STABILITY OF THE SENSORY QUALITY OF VIRGIN OLIVE OIL DURING STORAGE
AN OVERVIEW

Key words: minor compounds, oil storage, phenols, sensory stability, virgin olive oil, volatiles

INTRODUCTION

The assurance of the stability and quality of food products is a matter of great concern for producers and sellers. In the case of fats and oils, oxidation (one of the most fundamental reactions in lipid chemistry) is the main cause of quality deterioration and its reaction rate determines the shelf life of this type of food product. To maintain the phenol and volatile molecule content responsible for the highly appreciated organoleptic and nutritional properties in newly produced virgin olive oil during storage, it is absolutely essential to control all the factors that promote lipid oxidation.

The high oxidative stability of virgin olive oil with respect to other vegetable oils is mainly due to its fatty acid composition, in particular, to the high monounsaturated-to-polyunsaturated ratio, and to the presence of minor compounds that play a major role in preventing oxidation. In spite of its high stability, virgin olive oil is also susceptible to oxidative processes, such as enzymatic oxidation, which occurs when the oil is in the fruit and during the extraction process, photo-oxidation, when the oil is exposed to light, and autooxidation which mainly occurs during processing and storage when the oil is in contact with oxygen (Frankel, 1985). This review is mainly concerned with the autooxidation process, the main cause of deterioration of virgin olive oil during its shelf life. Lipid oxidation occurs through the interaction of the triacylglycerol fatty acids with molecular oxygen which yields hydroperoxides by a free radical mechanism. The activation energy of this reaction is high and the initiation of lipid oxidation requires traces of transition metals or exposure to light; the reaction is accelerat-
ed by an increase in temperature. These factors can catalyse the decomposition of hydroperoxides, the primary oxidation products. The unstable hydroperoxides decompose to produce a range of volatile and non-volatile products. Some volatile components, mainly aldehydes, are the major cause of the sensory perception of the rancid defect in vegetable oils (Angerosa, 2000). During the autoxidation reaction, a series of compounds are formed in virgin olive oil (VOO), while minor components are degraded, causing rancidity and off-flavours, loss of nutritional value and finally consumer rejection. Some major factors influencing lipid oxidation are: the amount of oxygen dissolved in the oil that cannot be removed, the oxygen permeability of the packaging materials, the storage temperature, exposure to light and the fatty acid composition. Lipid oxidation is accelerated by the presence of free fatty acids, mono- and diacylglycerols, thermally-oxidized compounds and metals such as iron. In contrast, phenolic compounds and carotenoids decrease autoxidation in oil, while tocopherols, chlorophylls and phospholipids demonstrate both antioxidant and pro-oxidant activity depending on the oil system and storage conditions (Choe and Min, 2006).

### Table 1

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<td>- Opacity to light of packaging material</td>
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### The Importance of Controlling the Pro-Oxidizing Factors

Key facts regarding the major external factors and olive oil constituents that influence lipid oxidation are shown in Table 1.

### Physical factors

As mentioned above, several physical factors play a key role in controlling oxidation in virgin olive oil during storage. All of these must be carefully monitored to prevent alterations in the oil and to extend the shelf life.

### Oxygen availability

The great effect that oxygen availability has on the oxidation reaction rate is directly related to its partial pressure. The level of oxygen in the oil depends on the conditions used in some technological operations such as
centrifugation, and/or decanting and filtration. In the case of bottled or tank-stored olive oil, in which the surface-volume ratio in contact with the atmosphere is relatively small, the diffusion of oxygen into the bulk oil is a limiting parameter, and therefore the oxidation rate is controlled by diffusion (Yanishlieva, 2001). The head-space in the container and the oxygen permeability of the packaging material are two variables to be considered since they play a major role in oil stability during storage (Gutiérrez et al., 1988).

The basic factors that affect the shelf life of olive oil in different packaging systems and the main oxidative degradation mechanisms for them have been reported. Published results on plastic packaging material permeability show the following ranking of oil sample stability: PVC (polyvinyl chloride) ≥ PET (polyethylene-terephthalate) > PP (polypropylene) ≥ PS (polystyrene) (Tawfik and Huyghebaert, 1999). Moreover, the differences in the shelf life observed in oils bottled in PET or in glass are attributable to differences in the initial dissolved oxygen content in the oils (Sacchi et al., 2008). While the decay kinetics of bottled virgin olive oil depend on the shape and size of the bottle, it mainly depends on the material used to make the bottle, and on the initial value of the oxygen partial pressure in the headspace (Del Nobile et al., 2003). The shelf life of packaged olive oil under various storage conditions can be predicted by applying mathematical modeling and simulations (Kanavouras and Couteliers, 2006a; Kanavouras et al., 2006b). The feasibility of improving the stability of extra virgin olive oil by using inert gases, mainly nitrogen or argon in the head-space of the container, as a conditioner gas during storage to reduce dissolved oxygen, has been studied. The use of nitrogen as conditioner gas helped to avoid the risk of oxidation during storage (Di Giovacchino et al., 2002).

