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Mechanical Strategies to Increase Nutritional and Sensory Quality of Virgin Olive Oil by Modulating the Endogenous Enzyme Activities

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Abstract: This monograph is a critical review of the biological activities that occur during virgin olive oil (VOO) extraction process. Strategic choices of plant engineering systems and of processing technologies should be made to condition the enzymatic activities, in order to modulate the nutritional and the sensory quality of the product toward the consumer expectations. "Modulation" of the product quality properties has the main aim to predetermine the quantity and the quality of 2 classes of substances: polyphenols and volatile compounds responsible of VOO nutritional and sensory characteristics. In the 1st section, a systematic analysis of the literature has been carried out to investigate the main olive enzymatic activities involved in the complex biotransformation that occurs during the mechanical extraction process. In the 2nd section, a critical and interpretative discussion of the influence of each step of the extraction process on the polyphenols and the volatile compounds has been performed. The effect of the different mechanical devices that are part of the extraction process is analyzed and recommendations, strategies, and possible avenues for future researches are suggested.

Practical Application: In the field of virgin olive oil industry, time and energy should be spent on developing innovative processing plants and equipment able to better modulate the physical parameters that influence endogenous olive enzyme activities, such as temperature, time, amounts of processing water and oxygen. This review paper can be a useful resource to design and develop innovative equipment by offering an exhaustive analysis of mechanical effects of industrial devices and biological effects of endogenous enzymes on the sensory and nutritional properties of virgin olive oil.

Introduction

Virgin olive oil (VOO), extracted from fresh and undamaged olive fruits (*Olea europaea* L.) and properly processed, constitutes a key staple in the Mediterranean diet (Bedbabis and others 2010) due to its nutritional, therapeutic (Visioli and Galli 1998; Stark 2002; Psaltopoulou and others 2004), and sensory properties (Angerosa and others 2004; Servili and others 2004; Bendini and others 2007) and is highly appreciated by consumers (Caporale and others 2006). VOO is exclusively extracted from fruits by means of mechanical techniques (Clodoveo 2012) and it is consumed, without refining, either in its crude form or as a food ingredient (Caponio and others 2008). The quality of VOO depends on physical, chemical, and biochemical reactions which occur during the extraction process, its storage, and its bottling (Jiménez Márquez and others 1995; Alba Mendoza and others 1997; Servili and others 2003; Morelló 2004a, 2004b; Parenti and others 2006a, 2006b; Clodoveo 2012; Frankel and others 2013). A strategic choice and an appropriate use of the different combinations of equipment, which can be found in a VOO extraction plant, allow to either enhance or inhibit the activity of enzymes naturally present in olive tissues. During the VOO extraction, after the breaking of the olive cells, various enzymes, originally trapped within the cell compartments, are released and catalyze both desirable and undesirable reactions (Salas and others 1999; Lorenzi and others 2006; Mazzuca and others 2006; Saraiva and others 2007; Ortega-García and others 2008; Romero-Segura and others 2009; Taticchi and others 2013). Not all the olive enzymes are useful for the best quality of VOOs (Clodoveo 2012). Determining the VOO quality requires a deep knowledge of tissue localization of enzymes in the olive fruit and how the activity of these enzymes can be manipulated by choosing the different mechanical devices that constitute the VOO processing plant. In fact, the enzyme activities can be influenced by choosing different combinations of equipment, selecting only some parts of drupes,

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or regulating some factors such as temperature, time and amounts of processing water and of oxygen (Servili and others 2003, 2007, 2008; Amirante and others 2006). So, the olive oil extraction process is extremely important for sensory and nutritional quality of the product. During each step, the contents of some components, such as polyphenols and volatile compounds, are significantly altered, depending on the extraction machines employed (Angerosa and others 2004; Servili and others 2004, 2008; Esposto and others 2009; Clodoveo 2012). The different enzymatic reactions can cause the formation of a great number of pleasant compounds of the VOO aromatic fraction (Salas and others 1999; Lorenzi and others 2006; Kalua and others 2007, 2013) and the hydrolysis and the oxidation of the phenolic compounds (Servili and others 2004, 2007, 2008; Ortega-García and others 2008; Romero-Segura and others 2009) in the final product.

With regard to the VOO aromatic fraction, approximately 180 compounds belonging to several chemical classes (aldehydes, alcohols, esters, ketones, hydrocarbons, acids) have been separated from VOOs of different qualities (Lerma-García and others 2009). Typical flavors and off-flavor compounds that affect the volatile fraction of a VOO originate by different mechanisms: positive odors are due to molecules that are produced enzymatically by the so-called lipoxygenase (LOX) pathway (Aparicio and Morales 1998; Angerosa and others 2004). Conversely, the main defects or off-flavors are due to sugar fermentation (winey), amino acids (leucine, isoleucine, and valine) conversion (fusty), enzymatic activities of molds (musty), or anaerobic microorganisms (muddy), and to auto-oxidative processes (rancid) (Angerosa 2002; Vichi and others 2003; Lerma-García and others 2010; Frankel and others 2013).

With regard to the phenol profile, VOO polyphenols belong to different classes: phenolic acids, phenyl ethyl alcohols, hydroxyisochromans, flavonoids, lignans, and secoiridoids (Bendini and others 2007). The amount of phenolic compounds is a crucial parameter when evaluating the quality of VOOs (Servili and Montedoro 2002). Phenols are closely related to both the resistance of oil to oxidation (Aparicio and others 1999; Velasco and Dobarganes 2002; Frankel 2010) and the typical bitter and pungent tastes (Servili and others 2004; Bendini and others 2007). Moreover, these compounds may provide a defense mechanism that delays aging and prevents carcinogenesis, atherosclerosis, obesity, liver disorders, and inflammations (Bendini and others 2007; Servili and others 2009; Frankel 2011). Phenolic composition of VOO is the result of a very complex multivariate interaction between genotype and agronomic, environmental, and technological factors (El Riachy and others 2011; Inglese and others 2011). In particular, the extraction process, mainly during the crushing and the malaxing steps, highly affects the phenolic composition of VOO due to the activity of endogenous enzymes of olive fruits, which are released into the olive paste during these steps (Di Giovacchino and others 1994; Morales and others 1999; Servili and others 2003; Amirante and others 2006; Clodoveo 2012).

So, during the crushing step the formation of pleasant volatile aldehydes and alcohols occurs due to cell destruction and results in the release of lipid-degrading enzymes that degrade membrane and storage lipid (Sánchez-Ortiz and others 2012). In this phenomenon a pathway involving LOX and hydroperoxide lyase has been demonstrated (Olias and others 1993; Salas and Sánchez 1999a; Luaces and others 2003, 2007a; Padilla and others 2009). Since free fatty acids (FFAs) do not accumulate in healthy plant tissues, the initial step in the degradation process should be the liberation of FFAs by lipolytic enzymes (Olias and others 1993). The presence of alcohols having the same chain length as the carbonyls found among the plant volatiles provides evidence for oxidoreductase activity in tissues (Olias and others 1993); finally, an alcohol acyltransferase is necessary for the formation of corresponding hexyl esters (Sánchez-Ortiz and others 2007).

At the same time, endogenous glycosidases and oxidoreductases such as polyphenoloxidase (PPO) and peroxidase (POD) can modify the profile of phenolic compounds during the oil mechanical extraction process (Vierhuis and others 2001; Servili and others 2004, 2007, 2008) After crushing, the concentration of secoiridoid glycosides, such as oleuropein and dimethyl oleuropein, decrease and their aglycon derivatives increase significantly in crushed pastes due to the activity of beta-glycosidases. PPO and POD catalyze phenolic oxidation in the paste and in the oil during the extraction process (Montedoro and others 2000; Servili and others 2004, 2007; Taticchi and others 2013).

This paper provides an overview of the involvement of enzymatic systems in the development of nutritional and sensory properties of VOO as well as the main strategies to selectively favor or inhibit certain enzymes by choosing different combinations of plant engineering systems.

Olive Fruit Enzymes

Enzymes are proteins that catalyze biochemical reactions (Narlikar and Herschlag 1997). Recently, scientists have singled out over 10000 different enzymes (Benkovic and Hammes-Schiffer 2003). So, a logical method of classification has been developed to ensure enzymes definition and identification (Bairoch 2000; Schomburg and others 2004). Enzymes are divided into 6 main classes according to the type of transfer which they catalyze (electrons, atoms, or functional groups) (Table 1).

The olive fruit consists of 50% water, 20% oil, 20% carbohydrates (pectic, cellulosic, and hemicellulosic substances), organic acids, pigments, phenolic compounds, and minerals (Najafian and others 2009).

Olives contain a complex system of so-called endogenous enzymes (Obergfoll 1997). Among others, pectinases, lipases, LOXs, hydroperoxide lyases, beta-glucosidases, PODs, and polyphenol oxidases have been detected (Olias and others 1993; Salas and Sánchez 1999a, 1999b; Montedoro and others 2000; Soler-Rivas and others 2000; Luaces and others 2003, 2007a, Servili and others 2004, Servili and others 2007; Padilla and others 2009; Taticchi and others 2013). The amount of those enzymes depends on fruit cultivar and on fruit ripening (Salvador and others 2001).

Pectinase

Olive cell walls are constituted of polysaccharides, mainly pectic polysaccharides rich in arabinose, glucuronoxylans, and cellulose, while xyloglucans, mannans, and glycoproteins occur as minor components (Heredia and others 1996; Mafra and others 2001; Vierhuis and others 2001). However, changes in cell wall polysaccharides occur during olive ripening (Jimenez and others 1995). In fact, when a fruit is unripe, pectin is bound to cellulose micro fibrils in the cell wall. Pectin is insoluble and so confers rigidity to the cell wall, while during ripening, the structure of pectin is altered by naturally occurring enzymes in the fruits (Fischer and Bennett 1991; Kashyap and others 2001). These alterations entail the breakdown of the pectin chain (Kashyap and others 2001). These changes are crucial in determining the olive fruit texture and softening when the pectin is converted to soluble products (Mafra and others 2001, 2006). Huisman and others (1996) and Vierhuis and others (2000) found that, during the ripening stages, the main

Table 1–Enzyme classes and types of reactions (courtesy of Novozymes A/S, Denmark).

Class of enzyme	Reaction profile
Oxi doreductases	Oxidation reactions involve the transfer of electrons from one molecule to another. In biological systems we usually see the removal of hydrogen from the substrate. Typical enzymes in this class are called dehydrogenases. For example, alcohol dehydrogenase catalyzes reactions of the type $R\text{-}CH_2OH + A \rightarrow R\text{-}CHO + H_2A$, where A is an acceptor molecule. If A is oxygen, the relevant enzymes are called oxidases; if A is hydro gen peroxide, the relevant enzymes are called peroxidases.
Transferases	This class of enzymes catalyzes the transfer of groups of atoms from one molecule to another. Aminotransferases or transaminases promote the transfer of an amino group from an amino acid to an alpha-oxoacid.
Hydrolases	Hydrolases catalyze hydrolysis, the cleavage of substrates by water. The reactions include the cleavage of peptide bonds in proteins, glycosidic bonds in carbohydrates, and ester bonds in lipids. In general, larger molecules are broken down to smaller fragments by hydrolases.
Lyases	Lyases catalyze the addition of groups to double bonds or the formation of double bonds through the removal of groups. Thus bonds are cleaved using a principle different from hydrolysis.
Isomerases	Isomerases catalyze the transfer of groups from one position to another in the same molecule. In other words, these enzymes change the structure of a substrate by rearranging its atoms.
Ligases	Ligases join molecules together with covalent bonds. These enzymes participate in biosynthetic reactions where new groups of bonds are formed. Such reactions require the input of energy in the form of cofactors such as ATP.

changes happened in pectic polysaccharides, as they became more branched and more soluble. The cell wall enzymes, responsible for the changes occurring in the pectic composition of fruits during ripening and processing, are pectinases which hydrolyze pectin by different mechanisms. These enzymes are divided into 2 broad classes: pectinesterase (PE) and pectin depolymerases (Alonso and others 1995; Ketsa and others 1998; Chin and others 1999).

