Chapter 9

The Malaxation Process: Influence on Olive Oil Quality and the Effect of the Control of Oxygen Concentration in Virgin Olive Oil

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9.1 INTRODUCTION

Olive oil was discovered by early man, as he accidentally crushed fallen olive seeds and noticed that the segregated oil moistened. Since ca. 5000 BC, people have collected and squeezed olives in stone mortars, but the Romans expedited the crushing operation with a millstone crusher, the *trapetum*, and improved the separation system with the introduction of presses (Kiourellis, 2005). Following the fall of the Roman Empire there were no innovations in olive oil processing for many years, which continued to be based on the screw press (Di Giovacchino, 2000). In 1795 the hydraulic pressing system was invented (Balatsouras, 1986). Since the second half of the 20th century many technological improvements and innovations have occurred (Kapellakis et al., 2008). Olive oil separators have replaced the traditional methods, and productivity has been increased with the widespread adoption of hydraulic pressing systems. The pressing process has been the most widespread method for processing olive fruit to obtain olive oil. The use of stone rollers or wheels on a slab of stone allows the grinding of olives and kneading the resulting olive paste. The olive paste was drenched with hot water, in order to achieve better separation of olive oil, and then placed in oil diaphragms. In 1960 a new process for separation of oil from olive paste was introduced, based on the density differences of the olive paste constituents (olive oil, water and insoluble solids). Separation is accomplished through a horizontal centrifuge. At the same time, the rotating wheels were replaced with metallic crushers (hammer crusher and disk crusher). In order to preserve the kneading operation that occurred using rotating wheels, a new machine was introduced: the malaxer machine. Thus, a new phase in the procedure was introduced: the malaxation phase.

Malaxing is an extremely important phase in olive oil extraction. During the malaxing phase the olive paste is subjected to a slow continuous kneading, aimed at breaking off the emulsions formed during the crushing process and facilitating adequate coalescence. It is necessary to heat the olive paste at a carefully monitored temperature during malaxation in order to diminish the viscosity of the product and to stimulate its enzymic activity, therefore increasing the extraction yields. This operation facilitates high extraction yields, by helping small oil droplets to coalesce. These can be separated subsequently using a decanter centrifuge (Di Giovacchino, 2000).

The malaxing process determines the balance between the quality and the quantity of the oil extracted, by varying a range of parameters (time, temperature and atmosphere in contact with the olive paste), as the olive paste is gradually heated and the enzymes within are activated. All this must be done without affecting the biochemical structure of the olive paste, as this would affect the flavor, shelf-life and nutritional properties of the oil (Amirante et al., 2006). This operation is one of the critical points in olive oil extraction. Many studies have been carried out to investigate its influence on the olive oil quality. Nowadays the olive oil consumer asks for healthy products. There has been a large increase in demand for high-quality virgin olive oil, attributed not only to its potential health benefits,

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but also to its particular organoleptic properties. In fact, the sensory quality plays an important role in customer preferences (Angerosa et al., 1998). The operating environment during malaxation affects the volatile and phenolic composition of virgin olive oil and, as a consequence, its sensory and healthy qualities. The sensory and health-related properties of virgin olive oil are closely linked to its volatile and phenolic composition (Reiners and Grosch, 1998; Servili et al., 2002). The presence of these substances in the oil is the end result of various endogenous enzymatic activities activated during processing of the olive fruit (Morales et al., 1999; Angerosa et al., 2001). Research on olive oil quality has also shown the effect of the oxygen concentration in the head space of malaxer on the activities of these enzymes and hence on the olive oil quality itself.

Processing conditions strongly influence rheological properties of the olive paste. These in turn determine the behavior of the paste during the sedimentation and can thereby influence the extraction yield (Di Renzo and Colelli, 1997). Extension of the malaxation time improves the yield but can damage the final quality of the product. In order to correct for this potential disadvantage modifications have to be made to the malaxing system (Amirante et al., 2002). Much research has targeted the efficiency of this innovative continuous system, with many changes to the malaxer machine, aimed at improving the eventual olive oil quality (Amirante et al., 2005, 2006, 2008a, 2008b).