Storage temperature
It is well known that storage temperature is one of the most relevant factors affecting lipid oxidation. A kinetic study of the autoxidation reaction in olive oil triacylglycerols stored in darkness at different temperatures (25°, 40°, 50°, 60° and 75°C), in the absence of pro- and antioxidant compounds to avoid confounding effects, confirmed that the reaction constant increases exponentially with temperature (Gómez-Alonso et al., 2004). The effect of temperature on the oxidation rate is quite complex; it increases the oxidation rate improving the formation rate of hydroperoxides, while it decreases the oxygen solubility in the bulk oil and increases the decomposition of hydroperoxides, changing the profile of the products formed (Ragnarsson and Labuza, 1977; Frankel, 1998; Velasco and Dobarganes, 2002). Therefore, it is very relevant to establish the relationship between storage temperature and VOO oxidation rate. The temperature-dependent kinetics of the oxidation indices and the unsaturated fatty acids are described well by the linear Arrhenius equation between 25° and 60°C (0.960 ≤ R² ≤ 0.999, p≤0.05). The time required to reach the upper limits for PV, K232 and K270 established for the extra virgin olive oil category in the current EU legislation, correlated well with temperature using an exponential equation (Mancebo-Campos et al., 2008).

Exposure to light
Luminous radiation is another external factor to be considered in the lipid oxidation process during storage, since it initiates auto-oxidation and produces photo-oxidation. To observe these effects, the oil must contain photosensitizers, like chlorophylls, that are excited by light absorption. Prevention of light exposure during storage of virgin olive oil is absolutely necessary to extend its shelf life (Jadhav et al., 1996); oils exposed to light are less stable than those kept in the dark (Caponio et al., 2005). However, VOO is usually protected from exposure to light radiation from the time of its production until it is exposed as bottled oil on the supermarket shelves. From that time onwards the opacity to light of the packaging material is of fundamental importance for its preservation (Gutiérrez et al., 1988; Méndez and Falqué, 2007). It has been observed that even small doses of UV radiation can induce oxidation in virgin olive oil (Luna et al., 2006).

Chemical factors
Apart from the external physical factors described above, the susceptibility of fats and oils to oxidation is influenced by their chemical composition, both major and minor constituents, including the oxidation products formed during the oxidation reaction itself, all of which can possess pro- or antioxidant activities. The well known high oxidative
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stability of VOO is related not only to the high monounsaturated/polyunsaturated fatty acid ratio of its lipid matrix, but also to the presence of minor components that have great antioxidant activity, particularly the phenolic compounds.

Triacylglycerols (TAG)
The susceptibility of lipids to oxidation increases as the unsaturation level of its fatty acids increases. As shown in a previous study (Holman and Elmer, 1947), linoleate is 40 times more reactive than oleate, whereas linolenate is 2.4 times more reactive than linoleate. The stability of the triacylglycerol matrix of olive oil has been demonstrated in experiments conducted in the presence of different antioxidants and compared to a mixture of fatty acid methyl esters (FAME) with the same composition (Marinova and Yanishlieva, 1996). The position of the fatty acid within the triacylglycerol moiety also affects its susceptibility to oxidation (Miyashita et al., 1990; Mateos, 2002); they are slightly less stable to oxidation when linoleate is in the 1,2- rather than the 1,3-triacylglycerol position. It has also been reported that the presence of mono- and diacylglycerols and of oxidized triacylglycerols has only a low pro-oxidant effect (Mistry and Min, 1988).

