contrasting results are due to the great variability of the experimental conditions and the evolution of malaxer machines. Servili et al. (2003a, 2003b, 2012) reported that the rise of temperature favors the oxidation of phenolics by the PPO and POD activities. These enzymes are active only in the presence of air and water; therefore, it is possible to inhibit or reduce their activity by controlling the atmosphere or by removing the olive pit before crushing.

For many years, malaxing machines were characterized by a nonhermetic closure. Amirante et al. (2006) found that this type of machine caused considerable loss of phenolic and volatile compounds. In fact, due to the stainless steel grill, the volatile compounds were dispersed into the air above the tank and, at the same time, phenolic compounds were oxidized by contact with the air. As a result of these findings, some equipment manufacturers accessorized their malaxing units with hermetic sealing (Clodoveo, 2012).

Parenti et al. (2008) studied the effect of malaxation temperature on the virgin olive oil phenolic profile in a hermetic malaxer. They found that both total phenol and secoiridoid concentrations, plotted as functions of the malaxation temperature, presented bell-shaped curves with maximums at 27 °C and 30 °C, respectively. At low temperatures, phenols are moderately soluble in the oily phase. Thus, initially, a rise of the temperature favors an increase of solubility and total phenol content until the maximum value of the bell-shaped curve is reached. Finally, the oxidative reactions prevail causing a detrimental effect on the total phenol content of the resultant virgin olive oil.

It can be hypothesized that contrasting results reported in the literature could be due to the heterogeneous experimental conditions (cultivar, maturity stage, agronomic and technological practices, etc.) and also to methodology based on extrapolation of curves from few and purely distributed samples.

*Atmosphere Composition: Control of Oxygen Concentration inside the Malaxer*

The control of oxygen contact with the olive paste during the malaxing phase is a key factor in the modulation of nutritional and sensory characteristics of the oil. The presence of the oxygen in the olive paste and in the headspace of the malaxer can cause both desirable and undesirable effects on triacylglycerols and phenolic compounds. The flavor improvement of virgin olive oils requires knowledge of the volatile biosynthetic pathways and demands the right tools for a controlled management of the temperature and the oxygen concentration.

Servili et al. (2009) and Taticchi et al. (2013) studied virgin olive oil phenols and the fruit endogenous enzymes responsible for the modification of their concentration in the final product. In particular, they tested the effect of exposure of olive pastes to air on the volatile and phenolic composition of virgin olive oil and, as a consequence, its sensory and healthy qualities. Their experiences and the results obtained by other research groups encouraged researchers to design modern hermetically sealed malaxers that can operate under inert gas (nitrogen and argon) (Clodoveo, 2012). For the moment, the malaxation under inert gas is not widely spread due to the high cost of nitrogen and argon and due to the effect that these gases have on the volatile compounds.

The presence of inert gas inside the headspace of the malaxer reduces the activity of oxidases and at the same time inhibits the synthesis of volatile compounds. In order to address these problems, Parenti et al. (2006a, 2006b) suggested taking advantage of the carbon dioxide emission that occurs simultaneously with the oxygen depletion during malaxation under sealed conditions. The initial concentration of $O_2$ (on average, 18% of the gaseous phase) guarantees the synthesis of an appreciable amount of volatile compounds. The subsequent emission of $CO_2$ due to respiration phenomena inhibits the enzymic oxidation of phenolic compounds.

In a very recent report, Servili et al. (2014) confirmed that the choice of the optimal temperature and the amount of $O_2$ during mixing is a strategy for the production of a high-quality extra virgin olive oil, but these parameters must be correlated to the olive cultivar. Although the strong impact of the $O_2$ concentration and the processing temperature on the bioactive phenol content is well known, the different values obtained with different malaxation conditions are cultivar-dependent. This indicates that the genetic biodiversity is one of the most important parameters that affects the phenolic concentration in extra virgin olive. So, the operative conditions of malaxation must be optimized according to the cultivar.

Jiménez et al. (2014), having studied the influence of the malaxation time and olive ripening stage on oil quality and phenolic compounds of Hojiblanca and Picual virgin olive oils, concluded, in accordance with Servili et al. (2014), that it is necessary to regulate the malaxation parameters such as time, temperature, and the composition of the atmosphere in contact with the olive paste according to the olive cultivar, also monitoring the chemical/biochemical changes of olive paste.