PE (EC 3.1.1.11) is a cell-wall-associated enzyme that presents a lot of isoforms facilitating plant cell wall modification and subsequent degradation. It is found in all higher plants as well as in some bacteria and fungi. In plants, PE plays an important role in cell wall metabolism during fruit ripening (Reca 2008). PE catalyzes the de-esterification of the methoxyl group of pectin forming pectic acid (Jayani and others 2005). The enzyme acts preferentially on a methyl ester group of galacturonate unit next to a nonesterified galacturonate unit (Kashyap and others 2001). *In vivo*, de-esterified pectins can bond with calcium ions to form gels, as it happens in peaches. Therefore, the cell wall is thickened and the amount of juice that can be extracted is reduced (Von Mollendorf and De Villiers 1988). Taylor and others (1994) observed the same phenomenon in plums and established a correlation between fruit texture and PE activity.

Pectin depolymerases readily split the main chain and they are further classified as polygalacturonase (PG) and pectinlyases (PLs) (Arunachalam and Asha 2010). The depolymerases catalyze the cleavage of glycosidic bonds via hydrolysis (hydrolases) or via β-elimination (lyases) (Hadj-Taieb and others 2002).

The PG catalyzes hydrolysis of α-1, 4-glycosidic linkages in pectic acid (polygalacturonic acid). There are 2 types of PG (Kashyap and others 2001):

(1) *Endo-PG*: also known as poly (1, 4-α-D-galacturonide) glycanohydrolase, hydrolyses α-1, 4-glycosidic linkages in pectic acid.
(2) *Exo-PG*: also known as poly (1, 4-α-D-galacturonide) galacturonohydrolase, catalyzes hydrolysis in a sequential fashion of a-1,4-glycosidic linkages on pectic acid.

The PLs catalyze cleavage of a-1, 4-glycosidic linkage in pectic acid by trans-elimination. There are also 2 types (Kashyap and others 2001):

(1) *Endo-PGL*: also known as poly (1,4-α-D-galacturonide) lyase, catalyzes random cleavage of a-1,4-glycosidic linkages in pectic acid.
(2) *Exo-PGL*: also known as poly (1, 4-α-D-galacturonide) exolyase, catalyzes sequential cleavage of α-1, 4-glycosidic linkages in pectic acid.

Mínguez-Mosquera and others (2002), studying the modifications caused by the endogenous pectinolytic activities in the pectic matter of Hojiblanca olive fruits, demonstrated that during ripening, PE activity increased until reaching a peak when anthocyanin synthesis in the fruit became marked (turning color stage). From then on, it decreased. Whereas, when anthocyanin formation began, PG activity in the ripe-green fruit decreased abruptly and then increased, reaching a maximum in the ripe-black fruit. This could justify the reduction of texture and pectin contents of the olives during ripening. Thus, on the whole, pectinases are hydrolytic enzymes which hydrolyze the pectin molecules and are readily soluble in water (Arunachalam and Asha 2010).

Lipase

Olive fruits, and the VOO, have been studied for many years from analytical viewpoints because of their commercial value and their potential health benefits (Macrae and others 1983; Tsimidou and others 1987; Christopoulou and others 2004; Di Bella and others 2007). Acidity is one of the main parameters concerning olive oil quality which exerts a great influence on olive oil stability (Frega and others 1999). Extra VOO, as indicated in the European Community Regulation 1513/2001 (EC 2001), must contain less than 0.8% of total FFAs (expressed as oleic acid). However, the quality of VOO and the amount of FFAs that they contain are mainly affected by olive characteristics and by the extraction procedures utilized. In fact, when olive fruits are damaged, before processing and/or during transformation, enzymes may cause an increment in FFAs level (Kiritsakis and others 1998; Gucci and others 2012). The enzyme responsible for the hydrolysis of the VOO triglycerides is the endogenous lipase which seem to be active when the fruit ripens (Kiritsakis and Markakis 1978). Lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) is the 1st enzyme in the degradation path of stored triacylglycerols (TAGs). It catalyzes the hydrolysis of ester bonds at the interface between an aqueous and nonaqueous phase: such a feature distinguishes them from esterase. Hydrolysis of TAGs by lipases can yield di- and mono-acylglycerols, glycerol, and FFAs. When the lipases act as hydrolytic enzymes, the FFAs released will act as a substrate for the LOX to obtain hydroperoxides. Few data are available on lipase activity in *Oleaeuropaea* fruits despite the importance of VOO for nutritional properties. Panzanaro and others (2010) analyzed

the biochemical characteristics of olive lipase extracted from olive mesocarp, evidencing, for the 1st time, an oil body-associated lipase activity in the olive fruit mesocarp. Results also confirmed those of Olías and others (1993) who had demonstrated that olive TAGs and soybean phospholipids can be hydrolyzed by olive crude extracts. Olive lipase was apparently not active below pH 3 and above pH 8; an optimal activity was recorded at pH 5 employing triolein as substrate. In contrast with these results, Olías and others (1993) showed that crude olive extracts had an optimal acylhydrolase activity around pH 8.5. This notable difference suggests the presence of 2 lipase isoforms, with different biochemical characteristics, in olive fruit.

Moreover, since the enzymatic activity depends evidently on temperature, Panzanaro and co-workers (2010) tested the catalytic activity of olive lipase at different temperatures (25 to 45 °C) and found a maximum of activity at 35 °C. Likewise, beyond such value, the activity rate decreased after 2 to 3 h suggesting that the enzyme is less stable at high temperatures. An increment of lipase activity was observed with the addition of calcium, whereas in the presence of copper, the activity was reduced by 75%. In addition, results showed that the olive lipase activity depends on the fruit stage: an increase in enzymatic activity was observed during the ripening process with maximum lipase activity at the spotted II stage and a lower value thereafter (Panzanaro and others 2010).

Furthermore, Panzanaro and others (2010) showed that hydrolytic enzymes, released during fruit milling, are active during the malaxation phase, so a long period and high temperatures during the malaxation step result in a higher yield of oil of lower quality. Moreover, during oil extraction, olive seeds lipase as well as exogenous lipases produced by fungi can hydrolyze TAGs (Vela 1971).

Olive oils contain diacyl-glycerol (DAG) more than other plant oils. The lipase in olive fruits is involved in DAG production and is directly related to the acidity of the olive oil. Shimizu and others (2008) studied for the 1st time the effect of lipase activity and specificity on DGA content of olive oil from the Shodoshima-produced olive fruits. Changes in the acidity and acylglycerol content of the oils extracted from the stored fruits were analyzed. Results showed an increase in the acidity and the DAG content of the olive oils owing to TAG hydrolysis during storage. Kinetic analysis revealed that olive fruit lipase was 4 times more selective for the sn-3 position than for the sn-1 position. In fact, sn-1, 2-DAGs preferentially increased during the early stages of storage. Regarding the thermal properties, results showed a gradual inactivation of lipase activity at temperatures of 30 °C or higher, and a ratio of the rate constant for inactivation to TAG hydrolysis at the sn-3 position about 0.2, 13, and 23 at 20, 30, and 40 °C, respectively (Shimizu and others 2008). Kumar and Prakash (2010) studied the potential applications of selenium and lithium as an inhibitor of lipase activity in rice bran. They found that selenium and lithium inhibit lipase activity. These inactivation phenomena of lipase could have implications for industrial application as well as in other food industries such as the VOO extraction process.

Enzymes responsible for determining the volatile compounds in oil

There is an increasing demand for olive oil all over the world (De Gennaro and others 2012) owing to its nutritional aspects as well as its sensory properties (Williams and others 2000). The aroma of extra VOO results from the complex mixture of volatile compounds, including aldehydes, alcohols, ketones, and esters (Ridolfi and others 2002) that generate a balanced flavor of green and fruity sensory characteristics (Aparicio and Morales 1998). These compounds have been determined by various techniques including headspace analysis and gas chromatography (Morales and others 1994; Angerosa and others 1996; Aparicio and others 1996). The use of headspace solid-phase microextraction (HS-SPME) is also commonly performed (Flamini and others 2003; Cavalli and others 2004; Vichi and others 2006; Baccouri and others 2008a). Volatile compounds are derived from polyunsaturated fatty acids through a cascade of enzymatic reactions known as the LOX pathway (Olias and others 1993; Salas and others 1999; Angerosa 2000; Williams and others 2000; Feussner and Wasternack 2002; Angerosa and others 2004; Kalua and others 2007) that is initiated by the liberation of enzymes when fruit tissues are broken (Olias and others 1993). Therefore, the concentration of volatile compounds depends essentially on the levels and activities of the enzymes involved in the LOX pathway (Angerosa and others 2000, 2004) which are genetically determined (Campeol and others 2001). Hexanal, trans-2-hexenal, 1-hexanol, and 3-methylbutan-1-ol are the major volatile compounds of VOO. Olive cultivar, origin, maturity stage of fruit (Ridolfi and others 2002; Gómez-Rico and others 2006; Bedbabis and others 2010), storage conditions of the fruit (Kiritsakis 1998; Koprivnjak and others 2002), and olive fruit processing influence the flavor components of olive oil and, therefore, its taste and aroma (Servili and others 2003; Angerosa and others 2004; Kalua and others 2007).

Lipoxygenase. LOX (EC 1.13.11.12) is found extensively in the plant and animal kingdoms (Brash 1999). It is a nonheme iron-containing enzyme that catalyzes the oxygenation of the (E,E)-1,4-pentadiene sequence of polyunsaturated fatty acids (linoleic, linolenic, and arachidonic acids) to produce their corresponding hydroperoxides (6- and 10-hydroperoxide) in which the double bonds are in a cis-trans configuration (Porta and Rocha-Sosa 2002). This enzyme catalyzes the 1st reaction of LOX pathway, which mediates the biosynthesis of several regulatory molecules involved in plant responses to stress and wounding, such as traumatin acid, jasmonic acid, and abscisic acid (Blée 1998), and the formation of C5 and C6 volatile compounds (Hatanaka and others 1987; Liavonchanka and Feussner 2006).

LOX activity was measured in both olive pulp membrane fractions by Salas and others (1999) and callus cultures by Williams and others (2000). Specifically, LOX activity was detected in particulate fractions of enzyme extracts from olive pulp (Salas and others 1999) and also in oil bodies extracted from olive endosperms (Georgalaki and others 1998a) and pulp (Lorenzi and others 2006).