Free fatty acids (FFA)
Free fatty acids have a pro-oxidant effect when they are added to a purified lipid substrate (Miyashita and Takagi, 1986; Mistry and Min, 1987). Therefore, it is very important to have low levels of free fatty acids and hydroperoxides in newly produced oil as they may accelerate the oxidation of fats. The pro-oxidant action of free fatty acids seems to be exercised by a carboxylic group, which speeds up the decomposition rate of hydroperoxides (Miyashita and Takagi, 1986; Kiritsakis, 1992; Frega et al., 1999). In a recent work Scarpellini et al. (2005) confirmed the pro-oxidant effect shown by free fatty acids (FFA) added to refined peanut oil, as previously reported by other authors using different vegetable oils (Catalano and DE Felice, 1970; Miyashita and Takagi, 1986; Frega et al., 1999). In fact, the oxidative stability values of a neutralized oil decreased in proportion to increasing percentages of oleic acid. They already observed a substantial reduction of stability of oil samples at concentrations lower than 0.5% oleic acid.

Traces of metals
Transition metals, mainly iron and copper, can catalyze the decomposition of hydroperoxides according to their oxidation-reduction potential to yield lipid peroxyl and alkoxyl radicals that initiate free radical chain oxidation (Benjelloun et al. 1991; Angerosa and Di Giacinto, 1993). Angerosa and Di Giacinto (1993) studied the catalytic effect of Mn and Ni on the oxidation of VOO. They measured the peroxide value (PV) and the $K_{232}$ as indices of primary oxidation compounds and E-2-pentenal and E-2-heptenal as markers of secondary oxidation products and showed a significant increase in the oxidation of VOO in the presence of metals. In the experiments carried out by Polvillo et al. (1994), contamination with iron was detected in olive oil that had been in contact with carbon steel. The stability of VOO measured through the peroxide, $K_{270}$ and $p$-anisidine values was less after storage for one month in contact with a carbon steel sheet than when stored in the absence of the metal. Bendini et al. (2006) measured the primary and secondary oxidation products in VOOs stored in the presence and absence of copper; drastic increases of these products were observed in samples with traces of metal. These results clearly demonstrate the ability of copper to promote autoxidation.

Phenols
The direct relationship between the content in phenolic compounds in VOO and its oxidative stability has been well known for a long time (Vázquez-Roncero et al., 1973; Gutiérrez et al., 1977; Gutfinger, 1981; Montedoro et al., 1992; Baldioli et al., 1996; Litridou et al., 1997; Salvador et al., 1999). In the case of hydroxytyrosol and its secoiridoid derivatives, which show a similar antioxidant activity (Baldioli et al., 1996; Mateos et al., 2002; Carrasco-Pancorbo et al., 2005), the formation of a stable radical has been proposed to explain their antioxidant activity (Visioli and Galli, 1998). In contrast, tyrosol and its derivatives show a very low or non antioxidant activity (Baldioli et al., 1996; Mateos et al., 2002; Carrasco-Pancorbo et al., 2005), due to the absence of an electron donor group that has a high transition energy and a limitation in the formation of the phenoxy radical. The presence of phenolic compounds in VOO is therefore extremely important, because they combat li-
rapid oxidation in its initial stages. However, they cannot block the autocatalytic mechanism. This effect is clearly visible from the data described by Bendini et al. (2006) for an accelerated oxidation test (60°C) on virgin olive oils which differed only with respect to phenol contents. During the six weeks of testing, the peroxide and oxidized fatty acid (OFA) values increased in all the oils. There was a contemporary decrease of oxidative stability (OSI time), but with different rates that were proportional to the initial content in phenolic antioxidants. This effect was amplified when copper was added as a catalyst of lipid oxidation. Mancebo-Campos et al. (2007) studied the oxidation process of several virgin olive oils with different contents of natural antioxidants under accelerated oxidation conditions (Rancimat) and compared to long-term storage at room temperature. All the samples reached the peroxide number limit (20 meq O₂·kg⁻¹) but in very different times (28-56 hours vs 96-167 weeks) depending on the temperature (100°C for the Rancimat test and 25°C for the prolonged storage) and the initial content in antioxidants.

Tocopherols
Although α-tocopherol is considered to be the most relevant antioxidant in vegetable oils, as well as in the protection of the lipid structures in vivo, several researchers have reported a lower antioxidant activity than hydroxytyrosol (Le Tutour and Guedon, 1992; Baldioli et al., 1996; Mateos, 2002). This may be explained by the “antioxidant polarity paradox” which states that hydrophilic antioxidants are often less effective in oil-in-water emulsions than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants (Porter et al., 1989; Frankel et al., 1994). Moreover, it has also been reported that in the presence of α-diphenols, α-tocopherol gives rise to a synergic effect (Servili et al., 1996).

