Olive Oil Composition: Volatile Compounds

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1. Introduction

In general olive oil is defined on the basis of its sensory characteristics. European Union (EU) regulations establish the organoleptic quality of virgin olive oil by means of a panel test, evaluating positive and negative descriptors (EU regulations). For the organoleptic assessment, several volatile compounds are considered as the main responsible for negative and positive attributes. Volatile compounds, either major or minor, are crucial to olive oil quality; even when present below their olfactory threshold, they can still be important to understand their formation and degradation pathways and provide useful quality marker information.

Volatile composition of olive oils can be influenced by a number of factors, from agronomic and climatic aspects to technological ones. Cultivar, geographic region, ripeness, harvest and processing methods can affect the volatile composition of olive oil. Storage time is also critical for quality. In order to evaluate the volatile profile of olive oil, sensitive analytical techniques as well as extraction procedures were developed. The big issues on aroma analysis are, the loss of compounds during sample preparation steps, and the knowledge that some of the so-called “compounds of interest” (with higher aroma threshold) are, probably, present only in trace amounts. Due to its nature, olive oil is a difficult matrix; for these reasons several methods have been, so far, proposed. The advantages and drawbacks of these methods will be further discussed. One dimension-Gas Chromatography (1D-GC) analysis was, until recently, the most used method to analyze volatiles in different matrices. The increased development of 2D-GC, allowing higher sensitivity and enhanced separation power, is changing the 1D-GC approach. The type of 2D and/or 3D qualitative and quantitative information, provided by 2D-GC systems, promoted the development of powerful chemometrics tools allowing a useful, and potentially easy, way for data interpretation. Fingerprint comparison can be used on a routine basis, providing important and quick information concerning differences among the olive oils produced and, probably most important, also allowing frauds detection.
This work will be divided in four main parts: 1) a brief summary of the composition and biosynthesis of the volatile fraction of olive oil; 2) the role of volatile compounds in olive oil quality: nutritional and sensorial quality; 3) the effect of agronomic and technological practices on olive oil aroma; 4) analytical methodologies for quantification and identification of volatiles compounds: new analytical methods.

2. Composition and biosynthesis of the volatile fraction of olive oil

The wide variety of volatile compounds found in high quality virgin olive oil are produced through biogenic pathways of the olive fruit, namely the lipoxygenase (LOX) pathways (Hatanaka, 1993), and fatty acid or aminoacid metabolism, as depicted in fig.1 (Angerosa et al., 2004; Angerosa et al., 2002). Besides the contribution of several volatile compounds, related with the mentioned pathways, the role of other compounds, especially aldehydes derived from auto-oxidation processes, should also be considered to the final aroma of the olive oils (Angerosa, 2002). Other metabolized products, originated from possible fermentations, conversion of some aminoacids, enzymatic activities of moulds or oxidative processes, are closely related with off-flavour of virgin olive oil. As illustrated in fig. 1, several compounds namely carbonyl compounds, alcohols, esters and hydrocarbons contribute to the aroma profile of olive oil (Angerosa et al., 2004).

Fig. 1. The main pathways involved in the formation of the volatile profile of high quality virgin olive oils. Adapted from (Angerosa et al. 2004; Angerosa 2002).

The volatile compounds, responsible for virgin olive oil aroma, are usually: low molecular weight (<300 Da); high volatility, sufficient hydrosolubility, fair liposolubility and chemical features to bond with specific proteins (Angerosa et al., 2002).

During crushing and malaxation steps, considerable changes, in olive oil chemical composition occurs accomplished by the activation of olive fruit enzymes due to the
inherent disruption of cellular tissues. Consequently, the LOX pathway is initiated by the hydrolysis of triglycerides and phospholipids, mediated by acyl hydrolase (AH), leading to the release of fatty acids. Lypoxygenases, after their release, become immediately active and transform the unsaturated fatty acids, produced by the action of AH, linolenic (LnA) and linoleic (LA) acids, into their corresponding 9- and 13-hydroperoxides, as shown in figure 2. The subsequent cleavage of fatty acids 13-hydroperoxides is catalysed by specific hydroperoxide lyases (HPL) leading to the formation of C6 aldehydes (Z)-hex-3-enal and hexanal from linolenic and linoleic acids, respectively) and oxoacids. The unsaturated form of C6 aldehyde ((Z)-hex-3-enal) undergo rapid isomerisation to the more stable (E)-hex-2-enal. The action of alcohol dehydrogenase (ADH), catalyses the reversible reduction of aliphatic C6 aldehydes to the corresponding volatile alcohols (Benicasa et al., 2003; Angerosa et al., 1998a). Alcohol species are further transformed into esters due to the catalytic activity of alcohol acetyl transferase (AAT), producing acetates (Kalua et al., 2007) (figure 2). Several factors, such as cultivar and extraction process, including operating temperature, seem to play a relevant role on the improvement of AAT activity (Salas, 2004). When the substrate is LnA, LOX catalyses, besides the hydroperoxide formation, also its cleavage, via an alkoxyl radical, increasing the formation of stabilized pent-1,3-diene radicals. These compounds can suffer dimerization leading to the production of C10 hydrocarbons (pentene dimmers) or react with a hydroxy radical present in the medium, leading to C6 carbonyl compounds (Angerosa et al. 1998b, Pizarro et al., 2011). The most important fraction of volatile compounds, of high quality virgin olive oils, comprises C6 and C5 compounds, especially C6 linear unsaturated and saturated aldehydes. The presence of other volatile compounds, namely C7-C11 monounsaturated aldehydes, C6-C10 dienals, C5 branched aldehydes and alcohols and some C6 ketones, in relatively high concentrations, in the aroma of virgin olive oil, is associated with unpleasant notes. The presence, or lack of defects, in the aroma of olive oils is related with the contribution of the various pathways involved on volatiles formation.