### *Process Water: Partition of Virgin Olive Oil Phenolic Compounds between the Oily and Aqueous Phases*

Water addition is a very common practice in the olive mills because it reduces the process time and increases the oil yield. On the other hand, concentration of hydrophilic phenols decreases, depending on the amount of water added.

The distribution between the oily and the aqueous immiscible phases is a function of their partition coefficients and the processing temperature. The partition coefficient ($K$) is expressed by the following equation:

$$K = \frac{[P]\ \text{oily phase}}{[P]\ \text{aqueous phase}} \tag{16}$$

where $[P]$ is the concentration of phenol compounds expressed as mg/g. The partition coefficient $K$ has no units. In conditions of chemical/physical equilibrium, it depends only on the temperature. A temperature increase could lead to an increase of the partition coefficient. Of course, the law of the partition equilibrium is strictly valid for individual compounds only, and not for the total phenol content of oil and vegetable water (Rodis et al., 2002). It is, therefore, important to limit the quantity of water during oil extraction and to increase the temperature until the maximum value suitable for each cultivar, above which the oxidative reactions prevail (Servili et al., 2014; Jiménez et al., 2014).

### *Beyond the Traditional Malaxation: Emerging Technologies and Innovative Strategies to Develop Continuous Plants*

The whole approach to the study of the malaxing phase and its effects on olive oil yield and quality has been changed during the last few years because of changes of the malaxer equipment. The malaxation phase actually represents the "bottleneck" of the continuous extraction process because of the long duration of this step. Currently, the system used to guarantee continuity in the process without interrupting the activity of the machines upstream and downstream of the malaxer consists of several malaxing machines in parallel (Clodoveo, 2013c, 2013d; Clodoveo and Hachicha Hbaieb, 2013; Clodoveo et al., 2013); this system has the disantvantage of a heavy plant investment (Figure 7.5). One of the critical factors determining this long time is the period necessary for the crushed olive paste to reach the process temperature (27–32 °C) (Clodoveo,

**Figure 7.5** Virgin olive oil extraction plant equipped with plural malaxing machines in parallel. Photography courtesy of Alfa Laval Olive Oil S.p.A.

2013c; Clodoveo et al., 2013). Malaxers currently on the market are heat exchangers characterized by a low overall heat transfer coefficient. It is, therefore, important to find an innovative technology to improve heat exchange.

Recently, Esposto et al. (2013) and Fiori et al. (2014) tested the introduction of a heat exchanger before malaxation in order to revise the traditional thermal conditioning applied to the olive pastes. The duration of this fast preheating is no longer than 1–3 minutes (depending on the mass flow rate) and it is followed by a short malaxation (10 min on average). Both the research groups, after these preliminary tests, concluded that the overall quality of the extracted oil is good, but the phenols are negatively affected by the flash thermal conditioning of olive pastes.

The other advantages obtained (efficiency of the process, reduction of bitterness, pleasant aroma, increase of yield) led two of the main oil mill plant manufacture companies to bring to market a new model of heat exchanger placeable between the crusher and the malaxer. Moreover, preliminary results obtained from destoned olive paste of cv. Peranzana revealed a significant increment of oil yields, on average equal to 1 kg of oil per 100 kg of olives (Clodoveo et al., 2014c).

In order to increase virgin olive oil yields, it is necessary to reduce the process time and improve process efficiency. It is, therefore, important to introduce technologies able to determine both mechanical and thermal effects. Abenoza et al. (2013) proposed the application of pulsed electric fields in the virgin olive oil extraction process without malaxing and with promising results in relation to the increase of oil yield. Very recently, Puértolas et al. (2015) studied at pilot scale in an industrial oil mill the impact of the use of pulsed electric field technology on Arroniz olive oil production. They found that the application of a pulsed electric field treatment to the olive paste significantly increased

the extraction yield by 13.3%, with respect to a control. Furthermore, olive oil obtained by this treatment showed total phenolic content, total phytosterols, and total tocopherols significantly higher than the control (11.5%, 9.9%, and 15.0%, respectively). The use of pulsed electric field technology had no negative effects on general chemical and sensory characteristics of the olive oil, maintaining the highest quality according to EU legal standards. Therefore, pulsed electric fields could be an appropriate technology to improve olive oil yield and produce virgin olive oil enriched in health-promoting compounds, such as biophenols, phytosterols, and tocopherols.