Pigments
Carotenoids, especially β-carotene are efficient VOO protectors against photo-oxidation, since they are capable of deactivating the oxygen singlet giving back its triplet status (Cuppett et al., 1997; Wagner and Elmaţda, 1999). On the other hand, the capacity of the chlorophyll molecule to absorb light energy and transfer it to chemical substances, makes it very active in lipid photo-oxidation in VOO (Rahman and Saari-Csallany, 1998). Chlorophylls may also act as low antioxidants during oxidation in the dark – absence of light – probably due to its capacity to donate hydrogen (Endo et al., 1984; Gutiérrez et al., 1992; Psomiadou and Tsimidou, 2002).

Filtered vs. unfiltered VOO
The results of some studies have hown a gradual loss in stability during the storage of filtered oils mainly due to a significant decrease in the phenolic components. When unfiltered and the corresponding filtered virgin olive oils were stored for nine months at ambient temperature in the dark, a loss in oxidative stability in the latter was observed due to a lower total phenolic content (Tsimidou et al., 2005). Other researchers (Gómez-Caravaca et al., 2007) have reported that eight virgin olive oils after filtration through cotton in the laboratory showed a significant loss of hydroxytyrosol, a simple phenol. Endowed with high antioxidant activity. Consequently there was lower oxidative stability of the filtered oils than of the unfiltered ones. Generally, the formation of simple phenols, such as hydroxytyrosol and tyrosol, was greater in unfiltered olive oils due to the hydrolysis rate of their secoiridoid derivatives. These reactions appear linked to the presence of a higher content of dispersed water droplets that maintain a partial enzymatic activity. On the other hand, it is well known that the filtration step which removes the organic sediments, prevents anaerobic fermentation which produces unpleasant volatile components responsible for the muddy defect. At the same time it has also been reported that filtration and dehydration decrease the hydrolysis rate of the triacylglycerol matrix, especially during storage at the higher temperature (40°C) and in oils with a higher initial free acidity (e.g. free acidity > 0.6%). Moreover, the formation rate of simple phenols due to hydrolysis of their secoiridoid derivatives was also greater in unfiltered olive oils. Thus, from this point of view, filtration and especially dehydration could help prolong the shelf life of some high-quality but less stable virgin olive oils, (e.g. Arbequina and Colombaia varieties) (Fregapane et al., 2006).

Degradation of minor constituents in VOO
Another consequence of the
autoxidation reaction is the degradation of the naturally-occurring minor components in VOO (Gómez-Alonso et al., 2007). The rates of degradation of α-tocopherol, squalene and phenolics in olive oil under different storage conditions have been reported. The main changes in the concentrations of these compounds are associated with the higher oxygen level in the empty portion of the glass bottles. α-tocopherol is the first molecule to be oxidized, whereas squalene and α-diphenols are protected in the first months due to the presence of α-tocopherol and their content decreases significantly only after 6 and 8 months, respectively (Rastrelli et al., 2002). The secoiridoid aglycones, namely, the oleuropein and ligstroside derivatives, and α-tocopherol decreased following pseudo-first-order kinetics during 8 months of storage in closed bottles in the dark, at 40 and 25°C. In all VOOs, the oleuropein derivatives were consumed faster than the corresponding ligstroside derivatives and α-tocopherol (Lavelli et al., 2006). Moreover, the α-tocopherol content decreased slightly and apparently linearly during its shelf life, although there may be a lag phase at the beginning of storage (Gómez-Alonso et al., 2007). Recently, there has been an increased interest in oxidized minor compounds (e.g. phenols, sterols, pigments), especially in relation to determining the freshness/aging status of VOO. The natural antioxidants in VOO are important not only for their in vitro protection against rancidity, and therefore the shelf life of the product, but also for their in vivo biological activity which enhances the nutritional value of this oil. Moreover, some individual phenolic compounds play an important role in positive sensory attributes, like bitterness, and hence in consumer preference.