When the most active pathway is the LOX cascade the olive oil aroma will not be defective. LOX pathway is predominant in oils of high quality.

3. The role of volatile compounds in olive oil quality: Nutritional and sensorial quality

The International Olive Oil Council (IOOC), European Commission (EC) and Codex Alimentarius have defined the quality of olive oil based on several parameters, such as free fatty acid content, peroxide value, spectrophotometric absorbances in the UV region, halogenated solvents and sensory attributes (Boskou 2006; Kalua et al., 2007; Lopez-Feria et al., 2007). In order to evaluate olive oil quality, the Codex Alimentarius and IOOC include also the insoluble impurities, some metals and unsaponifiable matter determinations (Boskou 2006). The nutritional value of olive oil arises from high levels of oleic acid and minor components, such as phenolic compounds. It is well recognized that the consumption of some natural antioxidant phenolic compounds produce beneficial health effects. These substances possess strong radical scavenging capacities and can play a relevant role in protecting against oxidative damages and cellular aging. Together with their bioactivity, olive oil phenols have a significant role on the flavour and the bitter taste of olive oil (Boskou 2006; Servili et al. 2002). Sensory quality plays a crucial role in the acceptability of foodstuffs and
some characteristics such as colour and flavour are the main sensations which contribute to their acceptability among consumers. Hence, the evaluation of the sensory quality of olive oils involves perception of both favourable and unfavourable sensory attributes.

**Olive oil lipids**

![Diagram of Lypoxygenase pathway for the formation of major volatile compounds.](Image)

Fig. 2. Lypoxygenase pathway for the formation of major volatile compounds. (Source: Benincasa et al., 2003).

Olive oil possesses a highly distinctive taste and flavor due to specific volatile organic compounds, belonging to several chemical classes, namely aliphatic and aromatic hydrocarbons, aliphatic and triterpenic alcohols, aldehydes, ketones, ethers, esters and furan and thiophene derivatives (Kiritsakis et al. 1998). These compounds, retained by olive oil during the extraction process, stimulate human gustative and olfactive receptors giving rise to olive oil balanced flavour of green and fruity attributes. Such compounds stimulate the free endings of the terminal nerve located in all the palate and in the gustative buds promoting the chemesthetic perceptions of pungency, astringency and metallic attributes. During olive oil tasting, the stimulation of the olfactory epithelium, by a large number of volatile compounds can also occur explaining all other sensations perceived by consumers (Angerosa, 2002). The major volatile compounds of olive oil which contribute for the positive attributes of olive oil
aroma (fruity, pungent and bitter) include hexanal, \((E)\)-hex-2-enal, hexan-1-ol and 3-methylbutan-1-ol. Their concentrations, except for \((E)\)-hex-2-enal, varying widely, are generally very low reaching minimum levels of ppb. Thus, volatile compounds, which are responsible for most sensory properties of olive oils, play a significant role on the evaluation of the overall oil quality having a decisive influence on acceptability. The sensory defects are also associated with the volatile composition of the olive oil and are, usually, related with chemical oxidation and exogenous enzymes involved in microbial activity. Chemical oxidation is responsible for the formation of off-flavour compounds, such as pent-2-enal and hept-2-enal. The off-flavour compounds associated with unpleasant sensory notes can be assembled in five classes—fusty, moistness-humidity, winey-vinegary, metallic and rancid (Morales et al. 1997; Morales et al., 2005; Escuderos et al., 2007; Faria et al.; Angerosa 2002; Kalua et al. 2007). Moistness-humidity, which possesses the highest sensory significance, is related to the presence of \(C_8\) volatile compounds (e.g., oct-1-en-3-ol and to a lesser extent oct-1-en-3-one) and short chain fatty acids (Morales et al., 2005). Normally they are a characteristic flavour of oils produced from olives infested with fungi and yeasts as a result of an inappropriate storage. Fusty sensory defect is correlated with the presence of ethyl butanoate, propanoic and butanoic acids, a characteristic flavour of oils from olives stored in piles which have undergone an advanced stage of anaerobic fermentation (Morales et al. 2005). Moreover, the presence of acetic acid, ethanol, 3-methylbutan-1-ol and ethyl acetate contributes to winey-vinegary attributes due to the olives fermentation. The rancid negative attribute is due to oils oxidation, characterized by the absence of \(C_6\) aldehydes and alcohols produced from linolenic acid, the absence of esters and the presence of several aldehydes with low odour threshold (Morales et al, 1997). Metallic flavour is associated to oils that have been in prolonged contact with metallic surfaces, during processing, and is characterized by the presence of pent-1-en-3-one; this ketone has been proposed as a useful marker of metallic off-flavour (Venkateshwarlu et al. 2004). The occurrence of pent-1-en-3-one is also positively correlated with bitter and pungency taste while hexanal is negatively correlated with these characteristics, depending on the final amounts. \(Z\)-Hex-3-en-1-ol and \(E\)-hex-2-enal are negatively correlated with bitter and pungent characteristics, respectively. Other common defects of olive oils, such as muddy sediment and cucumber are related with olive oil preservation.