Clodoveo et al. (Clodoveo, 2013c; Clodoveo and Hachicha Hbaieb, 2013; Clodoveo et al., 2013) tested the employment of ultrasounds and microwaves, other emerging technologies that have already found application in the food industry, in order to obtain technological advantages in virgin olive oil extraction. Both these technologies showed mechanical and thermal advantages at a pilot scale (Clodoveo and Hachicha Hbaieb, 2013; Clodoveo et al., 2013). The mechanical actions caused the release of the fraction of the oil and minor compounds trapped in the fruit cell, which remained unbroken after crushing. The faster thermal effect, on the other hand, significantly reduced the duration of malaxation.

The very promising results are indicated in a recent patent relative to a method for microwave dielectric heating in the extraction of virgin olive oil (Clodoveo, 2014). The main advantages of the innovative method compared to the conventional one are:

- More effective and selective heating
- Considerable process time reduction
- Yield increase and reduction of oil losses in byproducts
- Faster and safer heating control
- Less space requirements of apparatuses
- Applicability even for biological productions with a smaller environmental impact

In an attempt to develop innovative ultrasound equipment for the extraction of virgin olive oil, Clodoveo (2013c) proposed a combination of an ultrasound probe with a double-pipe heat exchanger. The main idea was to obtain a more efficient heat exchange before pumping the olive paste inside the malaxers. In this way, a simplification in the construction of machinery is attainable because the jacket for heating the olive paste is excluded and the tank can be thermally isolated in a simpler and cheaper manner.

### *Separation of Phases*

The separation of oil from solid and liquid phases of olive paste is obtained by applying three different systems: pressure, percolation, and centrifugation (Amirante et al., 2000, 2010b; Baccioni and Peri, 2014). One of the main factors influencing the total phenol content of the resulting olive oil is the water used to dilute the olive paste. In the pressing systems no water is added. Thus, more stable oils with higher levels of antioxidants

are produced, as demonstrated by Di Giovacchino et al. (2002) (Figure 7.6), provided that very good quality olives are used and the mats are carefully cleaned.

*Pressure*

The pressure extraction system is considered as an obsolete technique because it is the oldest method for processing olive fruits. The oil may have a higher phenol content compared to the product obtained by centrifugation (Salvador et al., 2003) because the olive paste is not diluted by water; however, the low work capacity and the high labor costs have reduced its use.

*Percolation*

Olive oil extraction from olive paste by the percolation method, also called Sinolea, is based on the difference of the surface tension between olive oil and vegetable water. Its use is limited because of the high cost and large size of the plant. In this system, steel blades are plunged into olive paste and preferably coated with oil, which drips off and splits up from the other phases, thus creating a flow of oily must. The Sinolea is able to extract only 50–70% of the oil (first extraction oil). The remaining oily paste, after an additional malaxation for 20–40 minutes, is thinned with water and further centrifuged with a three-phase decanter in order to recover the main part of the re-