**EFFECTS OF OIL STORAGE ON VOLATILE COMPOUNDS: IMPACT ON AROMA**

Oxidation, an inevitable process that may start after the harvesting of the olives but certainly during the extraction of the virgin olive oil leads to a progressive deterioration of the product that becomes more serious during oil storage. Initially, lipids are oxidised to hydroperoxides, which are odourless and tasteless (Frankel, 1982) and do not account for sensory changes. However, they are susceptible to further oxidation or decomposition into products of secondary reactions, which, are responsible for the typical unpleasant sensory characteristics, identified on the whole as a rancid defect. This disagreeable sensory note is especially perceptible in oils that are strongly oxidized due to incorrect or excessively long storage. Decomposition occurs through a homolytic cleavage of the hydroperoxide group which produces various compounds, including aldehydes, ketones, acids, alcohols, hydrocarbons, lactones, furans and esters (Frankel, 1985). The C₆ and C₅ compounds are enzymatically produced from polyunsaturated fatty acids through the so-called lipoxygenase (LOX) pathway. Quantitatively, linear C₆ unsaturated and saturated aldehydes are the most important fraction of volatile compounds in high quality virgin olive oils (Angerosa et al., 2004). The qualitative profiles of these volatile compounds depend on the level and activity of each enzyme involved in this LOX pathway (Aparicio and Morales, 1998; Angerosa et al., 2001). The main contributors to virgin olive oil aroma are not necessarily the volatile compounds present in the highest concentrations. In fact, several factors, such as volatility, hydrophobicity, conformational structure of the molecules and type and position of functional groups may affect the odour intensity more than the concentration due to their capacity to establish bonds with olfactory receptor proteins. Therefore, the contribution of each volatile compound to the whole aroma and flavour is related to their concentration in the oil with respect to their sensory threshold (Morales et al., 1997).

The concentration and odour threshold of the volatile compounds are indeed crucial to virgin olive oil quality. Several researchers (Solinas et al., 1987; Angerosa, 2000; 2002) have reported that during oxidation the drastic reduction of the C₆ aldehydes, alcohols and esters from the LOX pathway and the increase of many saturated and unsaturated aldehydes (C₅-C₁₁) from chemical oxidation, including hexanal, reduces the perception of the positive attributes and pleasant sensory notes leading to the kind of off-flavour in virgin olive oil recognized as a rancid defect by assessors. In a recent
paper, Cerretani et al. (2008) discussed the results relative to correlations between the chemical and sensory analysis. Assessors belonging to four panels (two Italian and two Spanish) analyzed 16 monocultivar VOOs produced in Italy and Spain. They assessed pleasant flavours related to different perception routes (orthonasal, retronasal and gustative) such as green and ripe notes from olive and other fruits as well as negative attributes. The four panels were in agreement on the presence of a slight rancid defect in only two samples characterized by a higher content of saturated aldehydes such as heptanal, nonanal and decanal. The most advanced oxidation stages were characterised by the complete disappearance of compounds arising from the LOX cascade and by very high concentrations of the above-mentioned aldehydes. They contribute mainly to the undesirable defect perceptions due to their low odour thresholds (Guth and Grosch, 1990). Other contributors are the unsaturated hydrocarbons, furans and ketones. It should be noted that although the E-2-hexenal content, which gives the typical “green note” to extra virgin olive oil, is by far the major C₆ aldehyde compound in all fresh oils, hexanal seems to contribute more to the green odour than E-2-hexenal because of its lower odour threshold (75-300 vs. 420-1,125 µg kg⁻¹) (Reiners and Grosch, 1998; Angerosa, 2002; Aparicio and Luna, 2002; Morales et al., 2005; Kalua et al., 2006). In addition to fruity, the “green” sensation reminiscent of freshly cut grass, leaf, tomato, artichoke, walnut husk, apple or other fruits generally contribute to the aroma of high quality oil. “Green” notes include Z-3-hexenal, and Z-3-hexenyl acetate whereas alcohols such as E-2-hexen-1-ol, Z-3-hexen-1-ol and hexan-1-ol have less sensory significance than aldehydes due to their higher odour threshold values. Their sensory descriptions are associated with ripe fruity, soft green and aromatic sensory notes (Luna et al., 2006; Bendini et al., 2007). Esters are compounds associated with fruity nuances (Aparicio and Luna, 2002; Luna et al., 2006). Hexyl acetate and Z-3-hexenyl acetate, are present in the aroma of all fresh virgin olive oils but are minor components compared with aldehydes or alcohols. Different authors (Reiners and Grosch, 1998; Aparicio and Luna, 2002) indicate that Z-3-hexenyl acetate is linked to the pleasant green and banana notes (odour threshold 200-750 µg kg⁻¹). Among the C₅ compounds, 1-penten-3-one has been mostly associated with fruity, sweet and pleasant attributes such as tomato and strawberry (Angerosa, 2000; Aparicio and Luna, 2002; Luna et al., 2006; Morales et al., 1995). It has a very low odour threshold (0.7-50 µg kg⁻¹) so its contribution to the whole aroma can be considered important. Solinas et al. (1987) found that the concentrations of the aldehydes E-2-pentenal, hexanal and E-2-heptenal increase considerably in the oxidised oils. The authors suggested using E-2-heptenal as a marker for oxidation rather than E-2-pentenal and hexanal, since these two compounds are already present in the aroma of extra virgin olive oils. Morales et al. (1997) monitored the oxidation stages of a virgin olive oil during an accelerated thermoxidation process by determining the concentration of nonanal. The hexanal/nonanal ratio was found to be an appropriate way to detect the beginning of oxidation and follow its evolution, even if the hexanal is present in the original flavour (Morales et al. 1997). In an interesting work Vichi et al. (2003) investigated the oxidative changes in virgin olive oil headspace by using SPME/GC-MS technique to select some compounds as possible markers of the oxidation process. Samples, analyzed weekly, were oxidized at 60°C for 4 months. The peaks corresponding to nonanal and hexanal increased most rapidly during oxidation. This demonstrated that whereas the amount of hexanal is due to both autoxidation and the lipoxygenase cascade (through the formation of 13-LOOH), the concentration of nonanal may be solely attributed to the autoxidation of oleic acid. 2-Alkenals, such as E-2-pentenal, E-2-heptenal and E-2-decenal were also formed. These unsaturated aldehydes originate from secondary reactions of the primary autoxidation products (13-LnOOH, 9-LOOH and 9-OOOH, respectively). Moreover, the concentrations of 2,4-heptadienal isomers and E,E-2,4-decadial (from 12-LnOOH and 9-LOOH decomposition, respectively) were positively correlated with the time of oxidation. Among the alkanes, octane (formed from 10-OOOH) increased the fastest. The hexanoic acid content also increased and was due to the secondary decomposition of hexanal and 2,4-deca-
During the oxidation process, 2-pentylfuran and 2-ethylfuran are formed due to the degradation reactions of linoleate and linolenate hydroperoxides, respectively. These substituted furan compounds, in particular 2-pentylfuran, increased rapidly from traces to considerable amounts during oxidation. Vichi et al. (2003) proposed using them as markers for distinguishing oils in the late stages of oxidation. Kanavouras et al. (2004) carried out a storage study based on three major contributors to the oxidative degradation in packaged olive oil: temperature, availability of light, and presence of oxygen. They selected a group of volatiles such as hexanal, 2-pentylfuran, E-2-heptenal, nonanal, and E-2-decenal, highly correlated with oxidation in packaged extra virgin olive oil under various storage conditions for one year (glass/PET/PVC bottles; 15°C/30°C/40°C temperature; light or dark conditions).