LOXs exhibit some substrate preference for one particular polyunsaturated fatty acid. In general, LOXs from seeds present a preference for linoleic acid (Hilbers and others 1995), whereas α-linolenic acid was the appropriate substrate for the enzymes from leaves, as has been reported for apples (Kim and Grosch 1979) and tea leaves (Hatanaka and others 1979). Salas and others (2000) discovered that LOX activity was maximal at pH acid. The enzyme also exhibited some specificity toward certain substrates because it catalyzed the oxidation of FFAs at a rate 100-fold higher than that of the esterified acids; this observation implies the involvement of an acylhydrolase. Furthermore, LOX oxidized both linoleic and linolenic acid but with preference to the latter one and presented clear region specificity for the n-6 position of both polyunsaturated fatty acids (Kalua and others 2007).This selectivity reflected the pragmatic enrichment of C6 aldehydes, compared to C9 ones, in olive fruits and oil volatiles (Salas and others 1999). The C6 aldehydes, deriving from either linoleic or linoleic acids, were produced during oil extraction process. However, Lorenzi

and others (2006) are the first ones who have purified the olive LOX from the Leccino variety at the black stage, studying its kinetic parameters. They found that olive LOX had a better affinity for linoleic acid (Km = 82.44 μM) than for linolenic acid (Km = 306.26 μM). Moreover, the enzyme had a molecular mass of 98 kDa and presented a maximal activity at pH 6. Furthermore, some substances like nordihydroguaiaretic acid (NDGA) and propyl gallate (PG) act as inhibitors of olive LOX. The reaction product was 13-hydroperoxy octadecadienoic acid when linoleic acid was used as substrate.

Ridolfi and others (2002) studied the LOX in 3 olive cultivars Ascolana Tenera, Kalamata, and FS17. Results demonstrated that, by using linoleic acid as substrate, LOX activity was noticeable in the pH range from 5.0 to 7.0 with a maximal activity at pH 6.0 in 0.2 M sodium phosphate buffer.

Concerning the effect of temperature on LOX activity, results showed that 30 °C was the optimal temperature of LOX.

Moreover, the results showed an inhibition of hydroperoxides synthesis at higher temperature. In fact, the LOX of the olive cultivars tested kept about 80% of their maximum activity between 20 and 40 °C. In addition, at 50 °C there was a 60% to 70% inactivation of LOX in the cultivars Ascolana Tenera and FS17, whereas the decrease was less marked in cv. Kalamata (approximately 40%). Data showed also a complete inhibition of the LOX activity during heating enzymatic extract at 80 °C for 15 min.

Luaces and others (2007a) also studied the thermal stabilities of the main enzymes involved in the biosynthesis of VOO aroma through the LOX pathway in crude enzymatic preparations from olive fruits of cultivar Hojiblanca at the green stage (maturity index 1). Kinetic parameters for LOX showed 2 LOX isoforms (*LOXlab* and *LOXres*) with different thermal stabilities and relative activities of 88% and 12%, respectively. So, the decrease in the contents of C6 and C5 compounds in VOO aroma, as a consequence of heat treatments of olive fruit, leads to differences in thermal stability of *LOXlab* and *LOXres*. Data indicate that the C6 compounds derived from *LOXlab* and *LOXres* activity in olive fruits, while C5 compounds were exclusively originated from *LOXres* activity.

Sánchez-Ortiz and others (2011) demonstrated that LOX activity is a limiting factor for the synthesis of the oil volatile fraction, which is significantly higher in the Picual cultivar than in Arbequina; in fact, the lowest content of volatile compounds has been found in the oils being obtained from Picual fruits. Moreover, this limitation of LOX activity takes place mostly during the milling step in the process of olive oil extraction.

Growth and development of olive fruit is a long process lasting 6 to 8 mo, from anthesis to ripening, depending on the cultivar and other factors. Oil accumulation in the pulp starts 12 to 13 wk after anthesis and continues for some 20 wk until fruit ripening. The evolution of the physiological role of the enzyme and changes in LOX activity with growth and development have been investigated. Salas and others (1999) measured LOX activity in olives harvested at different stages of ripening. Data showed a high LOX activity at early stages of development and then a stable decrease in the following 14 wk of the test period (Salas and others 1999). Similar results have been reported for other fruits like tomato and pepper (Mínguez-Mosquera and others 1993; Riley and others 1996). These results implied the crucial role of the LOX in the physiological response to stress at early stages of fruit development (Blée 1998).

Hydroperoxidelyase. Hydroperoxidelyases (HPLs) catalyze the cleavage of fatty acid hydroperoxides at the bond situated between the carbon atom carrying the hydroperoxide group and the adjacent (E)-double bond (Salas and Sánchez 1999a). In plant tissues, these enzymes can yield either C6 aldehydes and C12 ω-oxoacids, from the 13-hydroperoxides of linole(n)ic acid, or aldehydes and ω-oxoacids of 9 carbon atoms from the 9-hydroperoxide derivatives from the same fatty acids, depending on the substrate specificity of the enzyme (Salas and Sánchez 1999a, 1999b; Rotondo and others 2011).

Salas and Sánchez (1999b) partially purified HPL from olive pulp and discovered a strict specificity for the n-6 hydroperoxide derivatives of both linoleic and α-linolenic acids. This explains the absence of C9 volatile compounds in the aroma of olive oil (Morales and others 1995). Salas and others (1999) showed that olive fruit HPLs seemed to be membrane-bound and localized preferably in chloroplasts in agreement with results obtained from other plants. Patui and others (2010) studied LOX and HPL activities in olive pulp microsomes from 2 cultivars (Ghiacciolo and Nostrana di Brisighella) from Northern Italy and found a significant activity of HPL only in the microsomal fraction, and thus all the data concerning HPL activity were referred to it. Moreover, unlike to LOX, HPL activity was detected between pH 6.5 and 9.0, with a maximum at pH 7.5. Luaces and others (2007a) suggest the existence of just one HPL isoform using data on thermal stabilities of HPL. Thermal stabilities of LOX and HPL enzymatic activities in crude preparations seem to explain the observed decrease of volatile contents as a consequence of heat treatments of olive fruit.

Anthon and Barrett (2003) showed that HPL was a heat-labile enzyme and presented optimal activity in slightly acidic conditions. In fact, maximum activity has been observed at 15 °C with a clear decline at 35 °C. Salas and Sánchez (1999b) investigated the changes in HPL during fruit development from 13 wk after flowering to fruit senescence (34 wk after flowering). HPL activity was higher in green olives harvested at the early developmental stages. Thereafter, it decreased slightly with retaining a high level along the entire maturation period. This profile of HPL activity during olive ripening is similar to the one noted in tomato fruits (Riley and others 1996), demonstrating no significant changes in the HPL activity level during fruit ripening. Furthermore, results proved that only the availability of the HPL substrate could be the limit volatile aldehyde formation (Salas and others 1999).

Enzymes responsible for determining the phenolic compounds of oil

The increasing interest in VOO consumption is due to its content of phenolic compounds, since these compounds have potent antioxidant activity and contribute significantly to the extraordinary stability of VOOs against oxidation (Tura and others 2007). However, the concentration and composition of phenolic compounds in VOO are strongly affected by agronomical, biochemical and technological factors such as olive variety (Tura and others 2007; Baccouri and others 2008a), location (Cerretani and others 2006), altitude, maturation degree (Baccouri and others 2008a), the content of phenolic glycosides initially present in the olive tissues and the activity of various endogenous enzymes acting on these glycosides (Uccella 2000; Servili and others 2004; Romero-Segura and others 2010), and oil extraction procedure (Servili and others 2004; Amirante and others 2006; Cerretani and others 2006). The main phenolic glycosides identified in olive fruits from different cultivars and maturation stages (Amiot and others 1986; Ryan and Robards 1998; Gómez-Rico and others 2008) are oleuropein, ligstroside, demethyloleuropein, verbascoside, elenolic acid glucoside, luteolin-7-glucoside, apigenin-7-glucoside, rutin, and quercetin-3-rutinoside.

These phenolic glycosides were hydrolyzed during oil extraction by an endogenous β-glucosidase, producing secoiridoides compounds which constitute the most important phenolic fraction of VOO (Servili and Montedoro 2002; Romero-Segura and others 2012). These substances are represented in particular by the dialdehydic form of decarboxymethylelenolic acid linked to hydroxytyrosol or tyrosol (3, 4-DHPEA-EDA or p-HPEA-EDA), an isomer of oleuropeinaglycone (3, 4-DHPEA-EA) and the ligstrosideaglycone (p-HPEA-EA) (Lim 2012). Moreover, endogenous oxidoreductases, in particular PPO and POD improve phenolic oxidation during the crushing and malaxation steps in the olive oil extraction process (Servili and others 2008).

Beta-glucosidase. Olive leaves and fruit are rich on oleuropein, which is a bitter phenol glucoside (Soler-Rivas and others 2000), that prohibits phytophatogens development (Amiot and others 1989). In fact, highly reactive molecules (Bianco and others 1999) with antioxidant and antimicrobial activities are produced from oleuropein hydrolyzes after olive attack by phatogens or by mechanical hurt. The enzyme involved in this reaction is b-glucosidase (EC 3.21.1.21) belonging to the glucohydrolase enzyme family 1 (GH 1); many component enzymes have been identified in plants where they play important roles in growth, development, detoxification, ripening, and defense (Esen 1993).

During fruit ripening, the b-glucosidases are implicated in the debittering of fruit tissues by oleuropein degradation, and glucose and aglycone molecules liberation (Brenes and others 1992; Ryan and others 1999; Morello and others 2004a; Obied and others 2008).

Mazzuca and others (2006) studied the beta-glucosidase location during the ripening of olive fruit by *in situ* activity assay, finding that enzyme and substrate are present in different cell compartments. In fact, oleuropein is restricted in the vacuoles of olive mesocarp cells (Bitonti and others 2000), and beta-glucosidase activity was detected in mesocarp cell chloroplasts. Moreover, they proposed 2 different isoforms of beta-glucosidase which are able to segregate in 2 different cell compartments.

In addition, the authors showed that, during ripening, beta-glucosidase activity depends on oleuropein content. In fact, in the green stage, the level of beta-glucosidase activity increases proportionally with the oleuropein amount, whereas, in black stage, when the oleuropein concentration declined, the glucosidase activity was low. This change in beta-glucosidase activity during ripening is due to the different attitudes of single mesocarp cells to synthesize the 2 oleuropein-degradative beta-glucosidase isoforms, whose histological distributions appear strongly related to the fruit ripening stage.

Peroxidases. PODs are glycoproteins present in different cell organelles such as chloroplasts (Zámocký and Obinger 2010). These glycoproteins are thermo-resistant and could be regenerated after heat inactivation (Vámos-Vigyázó and Haard 1981). Moreover, they have multiple isoperoxidase forms which differ according to molecular mass, isoelectric point, pH and temperature optima, substrate specificity, amino acid and sugar compositions, and heat stability (Saraiva and other 2007). PODs are oxidoreductases which catalyze the oxidation of phenolic compounds using either hydrogen peroxide or organic peroxides as the oxidizing agent (Gajhede 2001; Kader and others 2002).