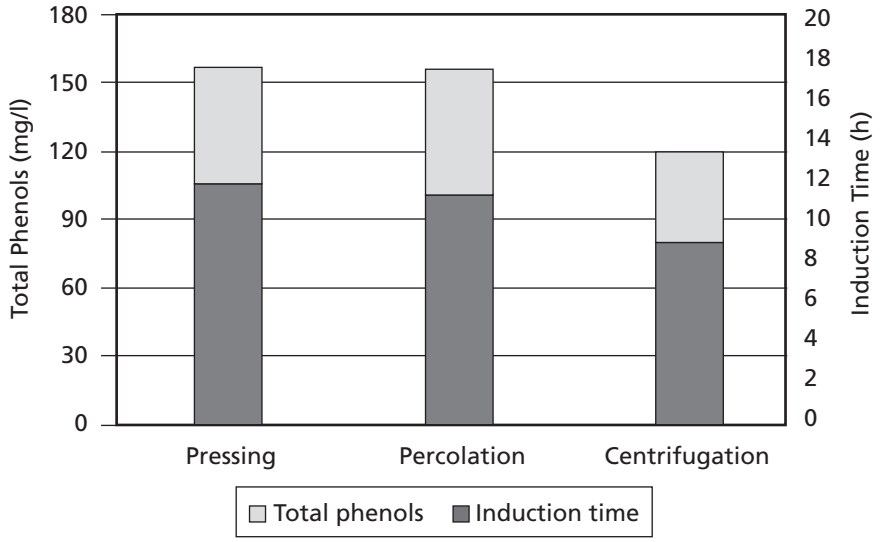


**Figure 7.6** Total phenols and induction times of oils obtained by pressing, percolation, and three-phase centrifugation. Data from Di Giovacchino (2002).

maining oil (second extraction oil). The Sinolea first-extraction oil is a high-quality virgin oil characterized by high phenol content. The second-extraction oil is of lower quality due to the additional malaxation and thinning with water.

#### *Centrifugation*

Centrifugation technology was introduced at the end of the 1980s and currently is the most applied extraction process. It is based on the differences in the density of the olive paste constituents (olive oil, water, and insoluble solids). Separation is accomplished through a horizontal centrifuge (decanter). Today, two different centrifugation systems are mainly used for olive oil production depending on the products produced at the end of processing: the three-phase and the two-phase centrifugation techniques. In the three-phase centrifugal decanter, water is added to dilute the incoming paste and, at the end of the process, it is divided into oil, vegetation water, and solids (olive pomace). In the two-phase process, paste is separated in oil as a liquid phase and a solid phase, composed of fragments and kernels, pulp, and vegetation water (humid olive pomace) (Amirante et al., 2010b). The two-phase process requires no dilution or only a little dilution during the malaxation phase. So, the main difference between the two types of machines is the amount of water added to dilute the olive paste: The two-phase process has low water consumption and low waste water production. As a consequence, the oils obtained after extraction by the two-phase centrifugal system have a higher content of phenols, longer induction times, and better sensory scores (Clodoveo et al., 2014b; Kalogeropoulos et al., 2014; Salvador et al., 2003).

### *Vertical Centrifugation and Filtration: Effect of the Dispersed Water on the Polar Antioxidant Level and the Product Stability*

The extracted oily phase can be further clarified in an automated discharge vertical centrifuge (disk centrifuge) with lukewarm tap water added. Vertical centrifugation separates the residual water and the solid impurities in order to obtain a clear oil, reducing the virgin olive oil humidity concentration to a mean value about 0.18% (Masella et al., 2009). However, the addition of water reduces the hydrophilic phenol content. DiGiovacchino et al. (1994) reported a decrease after vertical centrifugation both for total phenol and orthodiphenol concentrations as a function of increasing amounts of washing water (from 0 to 80% of the oily must). As recently reported (Masella et al. 2012), vertical centrifugation causes a strong oxygenation of the virgin olive oil, resulting in a marked increase of dissolved oxygen concentrations. This condition can lead to a noticeable shortening of the oil shelf life as a consequence of accelerated oxidation.

After this last centrifugation, some producers prefer filtering the oil using diatomaceous earth or cellulose fibers to achieve a more brilliant oil with reduced humidity, avoiding the risk of developing some sediment in the bottom of the bottle. Really, a high-quality extra virgin olive does not need to be filtered if deposition of a residue is

complete. Filtration may affect the phenolic content and positive flavor attributes. To overcome such problems, Italian and Spanish researchers (Lozano-Sánchez et al., 2012) proposed inert polypropylene filter bags and inert gas flows as filter aids.

