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Chapter 8

Technological Aspects of Olive Oil Production

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1. Introduction

Virgin olive oil (VOO) is obtained exclusively by mechanical extraction from the olive fruit and can be consumed crude without any further physical-chemical treatments of refining. Its sensory and health properties are intimately linked to its chemical characteristics, in particular to several minor components, which are strongly affected by the operative conditions of oil processing and can thus be considered as analytical markers of the quality of oil processing.

VOO contains different classes of phenolic compounds, such as phenolic acids, phenolic alcohols, hydroxy-isochromans, flavonoids, secoiridoids and lignans. The phenolic acids together with phenyl-alcohols, hydroxy-isochromans and flavonoids are present in small amounts in VOO (Montedoro et al., 1992). Secoiridoids which, in combination with lignans, are the main hydrophilic phenols of VOO, include the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA) an isomer of the oleuropein aglycon (3,4-DHPEA-EA) and the ligstroside aglycon (*p*-HPEA-EA). The main lignans found in VOO are (+)-1-acetoxypinoresinol and (+)-1-pinoresinol (Brenes et al., 2000; Owen et al., 2000). Both secoiridoids and lignans affect the quality of the sensory and health properties of VOO (Servili et al., 2004a), determining bitter, pungent sensations.

Many compounds, mainly carbonyl compounds, alcohols, esters and hydrocarbons, have been found in the volatile fraction of VOO.

The C_6 and C_5 compounds, especially C_6 linear unsaturated and saturated aldehydes alcohols and esters represent the most important fraction of volatile compounds found in high quality VOOs.

The C_6 and C_5 compounds, produced from polyunsaturated fatty acids by the enzymatic activities exerted by the lipoxygenase (LOX) pathway and their concentrations, depend on the level and the activity of each enzyme involved in this LOX pathway.

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The pathway (Figure 1) begins with the production of 9- and 13-hydroperoxides of linoleic (LA) and linolenic (LnA) acids mediated by lipoxygenase (LOX). The subsequent cleavage of 13-hydroperoxides is catalysed by very specific hydroperoxide lyases (HPL) and leads to C_6 aldehydes, of which the unsaturated aldehydes can isomerize from *cis*-3 to the more stable *trans*-2 form. The mediation of alcohol dehydrogenase (ADH) reduces C_6 aldehydes to corresponding alcohols, which can produce esters due to the catalytic activity of alcohol acetyl transferases (AAT).

Furthermore, an additional branch of the LOX pathway (Figure 1) is active when the substrate is LnA. LOX would catalyse the formation of stabilized 1,3-pentene radicals, which can either dimerize leading to C_{10} hydrocarbons (known as pentene dimers), or can react with a hydroxy radical, producing C_5 alcohols. The latter can be enzymatically oxidated to corresponding C_5 carbonyl compounds. These compounds are responsible for the most important sensory notes of VOO flavour, such as the "Green" and floral notes (Angerosa et al., 2001; Angerosa et al., 2004; Servili et al., 2009a).

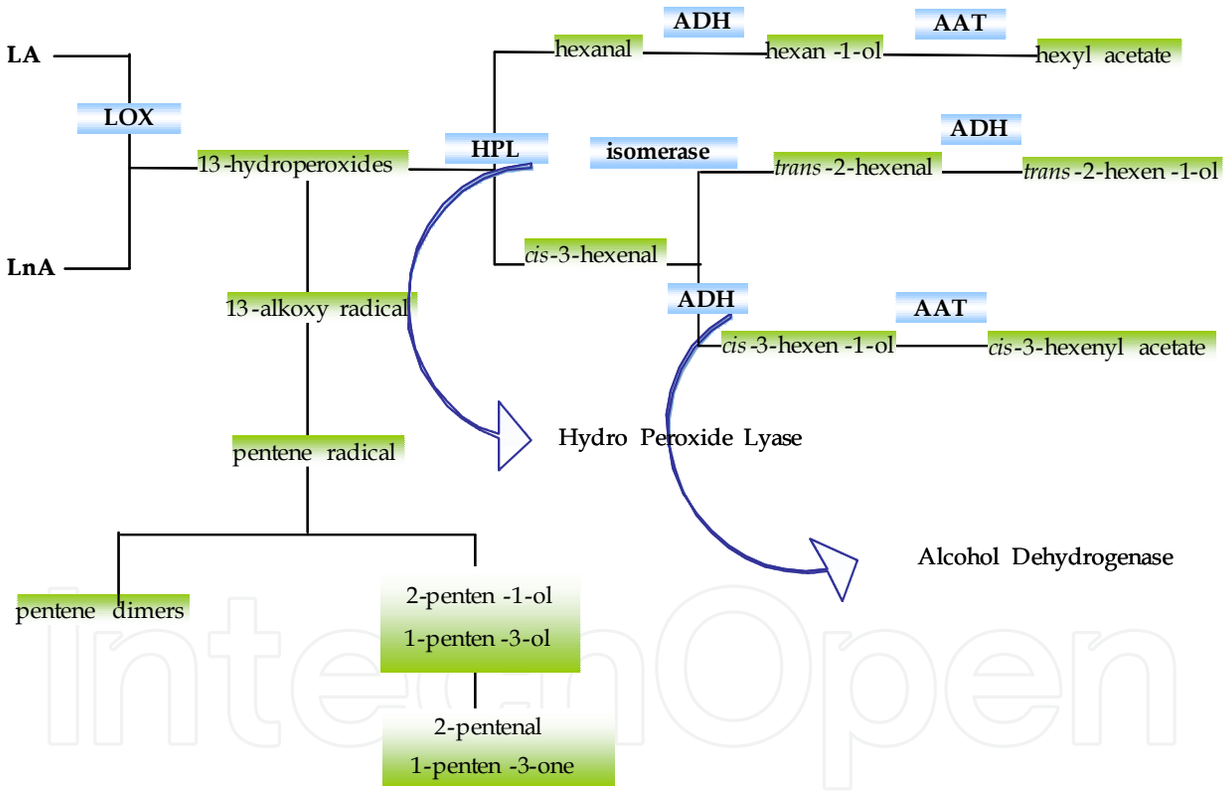


Figure 1. Lipoxygenase pathways involved in the production of C_6 and C_5 volatile compounds (Angerosa et al., 2004).

The new approach to VOO processing should include as its first objective the improvement of the quality of the sensory and health properties of oil. Since the presence of VOO hydrophilic phenols and volatile compounds is strictly related to the activities of various endogenous enzymes of olive fruit, their concentration in the oil is highly affected by the operative conditions of the mechanical oil extraction process. By taking into account the optimization of volatile and phenolic composition of VOO as the main goal of the new

approach to the mechanical oil extraction process, the conditions during crushing and malaxation can be considered as the most critical points (Capella et al., 1997; Caponio et al., 1999; Servili et al., 2002; Servili et al., 2004a; Angerosa et al., 2004; Servili et al., 2009a).