Kalua et al. (2006) carried out a real-time shelf life study for one year; virgin olive oil samples were stored under different conditions: in the light at ambient temperature, in the dark at ambient temperature, at low temperature in the dark, with or without headspace. All volatile compounds found in fresh oil decreased during storage in the light in the presence of oxygen, particularly E-2-hexenal. Under the non-accelerated conditions used in this study, neither nonanal nor 2-pentenal or 2-heptenal were identified as oxidation markers. Octane was the marker for storage in the light, whereas hexanal discriminated virgin olive oil stored in the light in the presence of oxygen. Only pentanal discriminated low-temperature storage. The major difference between oils stored with or without headspace was the appearance of longer chain volatile compounds, such as octanal and E-2-nonanal.

Luna et al. (2006) evaluated the effect of ultraviolet radiation on the production of off-flavours in bottled virgin olive oil. This study showed that it is possible to predict the value of the rancid attribute of a sample submitted to UV radiation based on its nonanal concentration. The intensity of positive attributes (green, fruity, bitter, and pungent) decreased along the process in both varieties under study (Arbequina and Picual), while the intensity of the rancid attribute increased greatly. During irradiation treatment with UV lamp for 12 days, E-2-hexenal was the volatile that decreased the most. On the other hand, 2-butenal, and 2-pentenal formed from hydroperoxides of linoleic acid and octane, nonanal, and 2-decanal formed from hydroperoxides of oleic acid were the compounds that showed the greatest variation.

The major compounds indicated by different researchers as markers of virgin olive oil oxidation, their sensory characteristics and odour thresholds are reported in Table 2.

In conclusion, E-2-heptenal, nonanal and 2-decenal are the most frequently used volatile markers of oxidation of virgin olive oil during storage. These three compounds are characterized by low odour threshold (5, 150 and 100 µg kg⁻¹, respectively) and by negative off-flavours namely oxidized, fatty and fish, that strongly contribute to the rancid defect perceived by assessors.