Unfiltered virgin olive oil, also called "cloudy," "veiled," or "natural," is produced in the form of an emulsion or dispersion that can persist for several months before deposition of a residue. Many chefs prefer this natural slight cloudiness in salads or in gourmet dishes, and many consumers consider this type of virgin olive oil to be more "green" and not overprocessed. However, this is not correct because the additional "processing" is only precipitation and filtering. Veiled oils seem to have longer induction periods compared to the filtered ones. It appears that the material in suspension dispersion plays a significant role against oxidation. The presence of emulsifiers may also have an impact. There are compounds in the oil with a low solubility in water that act as tensioactive solutes. Bianco et al. (1998) identified two digalactosyl glycosides in freshly produced oils, the α-1,6-digalactosyl derivative of the 1,2-glycerol diester of linolenic acid and the α-1,6-digalactosyl derivative of the glycerol linolenate-oleate diester. The physicochemical characteristics of such compounds and the stable emulsions formed may allow an increase in the transfer of hydrophilic phenolic compounds, which are strong antioxidants (Figure 7.7).

Tsimidou et al. (2005) found a higher total phenol compound content in veiled oils in relation to the filtered oils, and this may partly explain the higher stability. Lipoxygenase activity has been detected in freshly prepared olive oils (Georgalaki et al., 1998). Taking into consideration the higher stabilities of cloudy oil, it can be postulated that the polar phenolic compounds present may act not only as primary antioxidants but also as inhibitors of oxidizing enzymes.

Lozano-Sánchez et al. (2012) studied the retentive capacity of inorganic and organic filter aids on phenolic compounds and found that a large number of polyphenols were retained in filter aids, lowering the total phenol content in filtered oil. Bakhouche et al. (2014) conducted a study to monitor the moisture and bioactives over the industrial filtrations process. They concluded: "Although filtration can make virgin olive oil brilliant and can increase its shelf life by reducing its moisture content, filtration sacrifices certain phenolic compounds which could affect virgin olive oil oxidative stability and its nutritional quality. Consequently, to maintain olive-oil quality, producers need to take into account both moisture loss as well as the antioxidant content during virgin olive oil filtration."

## Storage and Packaging: Bioactive Micro-Constituents and Shelf Life of the Product

The conditions of virgin olive oil storage (either in large tanks or in small packages) are critical for preserving quality and health properties (Boskou, 1996). All the strategies applied in the orchard and in the olive mill to produce virgin olive rich in phenols

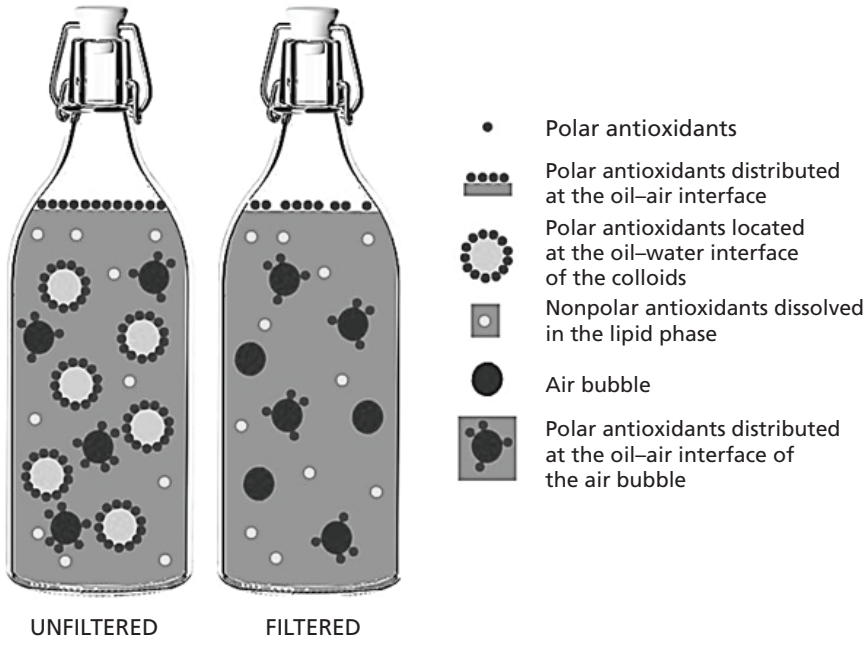


**Figure 7.7** Distribution of antioxidants in unfiltered and filtered virgin olive oil.

can be undermined by improper storage conditions. To slow down the oxidation rate during storage, certain factors such as the presence of oxygen and traces of metals, exposure to light, and the binomial storage time/temperature must be kept under control (Bendini et al., 2010).