9.2 ENZYMES INVOLVED DURING MALAXATION AND THEIR INFLUENCE ON PHENOLIC CONTENT AND VOLATILE COMPOUNDS

Fresh and good-quality virgin olive oil is appreciated by consumers for its delicious taste and aroma. These qualities are partially due to non-volatile compounds, i.e. complex phenols (bitter taste) (Angerosa et al., 1998), and a number of volatile chemical compounds generated during the crushing-malaxation steps of oil production (Morales et al., 1994). Phenolic compounds originate from endocellular oil; malaxation modifies their qualitative and quantitative composition (Montedoro et al., 1992; Servili et al., 1992). The distribution of phenolic compounds in oil and its by-products during mechanical extraction is strictly linked to endogenous enzyme activities (Servili et al., 2003). During extraction, the content of some components is significantly modified, depending on the extraction technique employed (Amirante et al., 2005). New components such as volatile compounds can be formed as a result of chemical and/or enzymatic pathways (Ranalli et al., 2001). The relationship between the volatile and the phenolic components in virgin olive oil is based on the activity of the polyphenoloxidase (PPO), peroxidase (POD) and lipoxygenase (LOX) pathways. Polyphenoloxidase (PPO) and peroxidase (POD) can oxidize phenolic compounds (i.e., secoiridoids), resulting in reduced phenolic concentration of the oil. Potential effects on product quality characteristics include a decrease in the 'bitter' and 'pungent' sensory attributes, oxidative stability and a reduction in the nutritional value of virgin olive oil (Georgalaki et al., 1998; Ranalli et al., 2001).

Volatile compounds, which help make up the aroma of many fruits and vegetables, are produced from polyunsaturated fatty acids via a cascade of enzymatic reactions known as the lipoxygenase pathway. Lipoxygenase is a non-heme iron-containing enzyme that catalyzes the oxygenation of the 1,4-pentadiene sequence of polyunsaturated fatty acids to produce their corresponding hydroperoxides (Hatanaka, 1993). The lipoxygenase pathway involves the oxidative degradation of the polyunsaturated linoleic and a-linolenic acids, which are split into volatile C6 or C9 carbonyl fragments. These fragments can be further modified by isomerization, reduction and esterification. The variety of volatile compounds thus produced constitutes the volatile fraction of the oil, so this pathway is important in helping determine the quality of the oil produced (Angerosa et al., 2001). Many of the volatiles produced are incorporated into the oil phase, adding to its characteristic aroma.

The mentioned enzymes are triggered by the milling of olives, and are active during the malaxation step. Both classes of enzymes have oxygen as a co-factor. Hence, the presence of oxygen in the head-space of the mixer might favor oxidation of phenols during malaxation, reducing their concentration in the pastes and in the oil. On the other hand, oxygen may facilitate the LPO pathway activity, determining the sensory note intensity in virgin olive oil. The latest research indicates that the choice of crushing system can limit the presence of enzymes such as PPO and POD. Strategic use of oxygen concentration during the malaxing phase helps to obtain high phenol concentrations. This in turn leads to well-balanced sensory characteristics in the resulting olive oil (Amirante et al., 2008a).

9.3 MALAXER MACHINE AND ITS EVOLUTION IN ENHANCING OLIVE OIL QUALITY

The malaxer machine consists of a stainless steel tank containing the olive paste, a malaxing central screw stirring the paste slowly and continuously and a mono screw pump conveying the paste to the separation phase utilizing a decanter machine. The malaxer is equipped with a circulating hot water jacket to heat the paste. Every tank is also equipped with a temperature switch. For many years the malaxer machine was characterized by a non-hermetic closure, i.e. an inox grill. The traditional tank has typically been a cradle shape. There was considerable loss of the phenolic and volatile compound content of the oil using this type of machine due to the lack of an hermetic seal

(Amirante et al., 2006). In fact the inox grill allowed the loss of volatile compounds into the air above the tank. At the same time, stirring the olive paste whilst in contact with the air caused oxidation of the phenolic content and thus their loss to the oil phase (Amirante et al., 2006).

The malaxing phase has been the subject of much research, which has stimulated the search for new technologies (Angerosa et al., 2001; Di Giovacchino et al., 2002; Amirante et al., 2005). In recent years improvements in malaxer machine technology have included new models with inert gas processing (nitrogen or argon) and oxygen concentration control using a hermetic cover cap. From an analytical point of view, processing in an atmosphere of inert gas has lessened the amount of peroxide, drastically reducing oxidation phenomena during the malaxing phase of the extraction process. Continuous nitrogen flow during malaxing reduces the oxidation of phenolic compounds, increasing oxidative stability of the product (Figure 9.1) (Amirante et al., 2006).