2. The structure and composition of the olive fruit

The first factor for high quality production in the VOO industry is the structure of the raw material, thus the quality and sanitary status of the olive are very important.

The olive fruit is a drupe, the weight of which varies between 0.5g and 20g. The constituent parts of the fruit, which include the skin, pulp and stone, represent respectively 1.5-3.5%, 70-80% and 15-28% of the weight of the fresh drupe. The stone contains the seed with a weight ranging from 2% to 4% of the whole fruit (VV. AA., 2003).

It is difficult to define the average composition of the olive fruit, due to its remarkable biodiversity, which produces high compositional variability. Water (40-70%) and fat (6-25%) are the main constituents of the fresh fruit.

The fruit contains water-soluble compounds, including simple sugars, organic acids, nitrogenous substances, phenolic compounds and an insoluble fraction of colloidal nature. Colloids of the drupe include the components of the cell wall or the middle lamella, such as hemicelluloses, celluloses, pectins, enzymatic and structural proteins.

The most important reducing sugars found in olives are glucose, fructose and sucrose, whereas citric acid, malic acid and oxalic acid are the main organic acids of the olive drupe (VV. AA., 2003).

In the composition of the olive, the phenolic fraction, which includes the precursors of natural antioxidants present in VOO, is of major importance (Amiot et al., 1986; Servili et al., 1999a, 1999b, 1999c; Servili & Montedoro, 2002). Phenolic compounds present in very high quantities of drupe (from 0.5 to 2.5% fresh weight) include oleuropein and demethyloleuropein. These substances are found mainly in the peel and pulp, whereas the seed contains nüzhenide, not found in the pulp and which is not considered a precursor of the phenolic compounds of VOO (Servili et al., 1999b). Lignans were found both in the pulp and in the woody core, but the latter cannot be released in VOO during oil processing (Brenes et al., 2000; Garcia et al., 2001; Servili et al., 2007).

The peel and the pulp together contain more than 90% of the total phenolic concentration of the fruit, which varies significantly according to the cultivar and stage of ripening of the olives (VV. AA., 2003; Servili et al., 2004a). The oil fraction is present in the pulp (16.5-23.5% fresh weight) and in the seed (1-1 .5% fresh weight).

Oilseed cells typically contain cytoplasmic and vacuolar oil. The compartmentalization of the oil in the olive pulp cells is, in this context, unusual when compared with that of oilseeds. In fact, the pulp cells of a ripe olive oil contain almost exclusively vacuolar oil, whereas the amount of cytoplasmic oil in the oilseed cells is remarkable.

Vacuolar oil in the olive pulp is the result of the mechanical process of oil extraction, which explains why even the most remote civilizations in the Mediterranean area have been using this fruit as a natural source of dietary fat.

3. New technological approaches towards the quality of virgin olive oil

3.1. Olive fruit storage

Storage of the olives before the mechanical oil extraction process is a critical point, which can reduce the quality of VOO. The first adverse effects are seen in the decrease in oil phenols and in the reduction of volatile compounds responsible for the flavour of VOO (Angerosa et al., 1996; Kiritsakis, 1998a; Kiritsakis et al., 1998b; Angerosa et al., 1999; Servili et al., 2004a; Servili et al., 2009a). The activation during olive storage of endogenous polyphenoloxidase (PPO) and peroxidise (POD), which catalyze the degradation of the phenols, can explain the loss of phenolic compounds. The reduction of volatile compounds responsible for the oil flavour can be due to the inhibition of the LOX pathway.

Moulds, yeasts and bacterial contaminations, and their corresponding metabolisms, are the underlying cause of the off-flavour biogenesis in VOO. In several operative conditions involving long-term storage of the olive and high relative humidity, mould contamination increases the free acidity due to the production of fungal lipase, and simultaneously forms the characteristic sensory defect of "mould" (Angerosa et al., 1999, 2004). Several mould species can also produce mycotoxins. Sugar fermentation produces the formation of acetic acid and ethyl acetate, which are considered responsible for the “vinegar” off-flavour.

During storage, the fatty acid alkyl esters of ethanol and methanol develop. The formation of these compounds is used as a marker to recognize low quality virgin olive oil. In fact, these compounds, combined with other sensory and chemical parameters, are used to classify virgin olive oil according to the internationals standards set by the International Olive Council (I.O.C., 1996).

These observations lead to the conclusion that olive storage should be avoided. To maintain the quality of VOO, the olives should be processed within twenty-four hours after harvesting. A thin, 30-40 cm thick layer of olives should be stored in perforated boxes on pallets, in order to minimize the fermentation processes, which underlie not only the formation of sensory defects, but also water condensation on the surface of the olive skin, which can promote the attack by moulds. Perforated boxes and pallets are also the most suitable to transport the olives from the olive grove to the mill (Angerosa et al., 1996, 1999; Servili et al., 2004a; Servili et al., 2009a).

Another aspect, which affects the storage premises, is related to the handling of the pallets or boxes. This process must be done by avoiding the use of forklift trucks run by petrol engines, which produce polycyclic aromatic hydrocarbons. These compounds can contaminate the olives, and subsequently, the oil (Angerosa et al., 2004).

3.2. Leaf removal and washing

Leaf removal is always recommended, especially when harvesting is done mechanically. The presence of leaves during the mechanical oil extraction process does not add any positive characteristic to the oil but, on the contrary, can change its taste and aroma.

Olives are generally washed by continuous washing machines. (Di Giovacchino et al., 2002; Perez et al., 2003). Olive washing has a more or less significant effect on the quality of VOO, depending on the characteristics and sanitary state of the olives. Fresh olives, harvested at the correct degree of maturity and properly transported and stored, showed no direct effect in the quality of the oil due to washing.

The most critical aspect of the washing process concerns the purity of the water used during this stage. The water should be changed frequently during processing to prevent the use of washing water containing too many earthy particles, which can release the compounds responsible for the "Earthy" sensory off-flavour into the oil (Angerosa et al., 2004).