Poor quality olive oils show remarkable modifications on their sensory basic characteristics, namely the decrease or absence of green, bitter and pungent notes. Generally, the intensity of stimuli elicited by volatile substances is related to their amount. Some other chemical factors, such as volatility and hydrophobic character, size, shape and stereochemistry of volatile molecules, type and position of functional groups as well as external factors, such as matrix effects, seem to affect odour intensity, more than their concentration, due to the influence of these chemical features on the interaction with olfactory and gustative receptors (Angerosa et al., 2004). Odour activity value, evaluated by means of the ratio between its concentration and its odour threshold, constitutes a useful tool to identify the main contributors to the olive oil aroma. The thresholds of several of these compounds are already presented in dedicated literature (Bouskou 2006; Angerosa 2002).

4. The effect of agronomic and technological practices on olive oil aroma

Factors affecting volatile composition of olive oils can be classified into four main groups: environmental (soil and climate); agronomic (irrigation, fertilization); cultivation (harvesting,
ripeness) and technological procedures (post-harvest storage and extraction systems) (Aparicio & Luna, 2002). It is generally accepted that volatile profile of virgin olive oils depends on the level and the activity of the enzymes involved in LOX pathway. As previously referred, the major volatile compounds responsible for odour notes of virgin olive oils are the C₆ and the C₅ volatile compounds which emerge from primary or secondary LOX pathway, respectively. The enzymatic levels are determined genetically, so they differ from cultivar to cultivar, but the enzymatic activity is influenced by all factors mentioned above. Apart endogenous plant enzymes, responsible for the positive aroma perception in olive oils, chemical oxidation and microbial activity (associated with sensory defects) should be considered.

4.1 Cultivars

Cultivars and harvest time must be carefully selected in order to correspond to the optimal level of fruit maturity (Esti et al., 1998; Caponio et al., 2001). Olives ripening is quite important for olive oil final composition. The cultivar influence depends on the activity of enzymes and is a genetic characteristic (Tena et al., 2007). The higher LOX activity for linoleic acid than linolenic acid supports the biogenesis of a higher amount of C₆ unsaturated volatile compounds the major constituents of olive oil aroma; usually olive fruits show the highest LOX activity 15 weeks after anthesis; activity decreases during development and ripening periods (Salas et al., 1999). Another enzyme involved is HPL that catalyses the cleavage of fatty acids hydroperoxides producing volatile aldehydes. The highest level of HPL activity is detected in green olive fruits, harvested at the initial development stages. Although there is a slight decrease at maturity, a high activity level is maintained throughout maturation. The decrease in C₆ volatile compounds concentration, in the olive oils of mature olives, is not attributed to HPL activity (Salas & Sanchez, 1999) rather to the availability of substrate. The behaviour of these two enzymes supports the decrease of C₆ volatile compounds content during fruit ripeness. At earlier ripening stages the amount of C₆ aldehydes and alcohols are very similar, and when olive skin colour changes from green to purple most of the C₆ aldehydes reach their maximum concentration (Angerosa & Basti, 2001). With the increase of ripeness a decrease is observed for most of the aldehydes formed from the lipoxygenase pathway, namely E-hex-2-enal (the main volatile compound in most European virgin olive oils), being Z-hex-3-enal an exception (Aparicio & Morales, 1998). Kalua et al (2007), however, state that the decrease in C₆ aldehydes, from the lipoxygenase pathway, might not be characteristic of all cultivars.