In general, PODs are involved in physiological processes in plants such as metabolism in the cell wall, stress, defense, tolerance, auxin catabolism (Vergara-Domínguez and others 2013), protection against pathogen attack, and so on (Veitch 2004).

Since olive POD plays an important role in the evaluation of phenolic compounds in VOO, many studies have investigated the biochemical characterization of this enzyme to understand the influence of the oil extraction on the enzyme activity.

Saraiva and others (2007) purified and characterized the POD from olive fruit (Douro variety) in the black ripening stage. Results showed 4 cationic and 4 anionic fractions. The predominant anionic fraction (PODa4) showed an isoelectric point of 4.4 and optimum pH and temperature of 7.0 and 34.7 °C, respectively. The apparent Km value was 41.0 and 0.53 mM, for phenol and H_2O_2, respectively.

Concerning the thermal stability, data established that POD enzyme lose 60% and 85% of its activity at 40 °C for 5 and 10 min, respectively. However, no measurable activity could be detected upon heating at 50 and 60 °C for 5 min.

Taticchi and others (2013) found that the POD activity in olives from the Moraiolo cultivar was maximal at 34.7 °C. This result was also reported by Saraiva and others (2007) in the Douro cultivar. However, the optimal value was approximately 30 °C in the Koroneiki cultivar (Tzika and others 2009). Moreover, the data concerning the thermal stability of POD showed the high stability of POD in the temperature applied in the malaxation steps. In fact, after 60 min of incubation between 20 and 40 °C, POD activity was almost constant. Additionally, Tzika and others (2009) found high POD stability over 45 min at 30 °C and a reduction in thermal stability of 34.5% at 40 °C. These results do not match the data found by Saraiva and others (2007) who observed a lower stability of olive POD at 40 °C.

The study of the oxidation of chlorophyll a (chl a) catalyzed by POD from the mesocarp of the olive fruit (Olea europaea L., cultivar Hojiblanca) in the presence of H_2O_2 and 2, 4-dichlorophenol (2, 4-DCP) was realized by Vergara-Domínguez and others (2013). Results showed that POD activity on chlorophyll was maximal at pH 7, when 2,4-DCP was used as the reaction cosubstrate. This result is close to that obtained by Laurenti and others (2003). In fact, they studied the effect of pH on horseradish peroxidase (HRP) activity using 2, 4-DCP as the substrate. Regarding the thermal stability profile of POD activity, the results demonstrated that POD activity on chlorophylls had a maximum rate at 30 °C after 50 min of the reaction. And for this time, the activity measured dropped to 68.5% and 44% at 40 °C and 20 °C, respectively.

García-Rodríguez and others (2011) studied the role of polyphenol oxidase and POD in shaping the phenolic profile of VOO. Results demonstrated that, in the 2 olive cultivars under study, Arbequina and Picual, the fruit mesocarp contains PPO activity, whereas most olive POD activity was concentrated in the seed. Moreover, olive seed POD activity was undetectable before 20 wk after flowering in both cultivars and then it was maximal at 28 wk after flowering, reaching 20.8 U/g FW and 15.21 U/g FW in Picual and Arbequina fruits, respectively, and afterward activity levels maintained constant.

Polyphenol oxidase. PPO (EC 1.14.18.1) is a copper-containing enzyme responsible for the enzymatic browning reaction occurring in many plants and vegetables damaged by improper handling, resulting in bruising, compression, or indentations (Zawitowski and others 1991). In the presence of molecular oxygen, PPO catalyzes the o-hydroxylation of monophenols to o-diphenols (monophenolase activity) and oxidation of the o-diphenols to o-quinones (diphenolase activity) (Chararra and others 2001).The products of these reactions are highly reactive molecules that can covalently modify and crosslink a variety of cell molecules to produce, by condensation and accumulation, black

or brown polymers. PPO was expressed in response to various biotic (Li and Steffens 2002; Thipyapong and others 2004a; Mayer 2006; Ortega-García and Peragón 2009) and abiotic (Thipyapong and others 2004b). Ortega-Garcia and others (2008) reported the kinetic and molecular characterization of PPO in fruits and leaves of the olive tree cv. Picual during ripening. Results showed an increase in PPO-specific activity during fruit ripening at all substrates tested such as catechol and catechin.

Moreover, a new 36-kDa PPO protein was detected in fruits during the last stages of fruit ripening which can indicate that a new PPO isoform could be present during this stage.

Recent studies have investigated the biochemical properties of PPO enzyme such as the optimum pH and temperature, and the thermal stability which depends on several factors like the olive cultivar.

In fact, Ünal and others (2011) found the optimum pH and temperature in olives (Domat cultivar) to be 4.5 and 30 °C, respectively, whereas Gençer and co-workers (2012) observed that PPO has its optimum activity at 40 °C and remains relatively stable up to 50 °C in several olive cultivars (Domat, Kiraz, Uslu, Gemlik, and Ayvalik). They found also that the optimum pH values were 6.5 with 4 cultivars except Domat one which the optimum pH value was 7.0. In addition, Taticchi and others (2013) found that the activity of PPO with respect to temperature had a maximum value at 50 °C of 4-methyl-catechol and at 60 °C of hydroxytyrosol. Regarding the thermal stability, Gençer and others (2012) observed high variability in the thermal stability of olive PPO according to cultivar and found that more than 50% of the initial activity was lost after 60 min of incubation at 40 °C for Donat and Gemlik cultivars. However, Taticchi and others (2013) found that PPO has a high stability at 20 °C, but a high degree of inactivation at 40 °C, with a large variation in stability according to the olive cultivar.

Segovia-Bravo and others (2007) characterized PPO from the Manzanilla cultivar and they found that the maximum activity was at pH 6.0. In addition, the enzymatic activity increased with temperature (8 and 25 °C) and was completely inhibited at pH values below 3.0 regardless of temperature. However, in alkaline conditions, pH inhibition depended on temperature and was observed at values above 9.0 and 11.0 at 8 and 25 °C, respectively.

Virgin Olive Oil Mechanical Extraction Process

Harvesting time and techniques, and olive delivering

Three main factors are crucial in establishing the final quality of VOO when harvesting the fruits: the harvesting time, the harvesting methods, and the post harvesting storage.

In fact, choosing the right time is useful to obtain the largest quantity and the highest quality of oil (Dag and others 2011), even if these 2 factors are polar opposites. Moreover, during fruit ripening in many plants, enzyme activity may change, varying the composition of fruits. Ortega-García and others (2008) studied the kinetic and molecular properties of PPO in olive fruits and its relationship with the oleuropein concentration during fruit ripening. They found that oleuropein concentration of olive fruit, and consequently in VOO, depended on β-glucosidase, PPO and POD activity. The activity of these enzymes may affect oleuropein concentration during the growth phase or green maturation. In the 1st period of the growth phase, El Riachy and others (2011) observed that the oleuropein concentration reached higher levels. Afterward, during the green maturation, it declined with the physiological development of the fruit probably because the activity of hydrolytic enzymes increased. This phenomenon is accompanied by the demethyloleuropein and elenolic acid glucoside increment. The decline of oleuropein continued rapidly during black maturation, suggesting a likely function of β-glucosidase in this metabolism confirmed by the appearance of oleuropein derivatives. The presence, in the olive fruit, of isomer of oleuropein aglycone and its dialdehydic form of elenolic acid, is important because these substances may subsequently be released into the oil during the mechanical extraction process, the determining appearance, the flavor, and the health-promoting properties of the resulting VOO. Olive POD activity was found to be low at early fruit developmental stages and to increase during the maturation process (García-Rodríguez and others 2011), contributing to the phenolic oxidation that takes place during the industrial process of obtaining olive oil (Luaces and others 2007a). Thus, depending on the cultivar characteristics, it is important to know the influence of the ripening stage on enzyme activities and related phenol composition and concentration in order to calibrate the choice of the fruit picking time and the most suitable technology to extract the VOO with the desired sensory and nutritional characteristics. Despite the several bibliographic sources already available, many researches in this field will soon become indispensable, owing to the great variability related to the influence of the cultivar and the region of the biosynthesis and catabolism reactions of polyphenols. Likewise, during fruit ripening, the enzyme activity responsible for the VOO aroma may change. In fact, Kalua and others (2007) reported that the highest level of Hydroperoxide lyase activity (the enzyme that produces volatile aldehydes and oxoacids) was identified in green olive fruits harvested in the initial developmental stages and it slightly decreased at maturity. Alcohol dehydrogenase activity also declined during the ripening process when the olive fruit color changed to purple, supporting the experimental data that showed a reduction in the content of C6 alcohols in the aroma of VOO as the fruit ripeness increased (Salas and Sánchez 1998). These considerations might encourage olive millers to estimate the maturity index of harvesting olives before choosing the combination of extraction techniques and conditions: the right selection of the mechanical equipment combined with the knowledge of the biochemical characteristics of the raw material is the key factor for determining the olive oil quality.

The choice of the harvesting method and its influence on VOO quality relate to its effect on preserving fruit integrity. If a particular method causes bruises on the fruit surface as a result of its mechanical impact or compression, olive respiration and the susceptibility to decay at a faster rate, in comparison to undamaged fruit, will increase. The oil extracted from these damaged olives can be high in acidity, low in stability (Garcia and others 1996), and poor in polyphenols and might develop off-flavors due to the enzymatic activities favored by the breakdown of the cells and the contact between enzymes and substrates that were initially compartmented differently. In the light of these considerations, hand picking appears to be the best method for preventing fruit damage (Jimenez-Jimenez and others 2013), yet, unfortunately, olive manual harvest is quite expensive. In the last few decades, in order to decrease harvest costs, mechanical harvesting has been introduced with the burden of an increase of fruit injury (Kader and Rolle 2004) that might cause a gradual disintegration of the cell structure (Koprivnjak and others 2000). When the amount of damage fruits is high, the extraction of oil should be made promptly, avoiding fruit storage at ambient temperature (Amirante and others 2000; Amodio and others 2005; Rinaldi and others 2005; Clodoveo and others 2007).