3.3. Olive crushing

The main hydrophilic phenols of VOO, such as secoiridoid aglycons, develop during crushing from the hydrolysis of oleuropein, demethyloleuropein and ligstroside, catalysed by the endogenous β-glucosidases (Servili et al., 2004a; Obied et al., 2008).The impact of crushing in the VOO phenolic and volatile compounds can be related to the differentiated distribution of the endogenous oxidoreductases and phenolic compounds in the constituent parts of the olive fruit (pulp, stone and seed). As reported in previous papers, the POD, in combination with the PPO, are the main endogenous oxidoreductases responsible for phenolic oxidation during processing. POD occurs in high amounts in the olive seed. The phenolic compounds, on the contrary, are largely concentrated in the pulp, whereas the stone and seed contain only small quantities of these substances (Servili et al., 2004a; 2007). As a result, the crushing methods, such as the olive stoning process or the use of mild seed crushers, which enable degradation of the seed tissues to be reduced by limiting the release of POD in the pastes, prevent the oxidation of hydrophilic phenols during malaxation, thus improving their concentration in the VOO (Figure 2) (Servili et al., 1999a, 2004a, 2007).

The operative conditions of crushing also affect the volatile composition of VOO (Table 1). As previously mentioned, almost all volatile compounds are responsible for the flavour of high quality VOOs when the olive pulp tissue is ruptured, thus the effectiveness of the crushing plays an important role in their production.

The traditional olive crusher used for many centuries was the stone crusher. The stone crusher consists of a basin formed by a plinth and a stainless steel edge with an opening for the unloading of olive paste at the end of milling. Two or four granite wheels rotate and revolve on a rough granite base at different distances from the centre of the tank. Rotation speed is normally 12- 15 rpm.

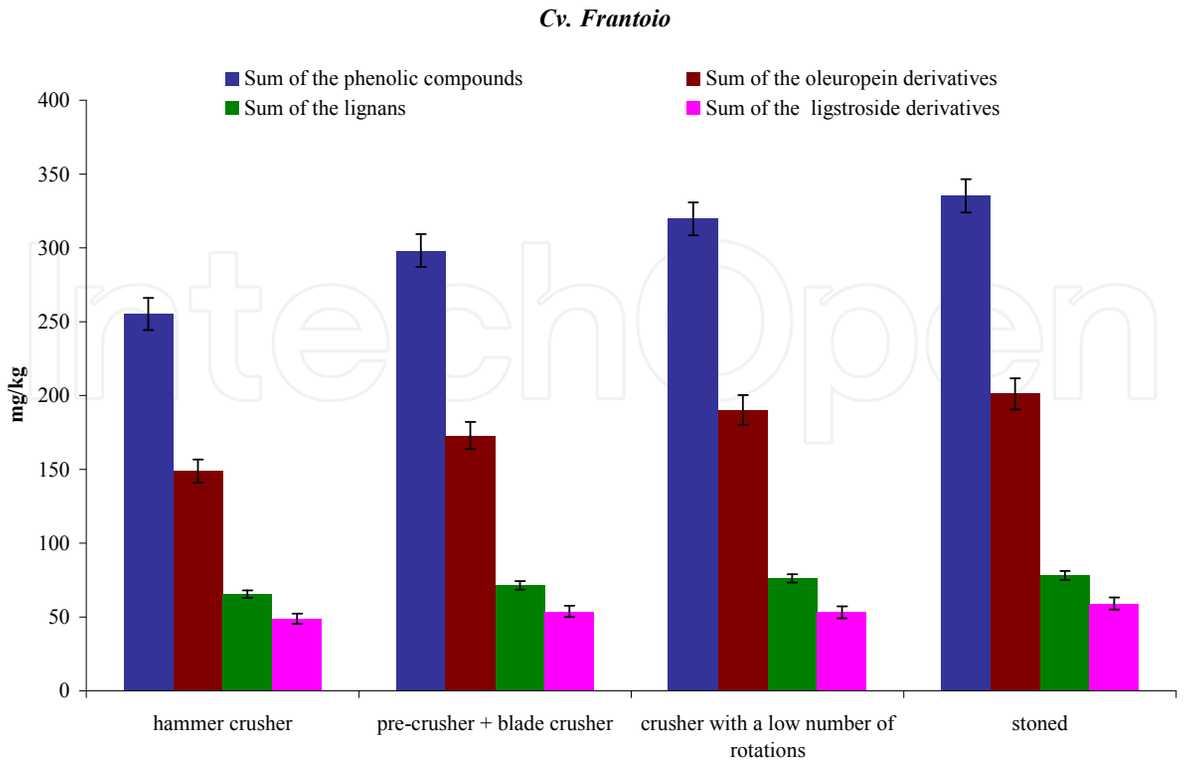


Figure 2. Effect of different crushing methods on the phenolic composition of VOO in Cv. *Frantoio* (mg/kg). The vertical lines are the mean value of three independent experiments ± standard deviation.

The popularity of the stone crusher extraction system using pressure gradually declined. In comparison with continuous crushers, this apparatus shows significant limitations in terms of olive oil quality. In particular, it reduces the phenolic concentration as the olive pastes are in long, extensive contact with the air during processing. Contact with the air stimulates PPO and POD, producing a high oxidation of phenolic compounds. Other weaknesses of the stone crusher are its low working capacity, the high hourly machine footprint, and its low ability to release the chlorophyll found in the olive skin, responsible for the green colour of EVOO, into the oil. This aspect is particularly relevant when the stone crusher is combined with a solid-liquid centrifugal separation. The crushing operation in oil extraction by centrifugation is generally replaced by the use of continuous crushers.

Continuous crushers include the hammer crushers, which were historically the first to be introduced as an alternative to stone crushers. These machines have some benefits that are attributable to their high working capacity, low footprint, and low installation costs compared to the stone crushers. At the same time, the hammer crushers show some disadvantages, such as the strong emulsifier effect produced on olive paste during crushing, a considerable increase in paste temperature and the high degradation of the seed tissues which, as mentioned earlier, can encourage phenolic oxidation (Angerosa & Di Giacinto., 1995; Servili et al., 1999a; Servili & Esposto 2004).