Studies have shown that extending the malaxation time improves yields but can damage the final quality of the product, causing dispersion of phenols into waste water and increasing degradative and oxidative phenomena (Amirante et al., 2002; Di Giovacchino et al., 2002). A few years ago, a heat exchanger was introduced between the crushing machine and the malaxing machine, bringing the olive paste instantaneously to the malaxation temperature and therefore reducing the malaxation time needed to warm the olive paste. This improved the phenol and volatile compound content of the product (Amirante et al., 2005). Recently, an innovative mixer for the malaxation process has been proposed. This new machine has been developed to improve the kneading and heating process of the olive paste, increasing the heat transfer surface in order to reduce the mixing time. The mixer was designed to guarantee perfect control of the atmosphere in contact with the paste. In order to improve the heating surface per volume with respect to standard cradle scutching machines, this innovative mixer has been designed with a circular and spiral-shaped interspace that covers the whole internal longitudinal surface of the tank. This means that paste can be conveyed and maintained at the desired temperature more quickly and effectively (Figure 9.2).

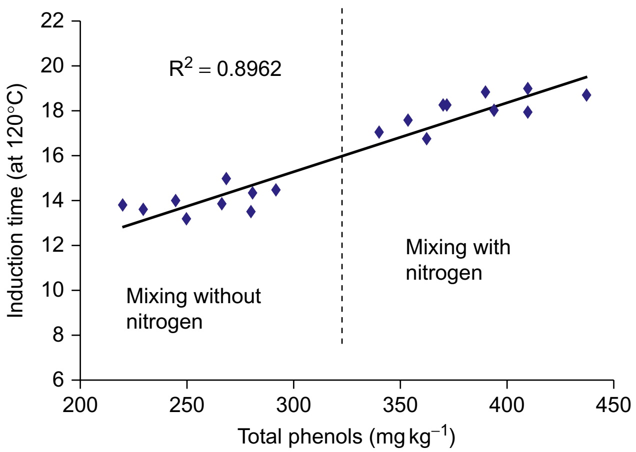

FIGURE 9.1 Correlation between total phenol content and induction time. The data show mean values (m) and standard deviations (sd). Phenolic compounds obtained from the oils extracted with a malaxer utilizing a continuous flow of nitrogen. Dates were correlated with the oxidative stability. Oils with a higher phenol content show reduced oxidation. Each point on the graph represents the mean of five values. Adapted with permission from: Amirante et al. (2006). 'New mixer equipped with control atmosphere system: influence of malaxation on the shelf-life of extra virgin olive oil', in Italian Journal of Food Science. Special Issue, 215–220.

The new malaxer is equipped with a more efficient reel designed to provide bidirectional thrust to paste, causing it to continuously rotate and bring new sections of paste into contact with the heating walls. Initial studies carried out on this machine have highlighted its importance when used in continuous processing regarding the health and organoleptic properties of the resulting olive oil. The new mixer allows better control of processing parameters such as temperature, time, and the atmosphere in contact with the olive paste, facilitating a combination that enhances the quality of the olive oil.

9.4 MIXING CONDITIONS AND THEIR EFFECT ON THE OLIVE OIL QUALITY

Malaxation temperature and time are processing-related parameters that can be controlled to change the sensory properties of the oil and increase its phenol content. Increasing the time and temperature of the malaxing process decreases the concentration of secoiridoid aglycons and phenolic alcohols in olive pastes. These changes are attributed to the oxidative reactions catalyzed by endogenous oxidoreductases such as polyphenoloxidase and peroxidise as well as of the distribution of hydrophilic phenols between the oil and the water phase (Table 9.1) (Angerosa et al., 2001).

Malaxation conditions play a crucial role in the formation and degradation of phenolic compounds and also influence the volatile compound levels. Malaxing olive paste at 30°C for at least 45 min (Ranalli et al., 2003) produces both pleasant 'green' extra virgin olive oil and acceptable oil extraction yields, but malaxing at 35°C introduces several defects into the oil without increasing oil yield (Ranalli et al., 2001). Volatile compounds were the most common discriminating variables for malaxation time and temperature. The increase of alcohols and of C6, C5 and carbonyl compounds, especially of hexanal, contribute significantly to the olive oil flavor resulting from the longer malaxation time, whereas higher malaxation temperatures lead to a reduction of C6 esters and C5 metabolites and an accumulation of hexan-1-ol and trans-2-hexen-1-ol,

FIGURE 9.2 An innovative malaxer in a industrial plant. This new machine has been used in experimental tests carried out during the 2007/08 season in order to study its effect on the healthy and organoleptic properties of olive oil.

TABLE 9.1 Total secoiridoid aglycons in the oils extracted at different malaxation times and temperatures (as ppm resorcin). Changes in secoiridoid compounds with respect to time and temperature changes in paste malaxation. Oils were extracted from the fruits of two Italian cvs (Coratina and Frantoio). Several possible beneficial properties of secoiridoids have been identified. These include enhancing the shelf-life of olive oil and a possible protective effect against thrombotic risk and atherosclerotic damage.