The olive cultivar influences also fatty acid composition and, particularly, the ratio of oleic to linoleic acid (C18:1/C18:2), triglyceride profile, and phenolic content of olive oil (Aparicio et al., 2002; Tovar et al., 2002; Beltran et al., 2005). Some differences can be found in the fatty acid content of varietal virgin olive oils (Aparicio 2000); they do not vary so much, however, as to be determinant for the volatile profile. In spite, C₆ volatile compounds (aldehydes, alcohols and acetyl esters) formed from 13-hydroperoxides of linoleic and α-linolenic acids, account for 60 to 80% of the total volatile compounds (Aparicio & Luna 2002). The concentration of C₆ volatile compounds, of 36 monovarietal virgin olive oils produced in countries from Mediterranean basin, show that aldehydes (hexanal, Z-hex-3-enal and E-hex-2-enal) and pent-1-en-3-one contribute, distinctly, to the sensory profile of these varietal oils, taking into account the odour thresholds of these volatile compounds (75, 3, 1125 and 50 μg Kg⁻¹, respectively) (Aparicio & Luna 2002). These authors found high concentrations of E-hex-2-enal in Italian cultivars, in accordance with results previously obtained by Solinas
et al. (1988), and they all suggest that monovarietal virgin olive oils could be distinguished by this compound. According to Solinas et al. (1988) octanal, nonanal and hex-2-enal contents are a cultivar characteristic; the presence of propanol, amyl alcohols, hex-2-enol, hexan-2-ol and heptanal seems also to be related to the olive cultivar. Nevertheless, olive oils from different cultivars, produced under the same exact conditions (extraction system, ripeness stage, pedoclimatic and agronomic conditions), exhibit different amounts of total volatiles, ranging from 9-83 to 35 mg kg\(^{-1}\) (Luna et al., 2006). Baccouri et al. (2008), when studying volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils, found that the overall amounts of C\(_6\) aldehydes were clearly higher than the sum of C\(_6\) alcohols in Chemlali and Sicilian samples, whereas, in Chetoui oils, the sum of C\(_6\) alcohols was generally higher than the C\(_6\) aldehydes. The explanation relays again in the differential activity of the enzymes involved. These authors also reported a decrease, in the amounts of C\(_5\) aldehydes and alcohols, during the maturation. Morales et al. (1996) studied the influence of ripeness on the concentration of green aroma compounds; the total content of volatile compounds decreases with ripeness; there are markers for monovarietal virgin olive oils obtained from unripe (E-hex-2-enal), normal ripe (hexyl acetate) and overripe olives (E-hex-2-enol) regardless of the variety (Aparicio & Morales, 1998).

D’Imperio et al. (2010), when studying the influence of harvest, method and schedule, on olive oil composition, found a remarkable decrease of E-hex-2-enal as was previously reported by Aparicio et al. (1998); an increase of hexanal seems to be related to the use of shakers for harvesting. A decrease in unsaturated fatty acids content was also observed relating these findings to the lipoxygenase pathway.

### 4.2 Environmental factors

Pedoclimatic factors depend on environmental conditions, soil, type and structure, and/or climatic conditions, temperature and rainfall (Beltran et al., 2005). Cultivars do not always grow at the same altitude, but olive grove zones are spread over a wide range of altitudes, where climatic conditions can be quite different. All these have impact on chemical and sensory profiles of olive oils. Monovarietal olive oils, obtained from olives grown at higher altitudes, are in general sweeter and have a stronger herbaceous fragrance, when compared to the ones produced with olives grown at lower altitudes. Lower temperatures, at higher altitudes, may influence the enzymes from lipoxygenase pathway, since hexanal (green-sweet perception) comes from increased levels of linoleic acid, and E-hex-2-enal (green odour and astringency taste) from lower levels of \(\alpha\)-linolenic acids (Aparicio & Luna, 2002). Temime et al. (2006) studied the volatile compounds from Chétoui olive oils, the second variety cultivated in Tunisia, and reported significant differences on volatile compounds when, just, environmental conditions were different. Dabbou et al. (2010) studied the quality and the chemical composition of monovarietal virgin olive oil, from the Sigoise variety, grown in two different locations in Tunisia, a sub-humid zone (Béjaoua, Tunis) and an arid zone (Boughrara, Sfax). Olive oils produced from olives grown at the higher altitude were characterized by higher contents of E-hex-2-enal (11.92 mg kg\(^{-1}\)) and hexanal (1.24 mg kg\(^{-1}\)), whereas the oils, from the lower altitude, were distinguishable by the higher content of Z-hex-2-en-1-ol (8.78 mg kg\(^{-1}\)) and hexan-1-ol (2.17 mg kg\(^{-1}\)). The sum of the products of the lipoxygenase oxidation pathways was higher in oils from Béjaoua (15.92 mg kg\(^{-1}\)) than in those from Boughrara (15.20 mg kg\(^{-1}\)). Among the LOX oxidation products, the amount of hexanal was higher in Béjaoua oils (1.24 mg kg\(^{-1}\)), whereas the content of Z-hex-2-en-1-ol was considerably lower.
In a recent study, concerning the behaviour of super-intensive Spanish and Greek olive cultivars grown in northern Tunisia, Allalout et al. (2011) found significant differences between oils; they consider, the majority of the studied analytical parameters, to be deeply influenced by the cultivar-environment interaction.

It seems there is an effect of genotype-environment interaction, responsible for olive oils characteristics.

### 4.3 Agronomic factors

Irrigation, a practice that has been adequately studied, seems to produce a decrease in the oxidative stability of olive oil volatiles due to a simultaneous reduction in oleic acid and phenolic compounds contents (Tovar et al., 2002).