The new approach to olive crushing is based on the differentiated crushing of the constituent parts of the fruit, such as the skin, pulp and seed. In other words, the

degradation process of the olive tissues should be strong for the skin and pulp, in order to facilitate the release of oil and pigments, whereas impact on the seed should be limited. This reduces the transfer of POD found in the seed to the olive paste pulp, which can increase the oxidation of phenolic compounds during malaxation. The new generation of mild seed crushers, which include the blade crusher, teeth crusher and double stoker crusher, reduce the phenolic degradation and simultaneously improve the concentration of volatile compounds, especially of hexanal, trans-2-hexenal and C_6 esters, with a consequent positive increase of the intensity of "cut grass" and "floral" sensory notes (Table 1) (Angerosa et al., 2004).

	hammer crusher	*pre-crusher + blade crusher*	*crusher with a low number of rotations*	*stoned*
Aldehydes				
Pentanal	236.5 ± 4	273.4 ± 2.1	17.9 ± 1	66.5 ± 6.7
Hexanal	280 ± 2.9	511.4 ± 35.7	553.7 ± 0.3	579.6 ± 5.3
2-Hexenal (*E*)	43600.6 ± 327	44718.9 ± 208	39811.6 ± 587	52228.1 ± 521
2,4-Hexadienal (*E*,*E*)	19.4 ± 0.1	42 ± 3.5	341.6 ± 14.4	88.9 ± 5.4
Alcohols				
1-Pentanol	167 ± 5.2	94.5 ± 4.7	23.3 ± 0.7	62.6 ± 1.7
2-Penten-1-ol (*E*)	166 ± 11.3	91.4 ± 5.1	52.4 ± 3.5	104.0 ± 2.1
1-Penten-3-ol	960.3 ± 53.2	899 ± 43.3	522 ± 49.2	300.0 ± 4.6
1-Hexanol	1788 ± 57	2152 ± 74	512 ± 41	1501.0 ± 18.4
3-Hexen-1-ol (*Z*)	88.4 ± 22.2	103.6 ± 10.1	49.2 ± 2.3	77.0 ± 8.0
3-Hexen-1-ol (*E*)	22.2 ± 0.2	20.2 ± 0.1	9.9 ± 0.2	20.4 ± 2.3

Table 1. Effect of different crushing methods on the volatile composition of VOO in Cv. *Frantoio* (μg/Kg). Results are the mean value of three independent experiments ± standard deviation.

Several researches have shown that olive stoning during the mechanical extraction process of VOO increases the phenolic concentration in VOO (Figure 2) (Angerosa et al., 1999; Mulinacci et al., 2005; Lavelli & Bondesan, 2005; Amirante et al., 2006) and, at the same time, modifies the composition of volatile compounds produced by the LOX pathway, increasing the concentration of those volatile substances correlated to the "green" sensory notes (Table 1) (Servili et al., 2007). These results are particularly important, because they would appear to demonstrate that the enzymes involved in the LOX pathway have a different activity in the pulp and in the seed of the olive (Table 1) (Servili et al., 2007).

The stoning process, moreover, produces a significant reduction in industrial oil yields. This problem is caused by stone elimination which, when present in the pastes, produces an important draining activity, which increases the efficiency of the separation of the oil from the olive paste during solid-liquid extraction.

It should be noted that the use of enzymatic preparations with depolymerizing activities, degrading the colloidal structure of fruit might partly solve the problem of low yields due to the extraction of pastes for stoning. So far, however, the European Union (EU regulation) does not currently allow the addition of enzymatic preparations.

3.4. Malaxation process

The mixing and heating (25-35 °C) of the olive pastes during malaxation causes the breakdown of water-oil emulsion, allowing oil droplets to form larger droplets, which separate easily from the aqueous phase during the solid-liquid and liquid-liquid separation processes.

The operative conditions applied during malaxation of the olive pastes largely affect VOO quality (Servili et al., 1994; Montedoro et al., 2002; Servili et al., 2004a; Inarejos-Garcia et al., 2009). As in the case of the crushing process, malaxation can also produce significant modifications in the minor components of VOO with particular emphasis on volatile and phenolic compounds. The technological parameters of temperature and oxygen management show the highest impact on the volatile and phenolic composition of VOO.

The problems concerning temperature management during malaxation have been widely studied for over twenty years and a substantial negative relationship between the processing temperature and the quality of the VOO has been shown (Garcia et al., 2001; Di Giovacchino et al., 2002; Servili et al., 2003a; Servili et al., 2004a; Kalua et al., 2006). However some aspects of the relationship between the operative conditions of malaxation and oil quality must be better defined.

As previously reported, the most sensitive quality markers linked to the effect of processing temperature are the phenols and volatile compounds with their sensory impact. The literature on phenolic compounds clearly shows that the phenolic concentration of VOO could be more or less drastically reduced in relation to the increase in the mixing temperature. In particular, the derivatives of oleuropein, demethyloleuropein and ligstroside are highly affected by the processing temperature, whereas lignans are less affected (Servili et al., 2004a).

The optimal temperature of activity for PPO and POD may explain the loss of phenolic compounds in the oil depending on the processing temperature. These enzymes catalyze the oxidative degradation of phenolic compounds during the mixing process and show an optimal temperature of activity at approximately 50° C and 45° C respectively for PPO and POD.

As a result, the oxidation of phenols by PPO and POD within the range from 20° C to 35° C would be progressively higher, depending on the operating temperature. This explains the widely published data about the differences in phenolic concentration between oils obtained at different temperatures of between 20° and 40° C (Sánchez & Harwood, 2002; Angerosa et al., 2001).

These results are, in any case, obtained by performing malaxation with the pastes under continuous contact with air, as shown in the traditional mixer (Servili et al., 1998, 2003a).

However, when the process is performed in the new-generation malaxer, known as a "covered malaxer", which can control contact of the olive pastes with oxygen during mixing, the results obtained in terms of relationships between phenol concentrations in VOO and the processing temperature are completely different.

During processing, the olive pastes release CO_2 and the dissolved O_2 is simultaneously consumed by the oxidoreductase activities. As a result, the reduction of the O_2 content obtained in the covered malaxer inhibits the PPO and POD activities, improving the concentration of hydrophilic phenols in the olive pastes and in the corresponding VOO (Figure 3) (Servili et al., 2008).