The main variables involved in the oxidative stability of virgin olive oil during storage are well known and documented (Bendini et al., 2010; Boskou et al., 2006; Piscopo and Poiana, 2012). This section is limited to specific aspects that are useful to highlight the main strategies to preserve virgin olive oil phenol content during storage. The choice of the handling procedures and the packaging material and shape should aim at avoiding:

- Adsorption of air (by minimizing the amount of air incorporated in the liquid by means of delicate procedures to fill the storage containers and by employing low gas permeability materials, e.g., metal cans or glass bottles)
- Excessive moisture (by bottling the product only after a full deposition of a residue)
- Contact with pro-oxidants (both tank and package should be made of chemically inert material)
- Exposition to the light (by choosing light barrier packaging materials)

- Packages with high thermal conductivity (by choosing packaging materials characterized by high resistance to temperature changes)
- Unnecessary agitation (agitation favors the molecule movement and renovates the liquid layers surrounding the reactive species, increasing the rate of oxidation)

The shape of the package can also have important effects on virgin olive oil stability. The best packaging is characterized by the lower headspace, which minimizes the amount of oxygen in contact with the product. Going (1968) demonstrated the importance of the area-to-volume ratio of the headspace of different containers during the storage of vegetable oils. They pointed out that, to minimize the oxidation rate, it is important to fill the container completely and reduce the contact area between the air and the product. A good alternative is to saturate the headspace of the package with inert gas, such as nitrogen.

## Olive Pomace and Olive Pomace Oil as Sources of Bioactive Compounds

Olive pomace is the solid residue obtained from the olive oil production process (during pressing or centrifugation). This residue is treated with solvents to extract the oil it contains to obtain *Crude Olive-Pomace Oil*. As it is not edible, it has to undergo a refining process to obtain *Refined Olive-Pomace Oil.* This oil, with no flavour, aroma or colour, is improved with edible Virgin Olive Oils (not including lampante) to obtain the oil called *Olive-Pomace Oil*. So, it is marketed in accordance with the following legal designations and definitions:

- *Crude olive pomace oil* is olive pomace oil with characteristics that are fixed for this category in this standard. It is intended for refining for use for human consumption, or it is intended for technical use.
- *Refined olive pomace oil* is the oil obtained from crude olive pomace oil by refining methods that do not lead to alterations in the initial glyceridic structure. It has a free acidity, expressed as oleic acid, of not more than 0.3 g per 100 g, and its other characteristics correspond to those fixed for this category in this standard.
- *Olive pomace oil* is the oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption as they are. It has a free acidity of not more than 1 g per 100 g, and its other characteristics correspond to those fixed for this category in this standard. In no case shall this blend be called "olive oil."

Olive pomace retains a significant amount of residual oil and can be a source of valuable compounds, mainly triterpenic acids (maslinic acid, oleanolic) and triterpene dialcohols (erythrodiol, and uvaol), all of which are pentacyclic triterpenes found in the nonglyceride fraction of pomace olive oil. Uvaol plays a protective role

in the oxidation of lipoproteins of low density in vitro and has a protective effect on induced hepatic injuries. Oleanolic acid (3β-hydroxy-olean-12-en-28-oic acid) and its isomeric, ursolic acid (3β-hydroxy-ursan-12-en-28-oic acid), have been studied for many pharmacological properties (Guinda et al., 2010). Numerous researchers confirm that pentacyclic triterpenes have antibacterial, antifungal, anticariogenic, antiallergic, anti-inflammatory, hepatoprotector, gastroprotector, hypolipidemic, antiatherosclerotic, and antidiabetic effects (Dzubak et al., 2006; Liu, 2005; Tian et al., 2002). They can also interfere in several phases of the development of different types of neoplasias (Juan et al., 2006; Kuo et al., 2009; Yamai et al., 2009). Recently, it has been reported that oleanolic acid also has beneficial effects on multiple sclerosis (Martín et al., 2010). Claims also exist indicating that triterpene acids may even become part of the fight against HIV, the cause of AIDS.