**THE EFFECTS OF OIL STORAGE ON PHENOLIC COMPOUNDS: THE IMPACT ON TASTE**

Virgin olive oil contains minor compounds that are of great sensory and biological importance; the molecules that have a phenolic structure are noted for their antioxidant properties. Among these the tocopherols are related to lipids due to the presence of a hydrophobic side chain in the molecule and the hydrophilic phenols characterised by greater polarity. The former are important for the antiradical and nutritional (vitamin E) properties, whereas the latter greatly influence the taste quality (bitter and pungency attributes) as well as the beneficial biological activity and oxidative stability of virgin olive oil (Servili, 2004; Bendini et al., 2007). Few individuals, except for trained tasters of VOO, know that bitterness and pungency perceived by taste are positive attributes of VOO. These two sensory characteristics are strictly connected by the quasi-quantitative phenolic profile of the product. Some phenols mainly elicit the taste perception of bitterness, while other phenolic molecules stimulate the free endings of the trigeminal nerve located in the palate and in the gustative buds giving rise to the chemesthetic perceptions of pungency and astringency attributes.
The major phenolic compounds identified and quantified in olive oil belong to five different classes: phenolic acids (especially derivatives of benzoic and cinnamic acids), flavons (luteolin and apigenin), lignans ( (+)-pinoresinol and (+)-acetoxyvinoresinol), phenyl-ethyl alcohols (hydroxytyrosol, tyrosol) and secoiridoids (aglycon derivatives of oleuropein and ligstroside) the latter are peculiar to virgin olive oil.

Several authors have associated some phenols with bitterness and have obtained models and determined relationships between individual phenols and bitterness intensity measured by a panel test or calculated from spectrophotometric absorbance at 225 nm known as the bitterness index (Gutiérrez et al., 1989; Gutiérrez-Rosales et al., 2003; Mateos et al., 2004). A clear example of the different sensory properties of secoiridoid derivatives was reported by Andrewes et al. (2003). They assessed the relationship between phenols and olive oil pungency; the dialdehydic form of ligrostoside aglycon was found to be responsible for the burning sensation present in many olive oils. In contrast, the dialdehydic form of the oleuropein aglycon, tasted at an equivalent concentration, produced very little burning sensation. Another study confirmed that the dialdehydic form of ligrostoside aglycon is the principal agent in VOO responsible for throat irritation. Andrewes et al. (2003) deduced that the pungent intensity could be measured by isolating this molecule from different VOO. Starting from this last work Beauchamp et al. (2005) compared the effect of the synthetic form (named “oleocanthal”, with oleo- for olive, -canth- for sting, and -al for aldehyde) to that of the purified compound from VOO.

Regarding bitterness, after isolation and water solubilization of the major peaks of the phenolic profile of VOO separated by preparative HPLC and purified, Gutiérrez-Rosales et al. (2003) concluded that aldehydic and dialdehydic forms of oleuropein were mainly responsible for the bitter taste of VOO. One year later, Mateos et al. (2004) reported a better correlation between the aldehydic form of oleuropein aglycon and bitterness. Using a trained olive oil sensory panel SI-NESIO et al. (2005) studied the temporal perception of bitterness and pungency utilizing a time-intensity (TI) evaluation technique. They showed that the bitterness curves had a faster rate of rising and declining than the pungency curves. The different kinetic perception is linked to the slower (2-30 m/sec) signal transmission of thermal nociceptors compared to other neurones (up to 100 m/s). During oil storage secoiridoids undergo modifications (decomposition such as hydrolysis and oxidation reactions) that result in their decline and, consequently, to a reduced intensity of the typical bitter taste and pungent note. Esti et al. (2009) recently carried out a study on intensity changes of bitterness and pungency perception over time in seven samples stored up to 18 months under two different temperature conditions (10° and 28°C). The results showed that all of the oleuropein and ligrostoside derivatives considered, except for hydroxytyrosol and tyrosol, were relevant predictors of the static and dynamic analysis for bitterness and pungency. In contrast, the dialdehydic form of the ligrostoside aglycon was only effective for predicting the pungency decrease in relation to storage time and temperature, but it was not correlated to changes in bitterness.

Even in small quantities, phenols in virgin olive oil are fundamental for protecting glycerides from oxidation; in fact, by virtue of their
favourable oxidation potential, they exert an intense protective action by exposing themselves to oxidation in the place of the lipid substrate. Phenolic compounds can inhibit oxidation through a variety of mechanisms based on radical scavenging, hydrogen atom transfer and metal-chelating. As chain breakers, they act by donating a hydrogen radical to alkylperoxyl radicals that are formed during the initiation step of lipid oxidation. The important role that phenolic compounds play particularly the o-diphenol structure, in protecting lipids from autoxidation, also when catalysed by metal such as copper, was evidenced in several works that, in particular, when catalysed by metal such as copper has been reported in several works that, in particular, deal with antioxidant activity of phenols in bulk oil and oil-in-water emulsion.