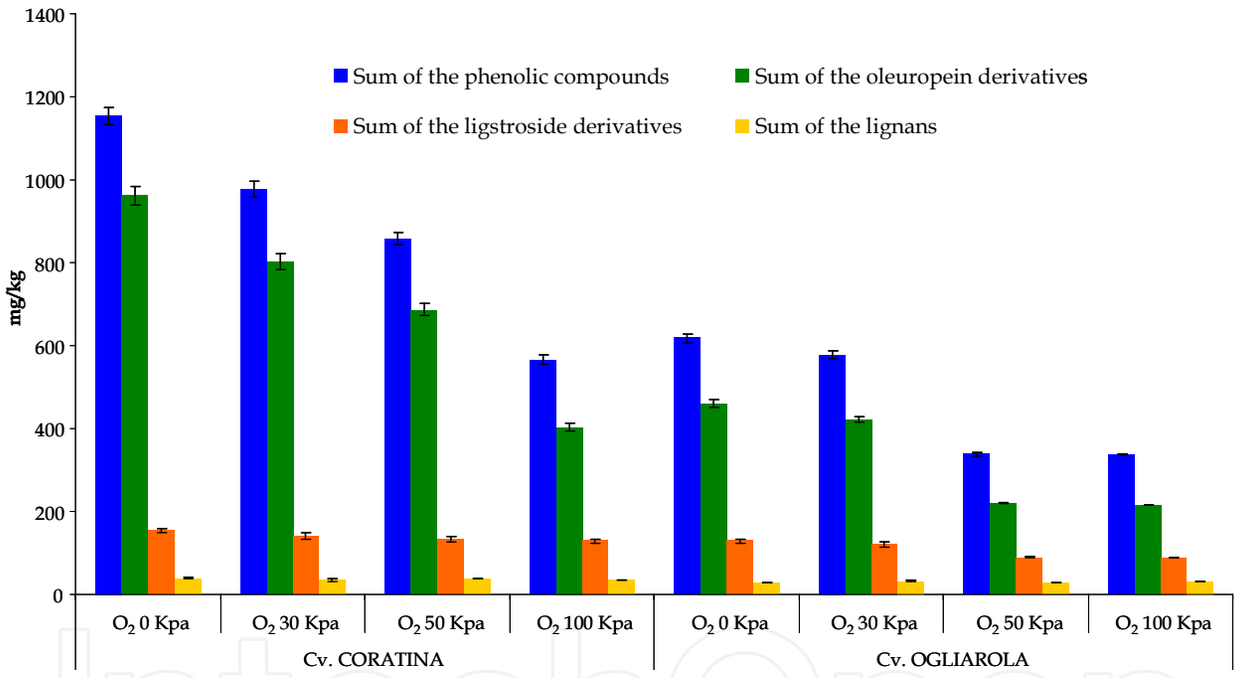


Figure 3. Phenolic composition (mg/Kg) of VOOs obtained after malaxation in different initial atmospheric compositions (Servili et al., 2008).
0 kpa saturated with N_2; 30 kpa corresponding to the air composition. The vertical lines are the mean values of three independent experiments, standard deviation is reported in brackets.

As a result, the oxidative reactions occurring in the pastes during malaxation can explain the relationships between VOO phenolic concentration and malaxation temperatures (Servili et al., 2004a; 2009a; 2009b). The O_2 dissolved in the pastes during malaxation, activate POD and PPO, which oxidize phenolic compounds according to the temperature and consequently reduce their concentration in VOOs obtained by pastes malaxed at high temperatures. The traditional malaxer, which contains a high amount of O_2 dissolved in the paste during the process due to contact with the air, represents a classical example of the aforementioned relationship between high temperatures and VOO phenolic loss. Low

amounts of O_2, on the contrary, inhibit the oxidative reactions of phenols during malaxation and, in this case, their concentration in the VOO increases according to the temperatures, because of a major release of phenols into the oil (Servili et al., 2008; 2009a; 2009b).

Thus, O_2 control during malaxation can be considered a new technological parameter which, in combination with the traditional ones (time and temperature of the process), can be used to optimize the VOO phenolic and volatile concentrations (Servili et al., 2004a, 2008, 2009a). In this regard, the time of exposure of the olive pastes to air contact (TEOPAC) was studied as a process parameter to regulate the O_2 availability in the paste and, as a result, the amount of phenols in the VOO (Servili et al., 2003a, 2003b).

Furthermore, the natural increase of an inert gas of this respiration catabolite, such as CO_2, released during malaxation after the destruction of the olive cell, may be combined with the use of nitrogen or argon to reduce the O_2 contact with the olive pastes during malaxation (Parenti et al. 2006a, 2006b; Servili et al., 2008).

The latter have to be carefully controlled according to the time of malaxation. Moreover, special attention must be paid to the traditional malaxers which work continuously in an air saturated atmosphere. In fact, in this case, the longer the time at the same temperature conditions, the greater the loss of phenolic compounds in the oil (Servili et al., 1994; Di Giovacchino et al., 2002; Servili et al., 2004a). On the other hand, there is not a direct link in the confined malaxer between the time of malaxation and the loss of phenolic compounds. However, malaxation times of over 35-40 minutes do not involve an extraction yield increase and, therefore, even though a loss in the quality of the oil is not observed, longer periods of malaxation are negative for a correct plant management.

Time and temperature of malaxation also affect the volatile profile and, therefore, the sensory characteristics of the resulting EVOOs (Angerosa et al. 2004; Servili et al. 2009a, 2009b). A large part of the volatile compounds, which explain the flavour of VOO, is due to the activity of the enzymes involved in the lipoxygenase pathway (Figure 1).

This group of enzymes promotes the formation of aldehydes, alcohols at C_5 and C_6 saturated and unsaturated and esters. The main effect of malaxation time is the increase of C_6 and C_5 carbonyl compounds, especially of trans-2-hexenal, which represent an important contribution to the flavour of olive oil due to their low odour threshold, whereas high temperatures of malaxation promote a fall of esters and cis-3-hexen-1-ol and an accumulation of hexan-1-ol and trans-2-hexen-1-ol, both considered by some authors as producing a not entirely agreeable odour (Angerosa et al., 2004; Servili et al., 2009a).

The enzymes involved in the LOX pathway such as lipoxygenase, hydroperoxidelyase, alcoholdehydrogenase and alcohol acyltransferase show an optimal temperature between 15 and 25 °C, whereas their activity decreases after 30 °C. Therefore, the malaxing process carried out at temperatures of over 35 °C can produce a reduction in the volatile compounds generation during malaxation.

From the aforementioned considerations, it is, therefore, clear that the mixing temperature control at levels lower than the 28-30 °C represents an advisable stage in the extraction process in order to get high quality VOO (Angerosa et al., 2001; Angerosa et al., 2004; Servili et al., 2009a). We must also take into account that even temperatures of paste below 22 °C in the new generation of confined malaxer lead to a decrease in the solubilisation of phenolic compounds and chlorophylls.

Considering the relationships between the volatile composition and O_2 concentration of VOO during malaxation, the results reported in literature indicate that the O_2 concentration in the pastes seems to have no effect on LPO activity during the malaxation (Table 2) (Servili et al., 2008).