Other factors causing damage to the fruits should be controlled, such as olive fly attacks or improper systems of transport and

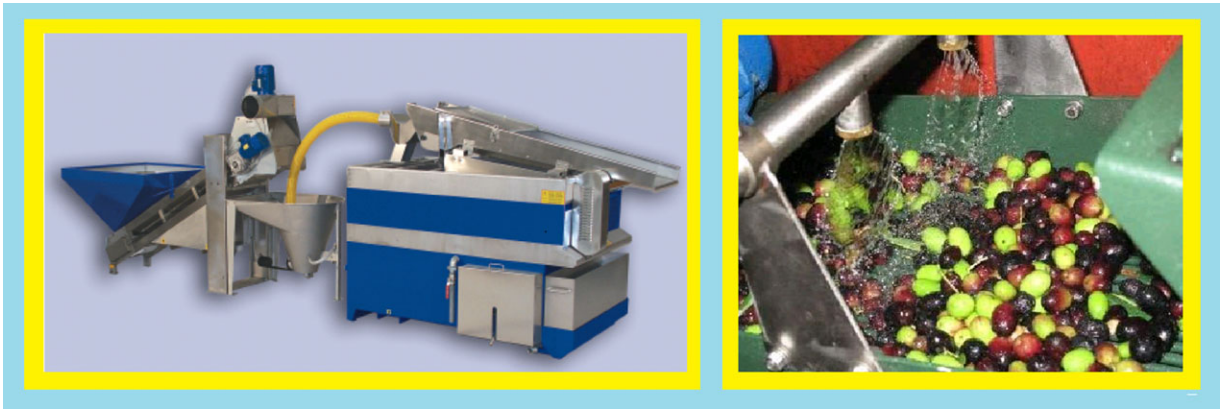

Figure 1–Washing machine (photos courtesy of Alfa Laval, Olive oil SpA, Italy).

storage of olives that can modify the VOO quality parameters (Kiritsakis and others 1998). Accordingly, prolonged storage of fruits in uncontrolled conditions produces volatile compounds that are responsible for off-flavors (Kiritsakis 1998; Koprivnjak and others 2000) due to the activity of endogenous or microbial enzymes that can find the optimal conditions for their activity (temperature, oxygen, substrate concentration). Olive oil obtained from stored olives is characterized by the absence of the C6 aldehydes, alcohols and esters from the LOX pathway and the presence of many aldehydes from chemical oxidation, including hexanal from both chemical and enzymatic reactions. These off-flavor compounds are potentially toxic and have low odor thresholds (Angerosa and others 2000).

In order to really modulate the oil composition during the whole extraction process and apply the best cultivation practices, without compromising the quality of fruits obtained, fruit storage before oil processing is not encouraged. Obviously enough, olives harvested from the ground, which usually give oil with defects, must be kept and milled separately from those harvested from the trees which can give virgin or extra virgin oils, due to the high level of FFAs produced by the lipase activity.

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 preserve 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 and others (2013) studied the effect of ripe olives crushed with olive leaves (from 1% to 10% of the total weight of processed olives). Likewise, if olive leaves are considered an excellent source of compounds with biologic properties, the resulting oils showed higher FFAs, peroxide value and K_{232}. The authors suggested 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 a leaf removal equipment in order to improve VOO quality.

Then, after the grading step, the olives can be also washed, especially if they have been harvested from the soil or have had spray residues (Figure 1). A critical point of the washing step is represented by the extra moisture of the resulting olive paste. The added water could determine 2 effects: the first one is a reduction of extraction efficiency, due to the formation of water/oil emulsions. The second one is the effect on the repartition of phenols between oil and water phases due to their amphipathic nature (the phenolic hydroxyl group is hydrophilic while the aromatic ring is hydrophobic in character). Moreover, olive washing machines are usually equipped with a water recycling system: this method might compromise the sensory quality of the final product due to the high microbial concentration in the recycled water and their fermentation activity: a good recommended practice should be the frequent renovation of the processing water. Based on their experience, several millers believe that oils made from washed olives are usually less desirable, with a reduction in bitterness and pungency, but also have a less fruity flavor. However, a systematic research in this matter is rather lacking and only scarce comments on the effect of this equipment are reported in the literature (Hermoso Fernández and others 1998; Civantos 1999; Vossen 2007). The reason for these organoleptic changes, unquestionably related to the phenolic content, is not yet clear and should thus be addressed by future research.

Crushing

The 1st step to extract VOO from fruits consists of crushing the drupes. The type of crusher chosen is critical to determine the quantity and the quality of VOO (Clodoveo 2012). In fact, the equipment used can influence various properties of the resultant olive paste. Depending on the different mechanical actions, the main aspects that can change are temperature, granulometry of fragments, exposition to the atmospheric oxygen, and differential crushing of olive tissues. These differences are critical for the release and start of endogenous enzymatic activities (Servili and others 2012). As a matter of consequence for these possible physical, chemical and biochemical variations, able to affect the endogenous oxidoreductases, the phenolic and volatile compounds of VOO are susceptible of great modifications (Servili and Montedoro 2002). The main endogenous enzymes involved in the determination of the final concentration of hydrophilic phenols in VOO are PPO, POD, and β-glucosidases. This last one transforms oleuropein and dimethyl-oleuropein into their stable aglycone product (Limiroli and others 1995). The aglycones are more soluble in the oil phase than the glycoside forms that remain in the water phase. This observation suggests that it is important to know the conditions useful to promote the β-glucosidase activity in order to enrich total phenol content of VOO. On the contrary, if the crushing system damages the β-glucosidases or does not

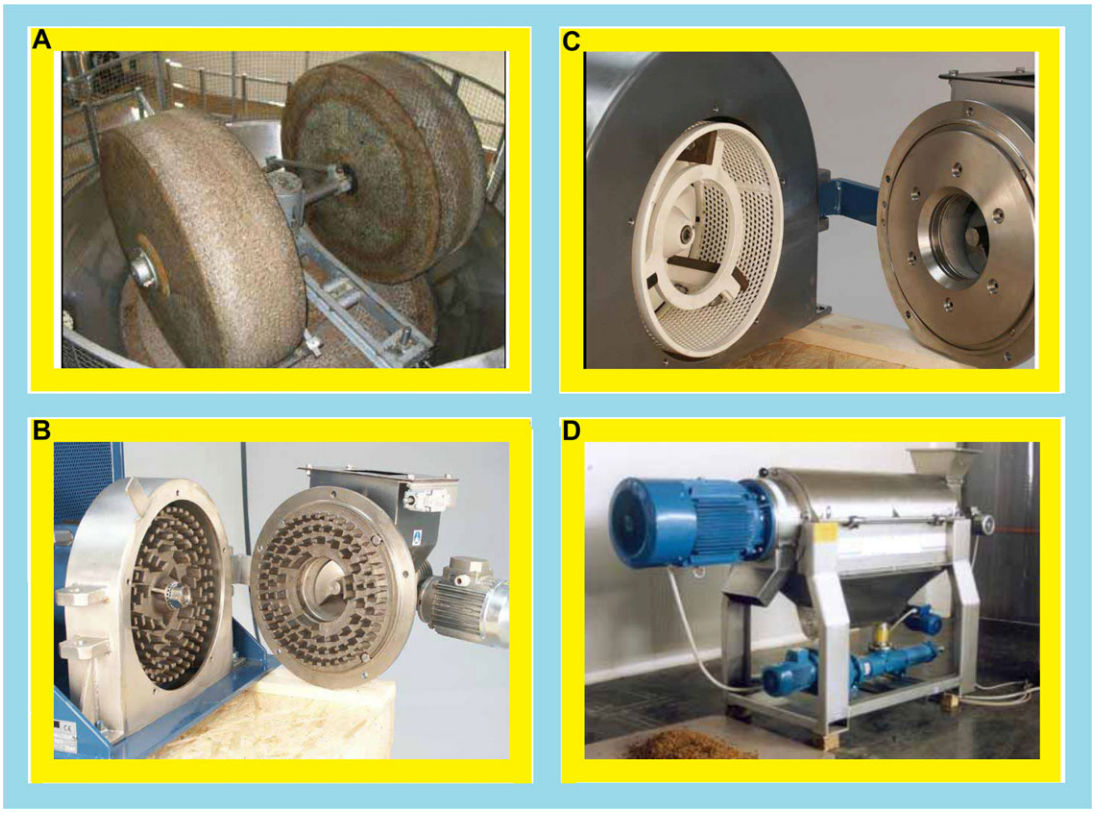

Figure 2–Crushing systems (A: stone mill; B: hammer crusher; C: disc crusher; D: de-stoner) (photos courtesy of Alfa Laval, Olive oil SpA, Italy; GEA Westfalia Separator Group, Germany; Pieralisi, Italy).

favor their activities, a large proportion of water-soluble phenols will be lost in the waste water.

With regard to the enzymatic development of the VOO aroma, this is determined by the activity and properties of the enzymes involved in the LOX pathway that acts immediately after drupe crushing (Sánchez-Ortiz and others 2012), when, due to the disruption of the cell walls and membranes, substrates meet enzymes synthesizing volatile compounds. The LPO, activated during crushing, catalyzes the genesis of C5 and C6 saturated and unsaturated aldehydes, alcohols, and esters that are related with the "cut grass" and "floral" sensory notes of VOO (Servili and others 2003). This enzymatic pool is sensitive to their environmental conditions, in particular the temperature, as this parameter can affect the level and the activity of enzymes involved in the pathway (Angerosa and others 1999; Angerosa and Basti 2001; Angerosa 2002). Ridolfi and others (2002), having studied the kinetic constants of the olive LOX enzyme, the substrate specificity, and the optimal catalysis temperature, reported that the maximum LOX activity was recorded at 30 °C. If the olive paste temperature rises up to 30 °C, the structure of such enzyme of LPO will begin to denature, interrupting the cascade pathway and the synthesis of the aromatic compounds, causing a decrease of volatile contents in VOO; in particular of concentration of C6 esters, which are very important contributors of delicate green perceptions, and of cis-3-hexen-1-ol, which gives pleasant real green sensations (Angerosa and others 2001). The study done by Anthon and Barrett (2003) demonstrated that the optimal temperature of HPL is 15 °C. Many researchers are studying strategies for a better control of temperature during the VOO extraction process in order to modulate the taste, the aroma, and the color of this product so as to meet consumer demands, but many researches are focused on the malaxation phase without considering the crushing effects. More attention should be dedicated to the influence of the crushing system on the physical, chemical, and biochemical modifications occurring in the olive paste during malaxation. In fact, the various apparatuses employed to crush olives have different effects by influencing the olive paste temperature due to the different mechanical energy spent to break the olive tissue.

The industrial apparatuses employed to crush the olives are the traditional stone mill, the hammer or disk crusher, and finally the innovative de-stoner (Amirante and others 2010a) (Figure 2). The stone mill comprises 2 to 3 stone wheels, which rotate in circles on a block of granite to crush the olives into paste (Figure 2A). It is a very expensive machine because of the granite required to build stone rollers. This device is bulky with a low working capacity and high labor cost. Moreover, it does not cut the skin and releases less chlorophyll. During the prolonged rotation, large drops of oil are formed**,** and, in some conditions, the malaxing step is unnecessary, thus minimizing extraction times. The energy released during breaking is low and olive paste isn't heated. The absence of any heating effect protects the pathway of LOX from denaturation (Padilla and others 2009); however, the products of this pool of enzymes, as soon as formed, vaporize readily at room temperature in the ambient atmosphere. The exposition of the olive paste to the atmospheric oxygen can promote the oxidation of polyphenols, causing a reduction of VOO stability but simultaneously a decrease in the bitter and pungent taste of the product

(Amirante and others 2006). In the light of this observation, the stone mill use is recommended for processing the bitter varieties rich in polyphenols (such as Coratina), which are not always accepted by consumers (Clodoveo and others 2013a).