According to Servili et al. (2007) the olive tree water status has a remarkable effect on the concentration of volatile compounds, such as the C₆-saturated and unsaturated aldehydes, alcohols, and esters. Put simply, deficit irrigation of olive trees appears to be beneficial not only due to its well-known positive effects on water use efficiency, but also by optimizing olive oil volatile quality. Baccouri et al. (2008) reported an enhancement of the whole aroma concentration of Chetouil oils obtained from trees under irrigation conditions when compared to similar ones from non-irrigated trees.

The effect of agronomic practices in oil quality is still controversial: data from Gutierrez et al. (1999) supports the hypothesis that organic olive oils have better intrinsic qualities than conventional ones. These olive oils usually present lower acidity and peroxide index, higher rancimat induction time, higher concentrations of tocopherols, polyphenols, α-diphenols and oleic acid. However, this work was carried out during 1 year, with one olive cultivar only, and results can not be generalized. Ninfali et al. (2008) in a 3-year study, comparing organic versus conventional practice did not observe any consistent effect on virgin olive oil quality. Genotype and year-to-year climate changes seem to have a proved influence.

### 4.4 Technogical factors

Volatile compounds are predominantly generated during virgin olive oil extraction, and are important contributors to olive oil sensory quality. Virgin olive oil quality is intimately related to the characteristics and composition of the olive fruit at crushing. Changes in olive fruit quality during post-harvest is considered determinant to the final sensory quality. Kalua et al. (2008) reported that low-temperature storage of fruits can produce poor sensory quality of the final oil. This decrease in quality might be due to lower levels of E-hex-2-enal and hexanal, associated with a decrease in enzyme activity, and a concurrent increase in E-hex-2-enol, which might indicate a possible enzymatic reduction by alcohol dehydrogenase (Olias et al., 1993, Salas et al. 2000) and reduced chemical oxidation (Morales et al. 1997). Inarejos-Garcia et al. (2010) studied the olive oils from Cornicabra olives stored at different conditions (from monolayer up to 60 cm thicknesses at 10 ºC (20 days) and 20 ºC (15 days)). E-hex-2-enal showed a Gaussian-type curve trend during storage that can be related to the decrease of hydroperoxide lyase activity. C₆ alcohols showed different trends, during storage, with a strongly decrease of the initial content of Z-hex-3-en-1-ol after 15 and 8 storage days at 20ºC and 10ºC under the different storage layers, whilst an increase of E-hex-2-en-1-ol was observed (except for mono-layer). Differences might be related to the
enhancement of alcohol dehydrogenase activity during storage. Besides the evolution and changes observed in the desirable LOX pathway, C$_6$ fraction, storage may give rise to undesirable volatile compounds, from metabolic action of yeasts, which was more evident when olive were stored at 20 °C. The effect of the extraction process on olive oil quality is also well documented (Ranalli et al., 1996; Montedoro et al., 1992; Di Giovacchino, 1996; Koutsaftakis et al., 1999; Servili et al., 2004).

Technological operations include several preliminary steps, leaf and soil removal, washing, followed by crushing malaxation and separation of the oil (and water) from the olive paste. This last step can be achieved by pressing (the oldest system), centrifugation (the most widespread continuous system), or percolation (based on the different surface tensions of the liquid phases in the paste).

Ranalli et al. (2008) studied the effect of adding a natural enzyme extract (Bioliva) during processing of four Italian olive cultivars (Leccino, Caroleo, Dritta and Coratina) carried out with a percolation-centrifugation extraction system. The improved rheological characteristics of the treated olive paste resulted in a reduced extraction cycle with good effects concerning olive oil aroma characteristics. Results have shown that enzyme-treated olive pastes always release higher amounts of total pleasant volatiles (E-hex-2-enal, E-hex-2-en-1-ol, Z-hex-3-enyl acetate, Z-hex-3-en-1-ol, pent-1-en-3-one, Z-pent-2-enal, E-pent-2-enal and others). For the individual C$_6$ metabolites, from the LOX pathway, a similar trend was generally observed, while for the total unpleasant volatiles, n-octane, ethyl acetate, isobutyl alcohol, n-amyl alcohol, isoamyl alcohol and ethanol, an opposite behaviour was found.

The fundamental step is, however, olive crushing. The release of oil from olives can be achieved by mechanical methods (granite millstones or metal crushers) or centrifugation systems. These different systems affect the characteristics of the pastes and the final oil (Di Giovacchino et al., 2002). Almirante et al. (2006) reported that the oils obtained from de-stoned pastes had a higher amount of C$_5$ and C$_6$ volatile compounds, when compared to oils obtained by stone-mills. This increment is due to stones removal, which possess enzymatic activities, metabolizing 13-hydroperoxides other than hydroperoxide lyase, giving rise to a net decrease in the content of C$_6$ unsaturated aldehydes during the olive oil extraction process. Servili et al. (2007) demonstrate that the enzymes involved in the LPO pathway have different activity in the pulp or in the stone. Stones seem to have a lower hydroperoxide lyase activity and a higher alcohol dehydrogenase activity when compared to the pulp. These authors also found higher amounts of C$_6$ unsaturated aldehydes olive oils volatiles (VOOs) obtained with the stoning process; the stone presence in traditional extraction procedure increases the concentration of C$_6$ alcohols (for Coratina and Frantoio cultivars).