	Initial O_2 partial pressure in the malaxer chamber headspace (kPa)							
	0[a]		30[b]		50		100	
	OGLIAROLA cv.							
ALDEHYDES								
2-Pentenal (*E*) [z]	291.5	(31.8)ab	343.0	(31.1)a	247.5	(11.7)b	269.5	(13.5)b
Hexanal	939.5	(9.2)a	1546.0	(200.8)b	1011.5	(27.6)a	1499.5	(16.3)b
2-Hexenal (*E*)	43645.0	(912.2)a	39130.0	(1054.7)b	37315.0	(233.3)b	38170.0	(1258.7)b
ALCOHOLS								
1-Pentanol	28.5	(2.1)a	128.0	(6.8)b	122.5	(3.5)b	158.0	(1.4)c
2-Penten-1-ol (*E*)	55.5	(3.5)a	63.0	(4.6)a	50.5	(9.2)ab	38.5	(6.4)b
1-Penten-3-ol	567.0	(17)a	871.0	(4.7)b	690.0	(1.4)c	809.5	(3.5)d
1-Hexanol	8357.0	(102.6)a	9699.0	(106.1)b	11660.0	(99)c	13675.0	(63.6)d
3-Hexen-1-ol (*E*)	35.0	(1.2)a	41.0	(3.5)a	47.5	(2.1)b	61.5	(2.1)c
3-Hexen-1-ol (*Z*)	286.5	(4.9)a	434.0	(20.6)b	341.0	(11.3)c	400.5	(7.8)d
2-Hexen-1-ol (*E*)	7662.5	(75.7)a	8616.0	(87.9)b	9355.0	(353.6)c	9780.0	(60.8)c
	CORATINA cv.							
ALDEHYDES								
2-Pentenal (*E*)	548.5	(16.3)ab	509.7	(5.8)b	636.7	(17.9)c	613.0	(51.2)ac
Hexanal	1187.0	(9.9)a	1624.3	(30)bc	1532.1	(27.3)b	1744.0	(121.2)c
2-Hexenal (*E*)	51565.0	(827.3)a	52900.0	(565.7)ab	54340.5	(355.7)b	53920.0	(332.1)b
ALCOHOLS								
1-Pentanol	40.0	(5.7)a	54.3	(5)b	39.4	(5)a	48.0	(3.2)ab
2-Penten-1-ol (*E*)	87.5	(0.7)a	67.0	(0.2)b	105.8	(5.7)c	105.0	(8.3)c
1-Penten-3-ol	890.0	(2.8)a	820.0	(1.2)b	1093.5	(33.7)c	1185.0	(91.2)c
1-Hexanol	2326.0	(49.5)a	3694.2	(2)b	1788.0	(57.2)c	2170.0	(123.1)a
3-Hexen-1-ol (*E*)	25.5	(0.7)ab	31.6	(3.8)a	20.0	(1.9)b	21.0	(1.9)b
3-Hexen-1-ol (*Z*)	561.0	(4.2)a	513.6	(9.6)b	486.3	(11.1)b	498.0	(31.2)b
2-Hexen-1-ol (*E*)	3654.5	(30.4)a	5905.0	(321)b	3350.1	(80.5)a	4185.0	(35.6)c

Table 2. Volatile composition (μg/kg) of EVOOs obtained after malaxation in different initial atmospheric compositions (Servili et al. 2008).

[a] saturated with N_2; b corresponding to the air composition. [z] Data are the mean values of three independent experiments, standard deviation is reported in brackets. Values in each row with different letters (a-d) are significantly different from one another at $p < 0.01$.

In conclusion, it can be considered that, as regards the process variables adopted during malaxation, the temperature should be set within the range of 24-27 °C, both for the traditional and for the new malaxer (i.e., the confined malaxer), while process times greater than 35-40 minutes may result in a large loss in terms of product quality (in particular in the traditional malaxer) without producing significant positive effects on the oil extraction yield.

Special attention must be paid to the control and regulation of oxygen percentage in contact with the olive paste during malaxation to optimize the phenolic content as well as the flavour of VOO. This new operating parameter can be used to act on the phenolic concentration of the pastes and, therefore, of the oils, excluding negative collateral effects on the volatile compound content of the product (Servili et al., 2003a; Migliorini et al., 2006; Servili et al., 2008). In fact, the traditional Italian *cvs* differ with respect to their content of phenolic substances and, because of this difference, the phenolic concentration in oil must be optimized; this optimization can be obtained by regulating their oxidative degradation level during malaxation. Thus, malaxation should be carried out without oxygen for the *cvs* with low phenolic concentration, whereas malaxation should be carried out with controlled supplementation of oxygen for those *cvs* characterized by higher phenolic concentrations (Figure 3, Table 2).

Moreover, it must be pointed out that no additional gases, such as nitrogen or argon, are required inside the head space in the confined malaxer to avoid the presence of oxygen. In fact, if the malaxer is filled with crushed paste during the process, the olive tissues of pastes naturally release carbon dioxide (CO_2) (Weichmann, 1987), whereas the limited amount of oxygen they adsorb during the crushing process will be consumed rapidly by endogenous enzyme activities. As a consequence, the malaxer head space will be naturally saturated by an inert gas, such as carbon dioxide (Servili et al., 2003a; Parenti et al., 2006a, 2006b; Servili et al., 2008).

3.4.1. Use of the technological co-adjuvants in malaxation

According to the UE 2568/1991 and 1989/2003 standards, co-adjuvants can be added during malaxation to break down emulsions and at the same time to guarantee a high oil extraction yield. In particular, the most frequently employed co-adjuvants are micronized talc and in some countries, although not in Europe, enzyme preparations are used.

Such enzyme preparations act on the structural colloids of the cells of not only the pulp, but also the skin and assist the activity of the endogenous enzymes (pectinases, cellulases and hemicellulases) resulting in an increase in the olive oil yield and in its quality (Vierhuis et al., 2001). In particular, it has been demonstrated that enzyme preparations tend to increase the phenolic content in VOO (Table 3). However, these effects are found only when working with olives characterized by an early ripening (Ranalli & Serraiocco, 1996; Ranalli & De Mattia, 1997; Vierhuis et al., 2001; Montedoro et al., 2002). In fact, the use of enzymatic preparations in olives when they have reached an advanced stage of ripening does not lead to any qualitative or quantitative benefit. This is due to the fact that the structural colloids in

mature olives, on which the aforementioned enzymes should act, have already been degraded by the endogenous enzymatic activity during the ripening of the fruit (Heredia et al., 1993). Therefore, the addition of enzymatic preparations would be useless in this case.