Cultivar	15′		30′		45′		60′		90′	
	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
Coratina	651	357	511	357	470		426	263	363	219
Frantoio	179	105	93	87	78	71	61	30	31	27

Adapted with permission from: Angerosa, F., Mostallino, R., Basti, C., Vito, R., 2001. Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chemistry. 72 (1), 19–28.

both considered by some authors to elicit unpleasant odors (Morales et al., 1999; Angerosa et al., 2001). In addition, high temperatures in the malaxation step activate the amino acid conversion pathway with production of considerable amounts of 2-methyl-butanal and 3-methyl-butanal, but without accumulation of corresponding alcohols correlated with a 'fusty' defect (Angerosa et al., 1998). A malaxing time of between 30 and 45 minutes at the lower temperature is recommended to obtain a good olive oil quality with acceptable yields.

In recent years several studies have investigated how the head-space composition changed during the course of malaxation, investigating the change in phenol and volatile compound content. Selective control of some endogenous oxidoreductases during the malaxing phase seems to be key to obtaining well-balanced olive oil. In this ambit previous studies tried using inert gas to remove oxygen in the headspace of the malaxer (Amirante et al., 2006, 2008a). They showed that using N_2 during malaxation reduced the oxidative degradation of phenolic antioxidants with little alteration to the volatile composition of oil. At the same time the O_2 level during the malaxation phase was monitored using an innovative malaxer with complete control of the mixing conditions such as time and temperature. Initially there was a rapid decrease in concentration of O_2 followed by a gradual reduction in its consumption rate. The O_2 concentration fell to below 5% after roughly 20 minutes, then gradually fell to 0. The overall effect is illustrated by the trend line in Figure 9.3.

Initially the O_2 concentration decreased by roughly $2\,ml\,L^{-1}\,min^{-1}$, probably caused by the oxidative activity of enzymes such as lipoxygenase, polyphenoloxidase and peroxidase. Some of this oxygen might be being consumed by cellular respiration resulting from the breakdown of

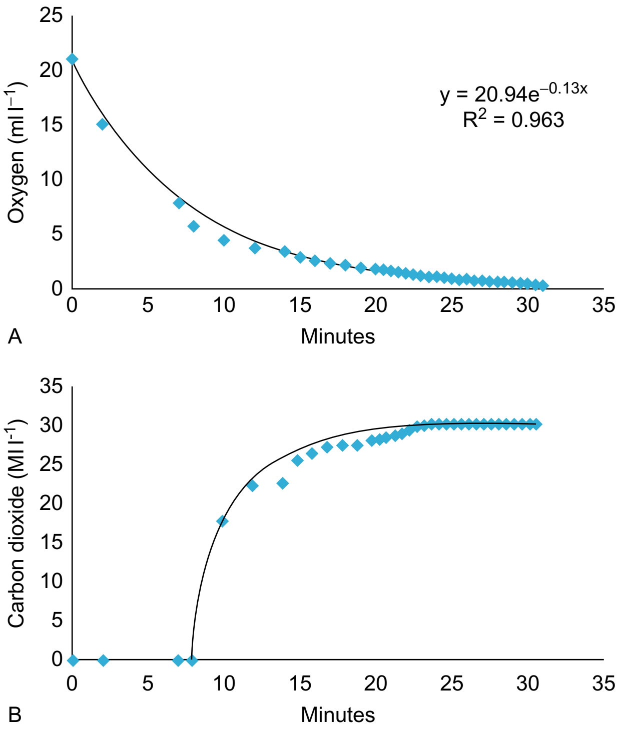

FIGURE 9.3 (A) Oxygen depletion during olive paste malaxation in the innovative malaxer. (B) Carbon dioxide emission during olive paste malaxation in the innovative malaxer. O_2 consumption and CO_2 emission measured during a set of trials. Each point represents the mean of five measurements. The O_2 represents concentration changes ($ml\,L^{-1}$) in the head-space of the malaxing machines. In these trials, an initial rapid decrease in concentration of O_2 was observed followed by a gradual decrease in consumption rate. The Innovative malaxer was used. Adapted with permission from Amirante et al. (2008a). 'Influence of three different atmosphere composition of head space of mixer on total phenol content of de-stoned virgin olive oil', Proceedings book of the International Symposium on Food and Bioprocess Technology. Brazil, 31 August to 4 September 2008. ISSN 1982-3797. CD printed.