It is clear from this information that edible olive pomace oil is not devoid of nutritional and biological active components. It has the same fatty acid composition and may contain higher amounts of squalene, α-tocopherol, and sterols. Besides, it is richer in triterpene dialcohols and probably in hydroxyterpenic acids, in spite of their elimination during refining with alkali. Probably through innovative methods for refining crude pomace oil, an oil richer in triterpenic acids suitable for consumption can be obtained.

Several extraction plants eliminate most of the moisture of fresh olive pomace and extract the oil from the cake by solvent extraction using hexane. Alternatively, "alperujo" (two-phase olive mill waste) is stored in large ponds for months and the residual oil is extracted periodically. In order to obtain an edible product, crude pomace olive has to be refined. The refining (physical or chemical) not only eliminates undesirable compounds (peroxides, degradation products, volatile compounds responsible for off flavors, free fatty acids, etc.) but also results in the loss of valuable bioactive compounds and natural antioxidants. Lama-Munoz et al. (2011) proposed the application of a new process based on the hydrothermal treatment of alperujo; the aim of their work was to obtain a final solid rich in oil and enriched in functional minor components (De Leonardis, 2014). The final treated solid had an increase in oil yield up to 97%, with a reduction in solids up to 35.6–47.6%. Sterols increased up to 33%, aliphatic alcohols increased up to 92%, triterpenic alcohols increased up to 31%, squalene increased up to 43%, tocopherols increased up to 57%, and oleanolic acid increased up to 16% through use of the new treatment. Additional studies are necessary to understand to what extent these increased levels are maintained in the edible oil after the refining process.

It is worth mentioning also that storage of alperujo in ponds for 7 months causes an increase in triterpenic acids and other bioactive compounds in the oily phase of the pomace. According to García et al. (2008), stored olive paste is a good source for crude pomace olive oil that is rich in triterpenic acids.

## Conclusion

Phenolic compounds are important for the sensory and nutritional qualities of virgin olive oil. For some health effects the evidence is already strong enough to enable the legal use of claims on the virgin olive oil label. During the extraction process, phenolic substances undergo chemical and biochemical changes that modify their structure and influence their presence in the final product. High-quality virgin olive oil can be produced only from healthy, fresh fruits at the right ripening grade. The quality of the oil present in the fruit cells can only be preserved during the elaboration process, but no technological solution is available that can create a quality product from poor-quality olives. The final quality of virgin olive oil and the level of bioactive compounds, arises, first of all, inside the orchard. The phenolic content depends both quantitatively and qualitatively on its genetic makeup. After choosing the best harvesting time for each cultivar in each particular geographical area, the other two main factors that are crucial for establishing the final quality should be considered: the harvesting methods and the postharvesting storage. If the harvesting system causes a high amount of damaged fruit, the extraction of oil should be made promptly, avoiding fruit storage at ambient temperatures. If every single step of the mechanical process is not rationally conducted, it can lead to a dramatic reduction of antioxidants, particularly phenols, which are molecules susceptible to chemical and biochemical oxidation reactions. The choice of crushing system depends on the raw material features (cultivar, ripening stage, water content) and the desired characteristics of the final product (phenol and volatile content, color, bitterness, and pungency). A deeper knowledge of the working parameters (process duration, temperature, atmosphere in contact with the olive paste, addition of lukewarm water) is necessary to diversify the oil quality. Malaxing conditions can modify the phenol contents in virgin olive oil and, as a consequence, its nutritional and sensory properties. The separation of oil from solid and liquid phases of olive paste is obtained by applying three different systems: pressure, percolation, and centrifugation. The main factor influencing the total phenol content of the resulting olive oil is the water used to dilute the olive paste. All the strategies applied in the orchard and in the olive mill to produce virgin olive rich in phenols can be undermined by improper storage conditions. It is important to develop innovative solutions to increase oil yields and improve quality, reducing at the same time the environmental impact of the process. Emerging technologies, such as pulsed electric fields, microwave and ultrasound are promising tecniques suitable for plant improvement and optimization. Innovations are also being developed in the sector of pomace olive oil aiming at obtaining a final product richer in functional minor components.

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