However, the EU regulations 2568/1991 and 1989/2003 do not allow the addition of enzymatic preparations, whereas they authorise the use of micronized talc as co-adjuvant. The use of talc as a co-adjuvant has been proved important to increase oil extraction yield without any interference to the quality of VOO. The amount of talc used ranges from 0.7 to 1.5% of the weight of the olives being milled after the first 10 minutes of the process. In fact, its addition to difficult pastes improves the paste structure and reduces emulsions. This product acts on the olive pastes by increasing the drainage effect and, therefore, improving the efficiency of solid-liquid separation during centrifugation of the crushed pastes (Servili et al., 2004b). The micronized talc can be added to the pastes during malaxation (0.7-1.5% of the paste weight) after the first 10 minutes of the trial. Several studies carried out on Italian cultivars show that the use of the micronized talc does not involve any negative effects on oil quality and, in some cases, its use leads to a meaningful increase in the extraction yield (Servili et al., 2004b).

	crushed paste			malaxed paste			malaxed paste + enzyme preparations		
3,4-DHPEA[a]	2.7	±	0.3^{a}	0.7	±	0.1^{b}	1.09	±	0.1^{c}
p-HPEA	2.3	±	0.4^{a}	1.2	±	0.1^{b}	1.02	±	0.1^{b}
3,4-DHPEA-EDA	515.0	±	23^{ab}	317.0	±	16^{c}	439	±	16^{d}
p-HPEA-EDA	24.8	±	1.9^{a}	25.8	±	1.4^{ab}	29.4	±	0.8^{c}
Lignans	32.5	±	1.4^{a}	24.2	±	0.8^{b}	28.5	±	0.9^{c}
3,4-DHPEA-EA	357.0	±	13^{a}	177.0	±	8.0^{b}	218	±	8.0^{c}

Table 3. Phenolic composition of virgin olive oil (mg/kg) with and without enzymatic treatment during malaxation (Vierhuis et al., 2001).

[a] The phenolic content is the mean value of three independent experiments (standard deviation). Values in each row bearing the same superscripts are not significantly different from one another ($P < 0.05$).

3.5. Olive oil extraction systems

Different extraction technologies, such as pressure and centrifugation and selective filtration (i.e. "surface tension" or "percolation") enabling the separation of oily must from the olive paste can be used (Boskou, 1996; Di Giovacchino et al., 1994, 1995).

3.5.1. Pressure extraction system

Pressing is one of the oldest methods of oil extraction and has evolved considerably over the centuries. In olive oil mills equipped with this system the press separation of the oil from the paste is currently carried out using open hydraulic presses, whereas close cage presses have almost disappeared not only due to high purchase prices, but also to their maintenance costs. The previously malaxed paste is subsequently stratified on stacked filter mats, each

covered with approximately 0.5 inches (1.25cm) of paste and interposed with metal disks. This operation is carried out mechanically thanks to a dispenser, which takes the paste from the malaxer and stores it on the nylon and/or polypropylene filter mats. Both types of filter mats have a central hole to allow the expressed oil and water (olive juice) to exit in both directions. From a theoretical point of view, this system guarantees intrinsic oil quality. However, its use presents a few problems, mainly due not only to its low working capacity per hour, in which case the storage of olives lengthens, but also to the proper use of the filter mats and to the types of materials used to build the equipment. The critical aspects of the process regarding the use of the press, which impacts on the quality of the oil, are concerned with both the proper management of the filter mats and the use of construction materials made of stainless steel. As regards the filter mats, it is important to point out that they can represent a source of contamination, due to fact that they may introduce fermentation and an oxidation defect into the oil, causing sensorial defects (Angerosa et al., 2004). This effect can arise both from the contamination with oils obtained from poor batches of olives and from fermentation processes of the vegetation water and pomace fragments, which remain in the filter mats, when they are kept in storage during the different processing stages. The latter problem occurs particularly when the oil harvest is interrupted by bad climatic conditions and it is impossible to work continuously. In order to minimize the risk of defects developing in the VOO, it would be desirable: i) to work in a continuous cycle; ii) to change the stacked filter mats frequently during the process and to clean them periodically using a pressure washer; iii) to store the aforementioned filter mats at a low temperature (0 °C-5 °C) to avoid fermentative processes during breaks in the oil processing.

As regards the materials used to construct the press, all the metallic parts which come into contact with the product must be made of, or at least covered with stainless steel to avoid the transfer of metals, especially those metals which can speed up the oil oxidation, to the oil during the extraction process.

3.5.2. Extraction by centrifugation

The majority of VOO is currently extracted by centrifugation in Mediterranean countries. The idea of exploiting direct centrifugation of the malaxed paste to extract the oil dates back to the late nineteenth century, when the use of the first decanter applied to food industries was widespread. In the olive oil sector, this idea determined technological innovations in the VOO mechanical extraction process, which were opposed to the traditional press. The first operating patents, including the patent by Corteggiani, date back to 1956, followed by new companies producing olive oil machines in the early sixties. This machine, called a decanter, consists of a drum containing a cylindrical and a conical part with a horizontal axis, inside which an additional cylinder worm is placed, which acts as a screw conveyor. The differential speed of the latter is slower than that of the outer drum in order to discharge the solid part. In recent years, this extraction system has evolved considerably in order to reduce the amount of water used during the process. In fact, the decanters can be classified as follows:

1. Traditional three-phases decanters, featuring water addition ranging from 0.5 to 1 m^3/ton.
2. Two-phases decanters, which can operate without the addition of water and do not produce vegetation water as a by-product of the extraction oil process.
3. New three-phases decanters, working at low water consumption ranging from 0.2 to 0.3 m^3/ton.

The traditional three-phases decanters, which allow the oil to be separated both from the vegetation water and from the pomace, feature a humidity level of between 50% and 55% and dilute the pastes produced to reduce their viscosity. In doing so, they facilitate separation of the oil-vegetation water with a dilution ratio ranging from 1:0.5 to 1:1 (from 50 to 100 l of water for every 100 Kg of paste to be decanted). In addition to the enormous amounts of vegetation water which have to be drained, this implies a decrease in the oil quality, principally due to the washing away of the phenolic compounds of the product, with massive decreases in this important antioxidant fraction (Ranalli & Angerosa, 1996; Servili et al., 1999c, 1999d; Stefanoudakii et al., 1999; Di Giovacchino et al., 2001; Servili & Esposto, 2004).