The next step is the malaxation. Malaxation is performed to maximize the amount of oil that is extracted from the paste, by breaking up the oil/water emulsion and forming larger oil droplets. The efficiency of this operation depends upon time and temperature. Pressing, percolation, or centrifugation, are finally used to separate the liquid and solid phases. Temperature and time of exposure of olive pastes to air contact (TEOPAC), during malaxation, affect volatile and phenolic composition of virgin olive oil, and consequently its sensory and healthy qualities. Cultivar still plays a fundamental role for the final composition (Servili et al, 2003). These authors showed that TEOPAC can be used to perform a selective control of deleterious enzymes, such as polyphenol oxidase (PPO) and
peroxidase (POD), preserving the activity of LPO. High malaxation temperature (> 25 ºC) reduces the activity of enzymes, involved in LOP pathway, reducing the formation of C₆ saturated and unsaturated aldehydes. A similar result is described by Tura et al. (2004). These authors found that changes in malaxation time and temperature produces differences in the volatile profile of olive oils. Increasing temperature and decreasing time led to a reduction in the amount of volatiles produced, but they also describe cultivar as the single most important factor in determining volatile profile of olive oils. The decrease of olive oil flavour, produced by high malaxation temperature, is due to the inactivation of hidroperoxide lyase (HPL) rather than lipoxygenase (LOX), as both enzymes have different behaviour regarding temperature (Salas & Sánchez, 1999b). LOX, when assayed with linoleic acid as the substrate, displayed a rather broad optimum temperature around 25 ºC and maintained a high activity at temperatures as high as 35 ºC, but HPL activity peaked at 15 ºC and showed a clear decrease at 35 ºC, in assays using 13-hydroperoxylinoleic acid as substrate. Similar results were obtained by Gomez-Rico et al. (2009) who observed a significant increase in C₆ aldehydes, in the final oil, as malaxation time increased; almost no changes in the content of C₆ alcohols were observed. Opposite results were found for the influence of the kneading temperature, where a drop in the C₆ aldehydes content as malaxation temperature increases is observed, especially for E-hex-2-enal and a slight increase in C₆ alcohols, mainly hexan-1-ol and Z-hex-3-en-1-ol.

The final step of olive oil production also affects olive oil quality. Separation of oil from water can be achieved using a two-phase or a three phase centrifugation system. Comparing monovarietal virgin oils obtained by both processes, the oils from two-phase decanters have higher content of E-hex-2-enal and total aroma substances but lower values of aliphatic and triterpenic alcohols (Ranalli & Angerosa, 1996).

Masella et al. (2009), when studying the influence of vertical centrifugation on olive oil quality, observed significant differences both in the total volatile concentration and in the two volatile classes from the LOX pathway involving LnA conversion. The observed decreased of C₆/LnA and C₅/LnA compounds can be explained by the volatiles partition between oil and water phases during vertical centrifugation.

Storage conditions also affect final quality. Light exposure, temperature and oxygen concentration, storage time and container materials are also determinant. A study by Stefanoudaki et al. (2010) evaluating storage under extreme conditions, showed subtle differences, in the pattern of volatile compounds, in bottled olive oils stored indoors or outdoors. When stored with air exposure the levels of some negative sensory components, such as penten-3-ol and hexanal, increased while other positives, like E-hex-2-enal were reduced. Filling the headspace with an inert gas can reduce spoilage.

5. Analytical methodologies for quantitation and identification of volatiles compounds: New analytical methods

5.1 Olive oil volatile compounds

In the volatile fraction of olive oils, approximately three hundred compounds have already been detected and identified by means of gas chromatography/mass spectrometry (GC/MS) methods (Boskou, 2006). Among these compounds, only a small fraction
contributes to the aroma of olive oil (Angerosa et al., 2004). The most common olive oil volatiles have 5 to 20 carbon atoms and include short-chain alcohols, aldehydes, esters, ketones, phenols, lactones, terpenoids and some furan derivatives (Reiners & Grosch, 1998; Delarue & Giampaoli, 2000; Kiritsakis, 1992; Boskou, 2006; Vichi et al., 2003a, 2003b, 2003c; Aparicio et al., 1996; Morales et al., 1994; Flath et al, 1973; Morales et al, 1995; Bortolomeazzi et al., 2001; Bentivenga et al., 2002; Bocci et al., 1992; Servili et al., 1995; Fedeli et al., 1973; Fedeli, 1977; Jiménez et al., 1978; Kao et al., 1998; Guth & Grosch, 1991). As all vegetable oils, olive oil comprises a saponifiable and a non-saponifiable fraction and both contribute for the aroma impact. As a result of oxidative degradation of surface lipids (Reddy & Guerrero, 2004) a blend of saturated and mono-unsaturated six-carbon aldehydes, alcohols, and their esters (Reddy & Guerrero, 2004; Matsui, 2006) are produced. As already mentioned they are formed from linolenic and linoleic acids through the LOX pathway, and are commonly emitted due to defence mechanism developed by the plant in order to survive to mechanical damage, extreme temperature conditions, presence of pathogenic agents, among others (Delarue & Giampaoli, 2000; Noordermeer et al., 2001; Pérez et al., 2003; Angerosa et al., 2000; Angerosa et al., 1998b). Volatile phenols are also reported as aroma contributors for olive oil and can play a significant organoleptical role (Vichi et al., 2008; Kalua et al., 2005).