In hammer-crushing machines, there is a 4-lobe rotor with wear-resistant metal plates that crush the olives against a stationary grid (Figure 2B). The dimension of the grid holes regulates the dimension of the olive fragments. The hammer-crusher is a continuous machine with high throughput. It may tolerate debris such as stones. The hammer-crusher, cutting deeply the skin, extracts more phenols, so the resultant oil has longer shelf-life than the oil obtained from employing the stone mill. The resulting oil can be characterized by a bitter, stronger, spicy taste due to these phenolic substances. However, the hammer-crusher, due to the violent mechanical action, produces some disadvantages: it may form an emulsion which impedes oil-water separation, causing a more intense fragmentation of the olive pits and determining a substantial increase in temperature of the olive paste, thus compromising the activity of the temperature sensing enzymes (Amirante and others 2010a). This increment in temperature has a detrimental effect on the LOX pathway, but oxidative enzymes, such as PPO and POD, seem to tolerate it.

Also, the disk crusher is a continuous machine with high throughput (Amirante and others 2010a) (Figure 2C). Olives fed into the crusher are flung away from the center and crushed as they meet the toothed disc. Similarly to the hammer crusher, it is less expensive than the stone mill, but it does not tolerate debris such as rocks and grit. It is possible to have precise regulation of olive paste particle size setting the distance between the disks, but it is not easily adjustable during the working of the machine. The use of the disk-crusher avoids the olive paste overheating if confronted with the hamer-crusher minimizing the risk of oxidation. It may give a longer shelf-life to the product compared to the oils extracted employing a stone mill due to the minor exposition of the olive paste to the atmospheric oxygen. However, this crusher may form an emulsion, which impede oil–water separation, but they are less abundant if compared to the hammer-crusher action. The use of the disk crusher may also affect the sensory characteristics: the oil can have a stronger, spicy taste, but less bitter than one obtained by the hammer-crusher, which may be an advantage for "mild taste" or "sweet" olives.

A relatively new approach to olive-crushing is based on differentiated crushing of the constituent parts of the fruit, such as the skin, pulp and seed. The de-stoner, also called "de-pitting" machine, crushes only the pulp tissues (Amirante and others 2006; Dugo and others 2007; Servili and others 2007; Rodríguez and others 2008) (Figure 2D). The resulting paste is solely made up of the fleshy part of the olive (mesocarp), without the stone (endocarp) that holds the seed. The resulting VOO has higher phenol content than those obtained by other crushing systems (Amirante and others 2006; Servili and others 2007). Considering that the seeds constitute about 25% of the total paste volume, the use of the de-stoner can improve the working capacity of the mill plant excluding about a quarter of the residual solid waste before the extraction process. Moreover, the seeds, rich in functional and bioactive minor compound, after the recovering, have a high economic appeal because of their residual value for the cosmetic and pharmaceutical industries (Amirante and others 2010a). Additionally, the de-stoned pomace is easier to use as an animal feed. However, in order to ensure good VOO extraction yield, the de-stoned olive paste requires long mixing times and a 3rd-generation decanter (Amirante and Catalano 2000) to separate the oil from the olive paste characterized by nonconventional rheological properties (the absence of stone fragments causes a change in olive paste viscosity). For this reason, the use of the de-stoner may give slightly lower oil yield in comparison to the other crushing systems (about 1.5 kg of oil per 100 kg of olives) (Amirante and others 1987). The impact of the de-stoner on VOO phenolic and volatile compounds can be related to the different distribution of endogenous enzymes and of phenolic compounds in the various parts of the olive fruit (pulp, stone, and seed). Considering the distribution of phenolic compounds in the various parts of the olive fruit, Servili and others (1999) found that oleuropein and dimethyl-oleuropein were present in all of the constitutive parts of the olive fruit, with the highest concentrations in the pulp, whereas luteolin-7-glucoside and rutin were present only in the peel; Nuzhenide was the most concentrated phenolic compounds in the seed. With regard to the distribution of enzymes in the differential part of the fruit, POD is highly concentrated in the olive seed (Amirante and others 2006; Servili and others 2007; Amirante and others 2010a). So, the exclusion of seed can reduce the enzymatic oxidation of the pulp phenol compounds. In fact, García-Rodríguez and others (2011) studied the fitness of seed POD to oxidize main phenolic compounds suggesting a significant role of this enzyme 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 phenolic compounds arising during the industrial process to obtain the oil. The modulation of olive POD employing the de-stoner could have a great impact on nutritional and sensory quality of VOO. Luaces and others (2007b) studied the effect of olive seed on the VOO phenolic compounds. In order to determine the role of the kernel, they mixed increasing seed proportion to the de-stoned pulp before the oil extraction. The de-stoned 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. Mechanical extraction of the olive oil from de-stoned paste emphasizes nutritional and sensory characteristics of the product not only because of the phenolic fraction but also to the volatile compounds produced by the LOX. The de-stoning leads to an increase in the concentration of those volatile substances correlated with the "green" (Servili and others 2007) and the cut-grass sensory notes of the oil (Amirante and others 2006) probably because the enzymes involved in the LOX pathway have different activities in the pulp and in the seed of the olive. In fact, the stoning process caused the increment of C6 unsaturated aldehydes while the olive seeds are considered responsible for the biosynthesis of 30% to 50% esters during the olive oil extraction process of intact fruits (Luaces and others 2003). These data demonstrate that the selectively crushing different parts of the fruit, such as the de-stoning, is the most efficient tool to modulate the nutritional and sensory characteristics of VOO, with the non-negligible advantage of optimizing the working capacity of the plant through the exclusion of a quarter of the solid waste before the extraction process. However, this technology is not widespread. The reason lies in the fact that the de-stoning technology produces minor quantities of higher quality VOO. Nevertheless, at the moment, the market does not recognize to the de-stoned VOO a sufficient premium price to offset the loss of yield. The main reason depends on the absence of an analytical tool able to demonstrate the effective employment of this technology, the so-called fingerprint of

Figure 3–Malaxer machine and its evolution. (A) Traditional malaxing machine characterized by a cradle shape and a nonhermetic closure. This type of machine causes considerable loss of phenolic and volatile compounds. (B) Innovative malaxing machine characterized by a cylindrical jacket that covers the whole internal surface of the tank for a quicker and more efficient heating of olive paste. The hermetic sealing ensures a perfect control of the atmosphere in contact with the paste in the malaxing machine through valves for inert gas (nitrogen or argon) (photos courtesy of Alfa Laval, Olive oil SpA, Italy).

the process. Future research will be needed to develop a method to certify that the oil has been extracted from de-stoned olives, in order to offer the best possible guarantees to the consumers and a proper profit to the millers.

Another poorly studied aspect concerns the influence of the atmosphere composition in contact with the olive paste during the crushing. Considering that the measure of the oxygen concentration inside the olive paste after crushing revealed an average value of 18% (very similar to the atmosphere composition) (Amirante and others 2008) it is important to develop innovative crushing systems able to modulate atmosphere composition inside the crushing chamber in order to control the oxygen concentration into the olive paste and to prevent undesirable oxidative reactions, mainly catalyzed by POD and POO, in the subsequent malaxing step where the process parameters are favorable for these enzymatic activities. An example of a plant that allows to modulate the atmosphere composition from the crusher to the malaxer has been described in a recent patent (Clodoveo 2013a), property of the Univ. of Bari. However, few data are available in the international literature on this topic, so, future researches may well clarify the relevance of this plant implementation.

Malaxation

Malaxation is one of the most critical points of the olive oil mechanical extraction process (Clodoveo 2012). The malaxer machine consists of a stainless steel tank containing the olive paste (Figure 3), and a malaxing central-screw stirring the paste slowly and continuously, at monitored temperature. From the point of view of olive millers, malaxation is useful to increase the oil yield since, during the milling process, pectic, cellulosic, and hemicellulosic enzymes are set free (Obergfoll 1997). These endogenous wall-degrading enzymes are also able to break the oil-water emulsions changing the rheological properties of the paste and to increase the minor compound concentration. In fact, in the olive paste an interaction between polysaccharides and phenolic compounds can occur (Rubio-Senent and others 2013). If these links persist, the release of the minor compounds in the oil could be inhibited (Servili and Montedoro 2002; Servili and others 2012). During malaxation of olive paste, a complex bioprocess occurs (Clodoveo 2012), modifying deeply the quality and composition of the final product in particular the phenol (Jimenez Marquez and others 1995; Ranalli and others 2001; Di Giovachino and others 2002) and volatile (Angerosa and others 2000; Ranalli and others

2001; Servili and others 2003) contents. It is possible to modulate the endogenous enzymatic activities by controlling 3 main process parameters: temperature, time and atmosphere composition inside the malaxer (Clodoveo 2012). Taticchi and others (2013) investigated the influence of the temperature on the olive PPO and POD activities, revealing that PPO is characterized by a lower thermal stability than the POD. This observation can explain the variation in phenolic concentrations in the paste during processing as a function of temperature, but it should most likely be considered to be only one of the concomitant factors affecting the final concentration of phenolic compounds in VOO. In general, an increase in temperature (from 25 to 35 °C) (Esposto and others 2013) can reduce the enzymatic oxidative reaction (PPO) causing an increase in both total phenolics and the pungent phenolic oleocanthal. Considering the volatile compounds, the main effect of an increase in the malaxing temperature is an increment of esters and cis-3-hexen-1-ol and an accumulation of hexan-1-ol and trans-2-hexen-1-ol, considered far from pleasant odor (Angerosa and others 2001; Kalua and others 2007).

Also, the duration of malaxation can influence VOO composition (Jimenez Marquez and others 1995; Angerosa and others 2001; Di Giovacchino and others 2002; Servili and others 2003). Regarding the polyphenols, these compounds are much more affected by the malaxation temperature than the malaxation time (Gomez-Rico and others 2009.). The main effect of an increase in the duration of malaxation is an increment of C6 and C5 carbonyl compounds, especially of hexanal, which represents an important contributor to the olive oil flavor (Amirante and others 2006).

The duration of malaxation significantly changes the volatile and phenolic profile of VOO. The increment of the duration of malaxation favors the activity of β-glucosidases which produce the aglycon molecules from the glycosides. However, POD and PPO are also involved in the oxidative reactions that occur**,** prolonging the duration of malaxation reducing the concentration of secoiridoid derivatives. Obied and others (2008) reported that an increase in the duration of malaxation determined a reduction of the 3,4-DHPEA-EDA and 3,4-DHPEA-EA concentrations, while the oxidative degradation of the 4-HPEA-EDA was lower because probably PPO and POD present different efficiencies in oxidizing o-diphenol and monophenol compounds.

A relationship between aroma components and malaxing time of olive paste occurred when the duration of malaxation increased (Angerosa and others 1997). The quantization of C5 and C6 components performed at different times, prolonging the duration of malaxation, showed that the most important increase was relative to the trans-2-hexenal content (Baccouri and others 2008b). An opposite tendency was recorded for the C6 ester content, which underwent a very considerable decrease after 30 min of malaxation. These differences were probably ascribable to the hydroperoxide lyase cascade, since trans-2-hexenal and hexanal represent the main accumulation products. The sensory evaluation of oils extracted at different malaxation times reveals a considerable decrease of the intensities of bitter and pungent descriptors and a weakening of some perceptions related to "green" notes, as the malaxation time was prolonged (Angerosa and others 2004).