The evolution of this technology has lead to the production of two and three-phases decanters with low water consumption. By using these new systems, the extracted oils feature a higher phenolic concentration than those extracted by means of the traditional centrifugation process, because the loss of these hydrophilic phenolic compounds in the vegetation water is reduced (Table 4) (Servili et al., 1999d). In this context, it is important to point out how these new extraction systems, which do not take into account the addition of water, enable high quality VOOs to be obtained. The focus of this problem consists in linking together the low processing temperatures with a reduced use of water dilution in the pastes: by using centrifugation systems, these two process variables should allow high

Phenolic compounds	*Coratina* cv.		*Ogliarola* cv.	
	two phases	**three phases**	**two phases**	**three phases**
3,4 DHPEA	0.87 ± 0.02	0.58 ± 0.08^b	0.66 ± 0.11^a	0.50 ± 0.11^a
p-HPEA	3.74 ± 0.07^a	2.34 ± 0.08^b	3.30 ± 0.10^a	4.22 ± 0.10^b
Vanillic acid	0.41 ± 0.01^a	0.19 ± 0.01^b	0.26 ± 0.01^a	0.14± 0.05^b
Caffeic acid	0.16 ± 0.01^a	0.12 ± 0.02^b	0.09 ± 0.01^a	0.21 ± 0.03^b
3,4 DHPEA-EDA	522.2 ± 13.5^a	427.2 ± 13.8^b	30.09 ±1.03^a	18.53 ± 0.68^b
p-HPEA-EDA	78.16 ± 0.52^a	67.26 ± 2.55^b	20.99 ± 0.82^a	22.40 ± 0.33^a
Lignans	38.41 ± 0.10^a	35.62 ± 1.11^b	48.00 ± 3.40^a	46.72 ± 5.78^a
3,4 DHPEA-EA	351.7 ± 11.0^a	244.9 ± 13.6^b	68.01 ± 6.00^a	52.04 ± 3.11^b
Total polyphenols	673 ± 4^a	585 ± 7^b	304 ± 5^a	263 ± 4^b
Induction period [h]	17.8 ± 0.1^a	15.5 ± 0.2^b	5.2 ± 0.1^a	4.6 ± 0.1^b

Table 4. Effect of water reduction during centrifugation on the phenolic composition of virgin olive oil (mg/kg) (Servili et al., 2002).
Data are the mean values of three independent experiments ± standard deviation. Values in each row, with cvs with different letters, are significantly different from one another ($p < 0,01$).

quality oils and machines featuring high yields to be obtained (Ranalli & Angerosa, 1996; Servili et al., 1999d; Stefanoudakii et al., 1999; Di Giovacchino et al., 2001).

As regards the by-products obtained by centrifugation, it is important to recall here that, whereas the aforementioned problem concerning the use of three-phases traditional systems is represented by the enormous amounts of vegetation water to be drained (0.7-1.2 m^3/ton), the main problem, when using the two-phases systems, is not only a decrease in oil quality, but also the high humidity level of the pomaces (50% for the traditional three-phases decanters, 55-60% for the two-phases). This last aspect implies two disadvantages: i) where to store by-products of the extraction process; ii) how to transport to the pomace oil factory and their subsequent use for residual oil recovery by solvent extraction.

The pomaces produced by two-phases system are generally employed for the production of compost or for spreading to improve agriculture soil, following a process similar to that used for the vegetation water of olives. In this context, three-phases decanters with low water consumption can represent an adequate alternative with respect to the two-phases extraction system, because they allow a quality of oil to be obtained which is comparable to that of oils obtained using the two-phases system, and pomaces with a reduced humidity content, similar to those produced by the traditional three-phases systems. On the contrary, they produce a certain amount of vegetation waters, which imply water draining procedures which comply with the regulations of the law.

3.6. Separation of the oil from vegetation water

The liquid coming from the extraction system is called "oily must", and consists of oil and vegetation water, which is separated by using vertical centrifuges. The oily must also contains solid particles and mucilage (0.5 to 1.0%). These substances are suspended if they are very small, whereas they are easily separated from the liquid if they are of an appreciable size (seed fragments and/or epicarp of olive fruit fragments). In particular, separation from the liquid is carried out by using a sieve (1~2 mm mesh) placed at the top of the olive oil storage tanks.

Disk stack centrifuges, suitable for separating solid impurities with a specific weight ranging from 1.050 to 1.150, are used to separate the oil from the oily must. The basic principles of centrifugation are well known. If a vigorous rotational motion is applied to the oily must (oil, vegetation water and impurities), the lighter part (i.e. the oil) is collected close to the axis of rotation, while the heavier part (i.e. the water) is collected further away. Finally, the impurities are collected even further away.

Moreover, spillways enable the oil as well as the vegetation waters and impurities to be recovered. A fraction of the impurities is deposited on the rotating drum, which must be periodically cleaned, even though self-cleaning decanters are widely used. In fact, these decanters work in a continuous cycle, providing a periodical and automatic discharge of the sediment, which could compromise the centrifugation process. The decanters most

frequently employed in the oil mills consist of a series of perforated, truncated cone-shaped disks, mounted on the hollow shaft-mounted drum in order to leave a free space of approximately 1 mm between the disks.

Centrifugal force forces the oily must poured in from the top through the hollow drum shaft upwards and it is divided into three different layers of oil, vegetation water and impurities, according to their specific weight. The drum diameter of decanters used in the oil mills ranges from 400 to 700 mm, with a rotational velocity ranging from 5000 to 12000 rpm. The working capacity of these machines in terms of litres of oily must poured in per hour is very high and it varies between 500 and 2000 l/h. The work carried out by the vertical centrifuges is qualitatively satisfactory, even though there is often a loss of oil in the vegetation waters. This loss cannot exceed 500 g/ton of processed olives if centrifugation process is to be considered adequate.

3.7. Olive oil storage

During storage, the phenolic composition of EVOO is modified by the endogenous enzymatic activities contained in the cloudy phase. These enzymes may reduce the "pungent" and "bitter" sensory notes, the intensity of which is strictly linked to the content of aglycon secoiridoids, and, at the same time, can produce olfactory and taste defects. Oil filtration partially removes the water and enzymes from EVOOs, and enables the EVOO phenolic content to stabilize during its storage. The filtration process of EVOO is a procedure carried out in two steps: first, the suspended solids are removed, and second, the elimination of humidity gives the oil a brilliant aspect. Normally, organic or inorganic materials are used in conjunction with a variety of filtration equipment to enhance or enable the separation of suspended solids and water-oil. The type of such equipment, often called filter aids, depends on the final objective (Montedoro et al., 2005).

The olive oil profile changes during its storage, due to the simultaneous, drastic reduction in compounds from the LOX pathway and to the neo-formation of volatile compounds, responsible for some common defects referred to as "rancid", "cucumber" and "muddy sediment" (Morales & Aparicio, 1997; Angerosa et al., 2004; Servili et al,. 2009a).

This runs parallel to the increase in saturated aldehydes nonanal, and above all hexanal in the oxidation process, but it cannot be considered a useful marker of oxidation, since it is also present in the aroma of high quality EVOOs (Angerosa et al., 2004; Servili et al., 2009a).

Furthermore, the presence of sediment as a result of the decantation of unfiltered olive oil during its storage can determine, under suitable temperature conditions, the production of unpleasant compounds responsible for the typical "muddy sediment" defect due to the fermentation which produces compounds, probably of the butyric type (Angerosa et al., 2004; Servili et al., 2009a).

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