5.2 Analytical methodologies

5.2.1 Sample preparation procedures

When the analysis of a volatile fraction, of complex matrices, is considered sample preparation cannot be underestimated. In biological samples, a wide chemical diversity, in a wide range of concentrations, must be expected (Salas et al., 2005; Wilkes et al., 2000). The chemical nature, and the amount of the detected compounds, strongly depends on the extraction technique used, to remove and isolate them from their matrices. The choice of a suitable extraction methodology depends on sample original composition and target compounds. However, an ideal sampling method does not exist and no single isolation technique produces an extract that replicates the original sample. In order to have enough quantity of each compound to be detected by chromatography, a concentration step must, usually, be considered. Sample preparation can be responsible for the appearance of artefacts, due to the chemical nature of the compounds extracted, and thus detected and quantified, and to a total or partial loss of compounds; this issues can, very strongly, determine the precision, reproducibility, time and cost of a result and/or analysis (Wilkes et al., 2000; Belitz et al., 2004; Buttery 1988; van Willige et al., 2000). These methods are revised in a recent manuscript (Costa Freitas et al.) where sample preparation procedures for volatile compounds are discussed as well as the advantages and drawbacks of each method.

In olive oil analysis, its oily nature strongly influences the choice of the extraction procedure. There are various techniques that can be used for the preparation of the sample analytes in biological material. From those so far applied, liquid extraction with or without the use of ultrasounds (Kok et al., 1987; Fernandes et al., 2003; Cocito et al., 1995) is probably the most used. Besides liquid extraction, simultaneous distillation extraction (SDE) (Flath et al., 1973) has also been widely used. The drawback of these methods is the use of solvents
Olive Oil – Constituents, Quality, Health Properties and Bioconversions

and consequently the need of compounds isolation from the solvent which represents an extra preparation step, as well as the dilutions steps during the extraction procedure. To avoid these steps, supercritical fluid extraction (SFE) (Morales et al., 1998) was also used for the isolation of volatile constituents of olive oil.

The methods based on extraction from the headspace are an elegant choice (Swinnerton et al., 1962). The more often used procedures are the so called “purge and trap” techniques (Morales et al., 1998; Servili et al., 1995; Aparicio & Morales, 1994) in which the compounds of interest are trapped in a suitable adsorbent, from which they can be taken either directly (using a special “thermal desorber” injector) or after retro-extraction into a suitable solvent which, once again, includes an extra extraction step. Another choice is direct injection of the headspace into the injection port of a GC chromatograph. This possibility does not include a concentration step, and consequently, the minor compounds are usually missing or not detected (Del Barrio et al., 1983; Gasparoli et al., 1986). A direct thermal desorption technique can also be applied, avoiding the use of any types of adsorbents, by just heating the target olive oil sample to a suitable temperature in order to promote the simultaneous, extraction, isolation and injection of the volatile fraction into the analytical column (Zunin et al. 2004, de Koning et al., 2008). The main advantage of this technique is its simplicity, although a special injection system is mandatory, which can be expensive. When SPME was introduced (Belardi & Pawliszyn, 1989; Arthur & Pawliszyn, 1990) several authors have focused their attention on adapting the technique for aroma compounds analysis (D’Auria et al., 2004; Vichi et al., 2003; Vichi et al., 2005; Ribeiro et al., 2008). The main advantages of this technique are: a) it does not involve sample manipulations; b) it is an easy and clean extraction method able to include, in just one step, all the steps usually needed for aroma extraction. The extraction step, in SPME, can be made either by headspace sampling or liquid sampling. Headspace sampling (HS) is usually the method of choice for olive oil aroma analysis. The fibre chemical composition is of main interest and determines the chemical nature of the compounds extracted and further analyzed. There are several coatings commercially available. Polydimethylsiloxane (PDMS) and polyacrylate (PA) coatings extract the compounds by means of an absorption mechanism (Ribeiro et al., 2008) whereas PDMS is a more apolar coating then PA. Polydimethylsiloxane/divinylbenzene (PDMS/DVB), polydimethylsiloxane/carboxene (PDMS/CAR), carbowax/divinylbenzene (CW/DVB), and divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) extract by an adsorptive mechanism. These second group of fibres have usually a lower mechanic stability but present higher efficiency to extract compounds with low molecular weight (Augusto et al., 2001). In both extraction mechanisms, once the compounds are expelled form the matrix, they will remain in the headspace and a thermodynamic equilibrium is established between these two phases (Zhang & Pawliszyn, 1993). When the fibre is introduced a third phase is present and mass transfer will take place in both interphases (sample matrix/headspace and headspace/fibre). When quantification is a requirement, equilibrium has usually to be achieved. Time and temperature are also very important issues to take in consideration, since they will affect equilibrium (Vas & Vékey, 2004) and thus extraction efficiency. Methods that consider quantification in non-equilibrium have also been developed (Ai, 1997; Ribeiro et al., 2008). In order to optimize the extraction procedures by HS-SPME, the efficiency, accuracy and precision of the extraction is also directly dependent on operational parameters like extraction time, sample agitation, pH adjustment, salting out, sample and/or headspace volume,
temperature of operation, adsorption on container walls and desorption conditions (Pawliszyn, 1997).