However, the oxidative enzymatic activities could be also regulated through a strategic control of the atmosphere composition of the head space of the tank (Servili and other 2003; Clodoveo 2012; Clodoveo 2013b). Considering the evolution of the malaxing machine, the early malaxers were characterized by a cradle shape and a nonhermetic closure: the olive paste was exposed to a great amount of oxygen which could promote oxidative reaction while the volatile compounds vaporized in the ambient atmosphere. Later, a hermetic sealing was added to ensure a perfect control of the atmosphere in contact with the olive paste, employing also inert gas such as nitrogen or argon. The presence of inert gas inside the head-space of the malaxer produces a 2-fold effect: it reduces the activity of the oxidase enzymes preserving the polyphenolic substances, but, at the same time, it inhibits the synthesis of volatile compounds. If the oxidative enzymes activity is inhibited under N_2, an extension of the duration of malaxing is possible without detrimental effect on VOO quality; prolonging the duration of malaxing under N_2, Migliorini and others (2006) found a higher extraction of phenol compounds, probably because of a higher b-glucosidase activity. In reality, the malaxation under inert gas is not widely widespread due to the high price of nitrogen and argon that cannot be ignored (Clodoveo 2013b). In order to solve this problem, Parenti and others (2006a, 2006b) suggested to benefit from the phenomenon of carbon dioxide emission coupled with the oxygen depletion during malaxation under sealed conditions. This technique is more advantageous than the saturation of the malaxation chamber with inert gas (N_2) (Servili and others 2003) since a partial oxidation of the fatty acid chains is necessary (especially in the initial part of malaxation) for the development of volatile compounds constituting the aroma through the LOX pathway. The strategic control of the oxygen is the key factor for balancing the possibility of promoting the synthesis of volatile compounds and of reducing the oxidation of polyphenol substances (Servili and others 2003; Clodoveo 2012; Servili and others 2012; Clodoveo 2013b). In fact, Sánchez-Ortiz and others (2008) reported that a reduction of oxygen during the industrial process to obtain VOO could produce—along with its beneficial effect on the nutritional quality and through a decrease of phenolic oxidation catalyzed by endogenous reductases—an improvement of the sensory properties of the oil. Furthermore, it has been observed that during the malaxation process the enzymes involved in the LOX pathway have remained active since the concentration of volatile compounds increases in the pastes (Angerosa and others 2004).

Further studies should be done in order to develop a more efficient equipment aimed at improving the efficiency of malaxation and reducing the duration of this stage. Actually, the so called "continuous system" is composed of a series of apparatuses able to work in continuous mode, with the exception of the malaxer which is a batch plant. It is clear that the weakest link of the chain in the VOO extraction process is the malaxation phase. In fact, the malaxation phase actually represents the "bottleneck" of the continuous extraction process. Currently, the system used to guarantee the process continuity, without interrupting the activity of the machines upstream and downstream of the malaxer, consists in placing several malaxing machines in parallel, with a heavy economic investment. Despite the time and the money spent, the research of an innovative technical solution to solve the conversion of malaxation from a batch process to a continuous process is still failing. The development of this issue should be a priority considering that a continuous process presents potential advantages such as minor operating costs, minor capacity limitations, faster return on investments, lower cost of production, reduced energy demands, reduced work-in-progress, faster and easier cleaning, real-time quality control and significantly reduced footprint facility. In order to realize this goal, 2 innovative solutions have been suggested which include ultrasound (US) and microwave (MW) pretreatment of olive paste (Clodoveo and Hachicha 2013). However, the applications of emerging technologies in the VOO

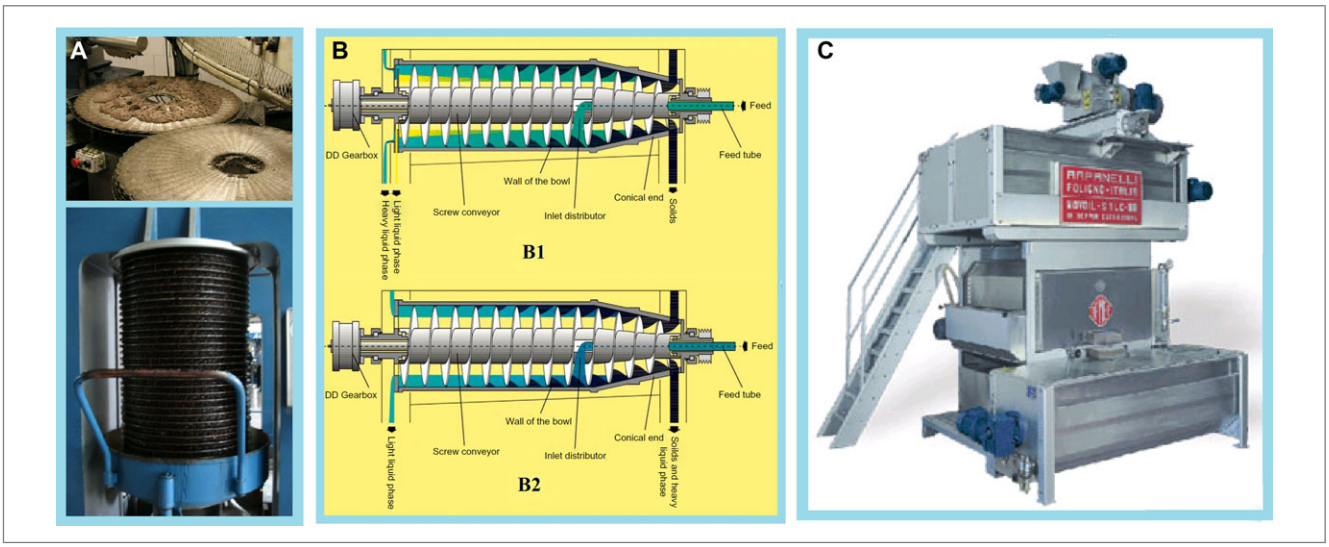

Figure 4–Separation of oil from solid and liquid phases of olive paste: A: pressure system; B: centrifugation systems (B1: 3-phase decanter; B2: 2-phase decanter); C: percolation system (photos courtesy of Alfa Laval, Olive oil SpA, Italy; GEA Westfalia Separator Group, Germany; Pieralisi, Italy; Rapanelli, Italy).

industry are still at the "embryonic stage": Clodoveo and others (2013a, 2013b) have suggested the design of a new US device (sono-exchanger) placeable between the crusher and the malaxer (Clodoveo 2013c).

Separation of oil

The separation of oil from solid and liquid phases of olive paste is performed by using 3 different systems: pressure, percolation, or centrifugation (Amirante and others 2010b) (Figure 4). The pressure extraction system is considered as an obsolete technique since it is the oldest method for processing olive fruit to obtain olive oil. The advantages of this method include the use of simple, reliable machinery and little initial investment; it has a low energy requirement and the resulting pomace is low in water content. The traditional method is a valid form of producing high-quality olive oil, if after each extraction, the disks are properly cleaned from the remains of paste in order to avoid the development of unpleasant odor notes arising from endogenous or microbial enzymatic activities. In this extraction method, the introduction of water is minimal when compared to the modern one, thus reducing the washing-off of the polyphenols. On the other hand, the exposition of olive paste to the action of oxygen and light is high.

The modern method of olive oil extraction uses an industrial decanter to separate all the phases by centrifugation (Amirante and others 2000, 2001). There are 2 types of decanters: a 2- or a 3-phase centrifugal decanter. In the 3-phase centrifugal decanter, the paste is divided into oil, vegetation water, and solids (olive pomace), including kernel and pulp fragments. In the 2-phase process, paste instead is separated into a liquid phase and a solid phase, composed of fragments and kernels, pulp, and vegetation water (humid olive pomace). The 2-phase process requires no dilution or only a little dilution during the malaxation phase. The main difference between the 2 typologies of machine is the amount of water added to dilute the olive paste: the 2-phase process has low water consumption and low waste water production (Di Giovacchino and others 2001). As a consequence, the oils obtained after extraction by the 2-phase centrifugal system exhibited a higher content of polyphenols, induction time values, and sensory score due to the little amount of water added to the olive paste. In fact, the amount of water added determines the dilution of the aqueous phase and lowers the concentration of the phenolic substances that are more soluble in the water. Due to the partition equilibrium law, the concentration of the same substances consequently diminishes in the oil.

The industrial machinery used for the percolation method is known as "Sinolea." The Sinolea method is based on the different surface tensions of the vegetation water and the oil. This method is not widespread due to the high cost of the plant and the low extraction yield obtained without coupling the horizontal centrifuge. During the extraction, the oil does not suffer any aggression, so the quality is very high because no water is added. VOO, obtained through percolation (1st extraction), has a higher content of phenols, o-diphenols, hydroxytyrosol, tyrosol aglycones, and tocopherols than oils obtained through centrifugation (2nd extraction) (Ranalli and others 1997, 1998, 1999).

Accounting for all these remarks, it is clear that the key factor, able to modify the VOO quality during the separation of oil from solid and liquid phases of olive paste, is the amount of added water, in which the phenols are more soluble than in the oily phase. The more suitable system seems to be the 2-phase decanter, but it is not widespread in all the countries which are traditional producers of VOO, due to the high moisture content of the resultant pomace which compromises the opportunity to extract competitively the pomace oil employing solvents. The centrifugal technology currently available still determines appreciable residues of oil in the pomace. For this reason, a great interest in this less valuable product still exists in the market. Pomace olive oil creates damages to the image of the VOO and competes with VOO determining a detrimental effect on the prices and on producer incomes. Future plant development should be oriented toward a system able to reduce the oily residues in the byproduct, increasing the oil yield and favoring the decline of the poor quality pomace oil in the market.