Paiva-Martins et al. (2006) studied the antioxidant activity and interactions with copper of oleuropein, hydroxytyrosol, monoaldehydic and dialdehydic forms of oleuropein, in virgin olive oil and oil-in-water emulsions stored at 60°C (Bonoli-Carbognin et al., 2008). They concluded that the formation of a copper complex with radical scavenging activity is a key step in the antioxidant action of the olive oil phenolic compounds in an emulsion containing copper ions. On the other hand, Bendini et al. (2006) working with a bulk oil system, reported a more rapid consumption of the tocopherols in samples that had the lowest o-diphenols content and these latter were able to chelate copper.

The storage of virgin olive oils at low temperature may have a positive effect by slowing the kinetics of the oxidative reactions; even if at the same time it decreases the solubility of the phenolic fraction that combats the lipid oxidation. The effects of storage at incorrect temperatures, whether too high or too low, may have significant repercussions on phenolic substances and indirectly on both shelf life and sensory characteristics of virgin olive oils. These excesses in temperature during winter or summer and lead to changes in the quality of the oil stored in tanks without a temperature regulator or in bottles kept in warehouses. Data obtained in a study by Cerretani et al. (2005) and Bonoli et al. (2005), illustrate an important technological consequence related to the storage conditions of virgin olive oils. If the storage temperature is subjected to a marked temperature decreasing (e.g. close to 0°C), the oil will change its physical state due to the crystallization of more saturated triacylglycerols and waxes. This could result in a destabilization of both micro-droplets of water and polar phenolic molecules and a loss of the total availability of the antioxidant compounds which would reduce its natural protection again lipid oxidation. Some interesting changes were found in defrosted oils after the freezing process. The oils showed a significant decrease in the oxidative stability value (by OSI test) that corresponded to the loss in the phenolic fraction. The loss of phenols, particularly the o-diphenols which are known to be the most active compounds for the shelf life of the oil, depends on their structure-activity relationship. A certain fraction of phenols, solubilised into the micro-droplets of water in the oil probably precipitated during the freezing/defrosting processes with a proportionate reduction in its oxidative resistance. Destabilization and precipitation of phenols also cause an unavoidable loss in positive taste attributes of the virgin olive oil.

The main effects observed in the phenolic fraction during oil storage are: hydrolysis of secoiridoids and oxidation of some phenolic molecules. The first mechanism, due to enzymatic or chemical reactions, leads to a breaking of ester bonds into molecules of oleuropein and ligstroside aglycons and to an increase in the amounts of elenolic acid, hydroxytyrosol and tyrosol. Furthermore, the oleuropein and ligstroside aglycons in their monoaldehydeic forms generally lose the carboxymethyl group that causes an increase in concentrations of the respective dialdehydic forms. These trends have been well documented in several studies. Cinquanta et al. (1997) studied the evolution of simple phenols during 18 months of storage in the dark. The tyrosol and hydroxytyrosol contents increased notably due to hydrolysis of their complex derivatives in a first stage, and a rapid loss of hydroxytyrosol compared with that of tyrosol at the end of the storage period, due to a higher antioxidant activity of the former. The partial transformation of secoiridoid derivatives into simple forms such as hydroxytyrosol, tyrosol and elenolic acid, leads to a decrease in bitterness and pungent intensity.

The formation of new compounds due to phenol oxidation has been proposed by some researchers.
According to Armaforte et al. (2007) it may be feasible to use the ratio of fresh phenols/oxidized phenols to determine the freshness/aging ratio of a virgin olive oil. This ratio appeared to decrease rapidly in samples that had an increased content of oxidized phenols. Oxidized phenols are produced by thermic and forced oxidative stress, as in the case of extended conservation (Rovellini and Cortesi, 2002; Ríos et al., 2005; Carrasco-Pancorbo et al., 2007).

Table 3 shows the major compounds reported by different researchers to be the major components responsible for taste perceptions such as bitterness and pungency. These reactions lead to a decrease in bitterness and pungent intensity, positive attributes that are characteristic of a fresh VOO.

From “Italian Journal of Food Science” nr. 4/2009

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