cellular structures by crushing and successive malaxation. The CO_2 levels emitted by paste malaxation are demonstrated in Figure 9.2 as changes in the head-space of the sealed chamber. The natural increase of CO_2 concentration coupled with the decrease of O_2 in the head-space of the hermetic malaxer might feasibly improve the quality of resulting oil. When air was used in the head-space of the mixer, the oils extracted contained much higher amounts of pleasant volatile compounds than the oils obtained using the nitrogen (Table 9.2). In fact, oxidative phenomena that take place during transformation result in the loss of compounds such as polyphenols, tocopherols and chlorophylls, which are important in both human health and conservation of the olive oil itself. In this experiment oxygen and carbon dioxide were measured in a full-scale plant. In a hermetic industrial mixer, oxygen was absent after 20 minutes of malaxation in the head-space. The atmosphere was saturated by carbon dioxide, facilitating a reduction in oxidative phenomena without using inert gases. This considerably reduces the cost of the extraction process. In order to obtain oil rich in the pleasant volatile compounds responsible for its fruity flavor, partial oxidation of fatty acid chains is necessary. These reactions largely take place in the initial stage of malaxation, where the lipoxygenase pathway generates volatile compounds that help make up the oil's aroma. The presence of a small concentration of oxygen is important, especially in the initial part of malaxation, to develop the typical aromas appreciated in the best extra virgin olive oil. Amongst the most important olive oil volatiles that arise from the lipoxygenase pathway include C_5 and C_6 compounds. The sensory qualities of unsaturated C_6 aldehydes and alcohols are related to the so-called 'green odor' of this product. The presence of these compounds

TABLE 9.2 The amount of C_6 and C_5 compounds, derived from the lipoxygenase pathway, found in virgin olive oil generated by the two different paste malaxation conditions being studied. The effect of the two different paste malaxation conditions on volatile compounds. When air was used in the head-space of the mixer, the oils extracted contained much higher amounts of 'pleasant' volatile compounds than when nitrogen was used.

Volatile compounds ($mg\,kg^{-1}$)	100% air		100% nitrogen	
	m	SD	m	SD
Hexanal	11.6	0.5	3.1	0.1
trans-2-Hexenal	147.3	5.8	43.5	2.1
1-Hexanol	45.2	3.1	3.9	0.7
trans-2-Hexen-1-ol	11.9	0.3	4.6	0.9
cis-3-Hexen-1-ol	7.7	0.4	1.4	0.1
cis-2-Penten-1-ol	3.6	0.1	nd	Nd

Data represent mean (m) and standard deviation (SD) values. Significant differences in the same row are statistically significant ($p < 0.01$).
Adapted with permission from: Amirante, P., Clodoveo, M. L., Leone, A., Tamborrino, A. 2008a. 'Influence of three different atmosphere composition of head space of mixer on total phenol content of de-stoned virgin olive oil'. Proceedings book of the International Symposium on Food and Bioprocess Technology. Brazil, 31 August to 4 September 2008. ISSN 1982-3797. CD printed.

in good-quality virgin olive oils is critical to provide the typical fresh 'green notes' that they are known for.

The natural increase of CO_2 concentration, coupled to the decrease of O_2, in the head-space of the innovative hermetic malaxer generates an inert malaxer head-space, potentiating its beneficial effect.

SUMMARY POINTS

- Malaxing is an extremely important phase in olive oil extraction in which the olive paste is subjected to a slow, continuous kneading to disperse the emulsions formed during the crushing process and to facilitate adequate coalescence.
- In recent years this operation has become one of the most critical points in the mechanical extraction process for olive oil. Many studies have been carried out to investigate this crucial phase and its influence on olive oil quality.
- Mixing conditions such as time, temperature and the composition of the atmosphere in contact with the olive paste can influence the activity of the enzymes that are responsible for the healthy and organoleptic properties of the product.
- A low malaxing temperature and a process time between 30 and 45 minutes are recommended to obtain good olive oil quality without compromising the yield.
- Much research has been carried out to optimize the malaxing phase. This in turn has stimulated the development of new technologies.
- In recent years new models of the malaxer machine have evolved with improvements that include the use of inert gases, optimized kneading and heating processes and optimized control of the atmosphere that is in contact with the paste.
- Using N_2 during malaxation reduces the oxidative degradation of phenolic antioxidants with little modification to the volatile composition of olive oil.
- The natural increase of CO_2 concentration coupled with the decrease of O_2 concentration in the head-space of the innovative hermetic malaxer generates an inert head-space of the malaxer potentiating its benefit effect.
- The strategic manipulation of oxygen concentration during the malaxing phase facilitates the generation of high phenol contents. This leads to well-balanced sensory characteristics in the resulting olive oil.

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