Vertical centrifugation

The oil phases are further clarified in an automated discharge vertical centrifuge (disk centrifuge) with lukewarm tap water

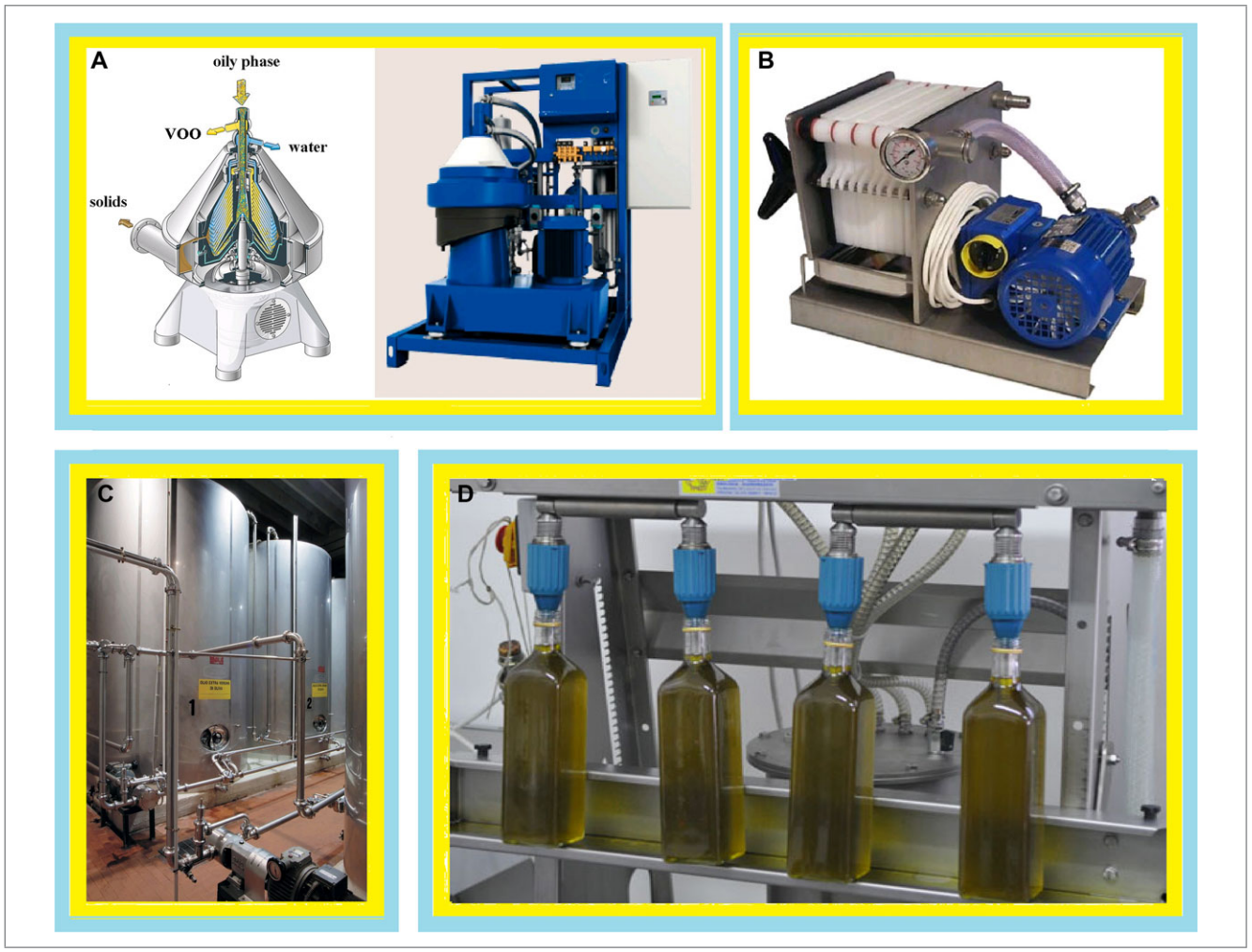

Figure 5–Vertical centrifugation (A), filtration (B), storage (C), and bottling (D) (photos courtesy of Alfa Laval, Olive oil SpA, Italy; GEA Westfalia Separator Group, Germany; Borelli Group, Italy).

added. Vertical centrifugation separates the residual water and the solid impurities in order to obtain a clear oil (Figure 5A) reducing the VOO humidity concentration to a mean value about 0.18% (Masella and others 2009). However, the addition of water reduces the hydrophilic phenol content. In fact, Di Giovacchino and others (1994) reported a decrease after vertical centrifugation both for total phenols and orthodiphenols concentration as a function of increasing amounts of washing water (from 0% to 80% of the oily must). As recently reported (Parenti and others 2007; Masella and others 2012), vertical centrifugation causes a strong oxygenation of the VOO 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. Considering the aroma of resulting VOO, they observed a decrease of C6 and C5 volatile compounds due to the partition of these substances between oil and water phases during the vertical centrifugation. As, the vertical centrifugation is the processing step that mainly contributes to the oil oxygenation, Masella and others (2012) recommended the need for engineering suitable vertical centrifuges designed to limit the oxygenation effect and the loss of aromatic volatile compounds. With this aim, they suggested blanketing the vertical separator with an inert gas which would be another useful way to reduce the dissolved oxygen concentration. VOO vertical centrifugation under inert gas causes a strong reduction of the oil oxygenation in terms of reduced dissolved oxygen concentration and oxidative indexes (peroxide values and K_{232}). This technical solution appears to be a practical solution to preserve VOO quality.

Storage

After the extraction process VOO should be stored in stainless steel and maintained at a constant temperature of between 10 and 18 °C before bottling (Jiménez Márquez and others 1995; Alba Mendoza and others 1997) (Figure 5C). The main cause of oil deterioration during storage seems to be the oxidative rancidity (Morelló and others 2004a, 2004b). This reaction occurs between unsaturated fatty acids and oxygen (Frankel 1991). There are 2 compositional factors able to determine the VOO susceptibility to oxidation: the fatty acid composition and the antioxidant compound concentration, such as carotenoids, tocopherols, and phenolic compounds (Psomiadou and Tsimidou 2002). Phenolic compounds are involved in VOO resistance to oxidation and are responsible for its bitter and pungent taste. During the storage, all the phenolic compounds tend to decrease in concentration except for the lignans that seem to be the most stable. On the contrary, the secoiridoid derivatives, 3,4-DHPEA-EDA, pHPEA-EDA and 3,4-DHPEA-EA are characterized by a more active participation in the oxidative processes. Due to the hydrolytic reactions on the

secoiridoid derivatives, simple phenols, such as 3, 4-DHPEA and p-HPEA, usually increase. As expected from the correlation between secoiridoid concentration and the shelf-life of the product, this last one decreases during storage (Morelló 2004a, 2004b). When VOO is improperly stored unpleasant odor or taste arising from the rancidity process could occur (Frankel 1998, 2005). These negative volatile substances, such as hexanal, octane and other C8 and C9 compounds, are formed through nonenzymatic oxidation during VOO storage, favored by high temperatures, oxygen, light, and pro-oxidants. Recently, the use of stripping nitrogen to remove the dissolved oxygen from the oil immediately after production has also been suggested, in order to increase VOO shelf life (Masella and others 2010).

Filtration

There is a dispute between the researchers about the so called "*veiled*" (not filtered) VOO and its stability along the time (Lercker and others 1994; Frega and others 1999; Velasco and Dobarganes 2002; Fregapane and others 2006; Lozano-Sanchez and others 2010). Cloudy, or veiled, VOO contains polyphenols, perhaps polymeric in nature, phospholipids and sugars, but it could contain too hydrolytic and oxidative enzymes, such as lipase, LOX, and polyphenol oxidase. Undoubtedly, during filtration, a loss of a significant part of polyphenols occurs favoring a reduction of oxidative stability; on the one hand, the conditions in the veiled VOO favor enzymatic reactions. Moreover, the filtration of VOO can avoid the fermentation of sugars or proteins producing volatile compounds responsible for an unpleasant muddy odor by butyric fermentation. On the other hand, some authors (Tsimidou and others 2005; Gómez-Caravaca and others 2007) observed a higher stability of the unfiltered oils in comparison to the filtered ones due to a higher total phenolic content in the emulsified water.

This matter remains unsolved for the time being. Efforts should be made to clarify which parameters are effectively able to discriminate when the filtration can be necessary to avoid a rapid quality decay or when a cloudy VOO can reveal a longer shelf-life due to its peculiar composition.

Future Trends in the Development of Innovative VOO Extraction Machines

Labor and energies should be spent to develop innovative equipment to better modulate the physical parameters that influence the endogenous enzyme activities, such as temperature, oxygen, and water activity (Clodoveo 2013c; Fregapane and Salvador 2013).

Many authors have studied the application of emerging technologies, such as pulsed electric fields (PEF), US, and MW in the VOO extraction system in order to develop innovative and sustainable plant engineering solutions useful to increase oil yields, process efficiency, and quality (Abenoza and others 2012; Clodoveo and Hachicha Hbaieb 2013). In particular, Clodoveo and others (2013a, 2013b) demonstrated that US pretreatment of olive paste before malaxation allowed a more rapid and efficient transmission of heat increasing extraction yield. The final product was richer in tocopherols, carotenoids, and chlorophylls than the untreated oils, showing a higher nutritional value (Clodoveo and others 2013a, 2013b; Clodoveo and Hachicha Hbaieb 2013). These results, in agreement with the already cited paper of Jimenez and others (2007), were ascribed to the effect of US which is caused by the phenomenon of acoustic cavitation. Acoustic cavitation has a strong impact on a solid surface and can disrupt biological cell walls. The mechanical effect of US promotes the release of soluble compounds from plant tissue by disrupting cell walls and thus improves mass transfer also in the olive tissues (Suslick and others 1997).

Therefore, the application of new emerging technologies, such as PEF, US and MW, in the VOO extraction process offers a number of advantages due to their mechanical and thermal effects. In contrast to the existing technology, the considered emerging ones cause the rupture of cell walls and the recovery of the oil and minor compounds trapped in the uncrushed olive tissue, increasing the work capacity of the extraction plant, reducing the process time. PEF, US, and MW can be retrofitted to existing VOO extraction plants or be engineering into new systems. The industrial application of these technologies could represent the 1st step toward a continuous malaxing phase. Moreover, as observed in other food processes, several reactions catalyzed by enzymes seem to be activated or inactivated by US (Sinisterra 1992), so this technology seems to be the most promising due to the possibility to modulate sensory and nutritional characteristics of VOO deriving from the enzymatic activities.

Conclusions

The quality of VOO depends on physical, chemical and biochemical reactions which occur during the mechanical extraction process that mainly modify the qualitative and quantitative composition of polyphenols and volatile compounds of VOO. There is a plethora of articles which analyze separately the effects of the endogenous enzyme activities and the mechanical actions on the nutritional and sensory characteristics of VOO. However, this is the 1st attempt to describe in a single paper all the enzymes technologically involved in the extraction process and the action of each machine on their activity. A strategic choice and an appropriate use of the different devices that compose the VOO extraction plant and the technological parameters allows to emphasize or inhibit the endogenous enzymes activities present in olive fruit tissues. These choices modify the nutritional and sensory properties of the final product. Management of the VOO quality requires a deep knowledge on the enzymes' cell and olive tissue localization and the way to modulate these enzyme activities by choosing the appropriate mechanical devices that are part of the extraction process. In fact, the enzyme activities can be influenced by opting for different devices' combinations, selecting only some parts of drupes, or regulating several factors such as temperature, time, amounts of processing water and oxygen. Furthermore, all the other operation subsequent to the extraction process, such as oil separation, clarification, filtration, storage and bottling can compromise the effects resulting from the best extraction conditions strategically chosen. The last big engineering revolution in the VOO technology was the introduction of the horizontal centrifuge, coupled to the malaxer. However, there are considerable opportunities to design innovative equipment expected to increase the extraction yield, to reduce the process time and to develop sophisticated technical tools to modulate the biological reactions into the olive paste. More information should be collected to better assess the physical mechanisms able to promote the coalescence phenomena and to reduce olive paste viscosity. This overview could find the right direction toward new engineering solutions, also considering emerging technologies such as PEF, US, and MW.

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Author's Contributions

M. L. Clodoveo conceived the design of the review paper, wrote the sections on the VOO extraction process, the abstract, the introduction, the future trends, and conclusion, also harmonized the different paragraphs and draw the illustrations. R. Hachicha Hbaieb wrote the section relative to the olive enzymatic activities. F. Kotti contributed to the manuscript revision. F. Kotti and M. Gargouri, in collaboration with M. L. Clodoveo, supervised Dr. R. Hachicha's activity.

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