5.2.2 Chromatographic methods for the analysis of olive oil volatiles

Capillary gas chromatography (GC) is the most used technique for the separation and analysis of volatile and semivolatile organic compounds (Beesley et al., 2001) in biological samples. GC allows to separate and detect compounds present in a wide range of concentrations in very complex samples, and can be used as a routine basis for qualitative and quantitative analysis (Beesley et al., 2001; Majors, 2003). Enantioselective separations can also be performed when chiral columns are used (Bicchi et al., 1999). The most common detector used is the flame ionization detector (FID), known by its sensitivity and wide linear dynamic range (Scott, 1996; Braithwaite & Smith, 1999). When coupled with Fourier transform infrared spectroscopy (GC/FTIR) or mass spectrometry (GC/MS) (Gomes da Silva & Chaves das Neves, 1997; Gomes da Silva & Chaves das Neves, 1999), compounds tentative identification can be achieved.

The most widely used ionization techniques employed in GC/MS is electron ionization (EI normally at 70 eV) and the more frequently used mass analysers, in olive oil volatile research, are quadrupole filters (qMS), ion traps (ITD) and time of flight instruments (TOFMS). The GC/TOFMS instruments allow the simultaneous acquisition of complete spectra with a constant mass spectral m/z profile for the whole chromatographic peak, while in qMS instruments the skewing effect is unavoidable. This fact enables the application of spectral deconvolution (Smith, 2004), and, potentially, a more accurate use of reference libraries for identification and confirmation of analytes may be possible. Nevertheless, for routine laboratory the development of TOFMS dedicated mass spectral libraries, to complement the libraries now generated by using qMS, should be considered. Spectral matching is usually better when qMS data are compared in some instances (Cardeal et al., 2006; Gomes da Silva et al., 2008).

In an ongoing research in our lab, HS-SPME was performed in order to identify volatile compounds in Galega Vulgar variety. Four fibres were used and the HS-SPME-GC/TOFMS system operated with a DB-wax column. In table 1 the complete list of compounds identified (using the four different fibres) is provided as well as fragmentation patterns obtained for those not yet reported in olive oils (table 2). Analysis were performed in two columns: a polar column (DB-WAX), usually recommended for volatiles analysis, and an apolar based column DB-5. The use of these two columns, of different polarity, was also very useful to detect co-elutions, occurring when the polar column was used, and helped the identification task, when associated to mass spectrometric and linear retention indices (LRI) data confrontation. Most identification were performed by comparing retention time and fragmentations patterns, obtained for standards, analysed under the same conditions, or by fragmentation studies, when standards were not available. The differences observed, in the LRI experimentally obtained for the DB-WAX column, compared to the literature were expectable since polar columns are known as being much more unstable, then apolar columns, and cross-over phenomena occur (Mateus et al. 2010). Their retention characteristics varies significantly among different suppliers, which suggest the need of LRI probability regions. This fact explains why few LRI data is available for polar columns. These results aims to fullfill some part of this gap.
<table>
<thead>
<tr>
<th>Compound name</th>
<th>LRI Experimental</th>
<th>SPME Fibres</th>
<th>Compound name</th>
<th>LRI Experimental</th>
<th>SPME Fibres</th>
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<td>Hexane</td>
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<tr>
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<tr>
<td>Ethyl 2-methyl-</td>
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<td>Tridec-6-ene  (isomer)</td>
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<tr>
<td>butanoate</td>
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<tr>
<td>Deca-3,7-diene (isomer)</td>
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<td>Styrene</td>
<td>1199 [1265]</td>
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<td>4-Hidroxy-4-methylpentan-2-one</td>
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<td>E-Non-2-enal</td>
<td>1494 [1502-1540]</td>
<td>PA D-C-P</td>
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Olive Oil Composition: Volatile Compounds
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<thead>
<tr>
<th>Compound name</th>
<th>LRI Experimental [Literature]</th>
<th>SPME Fibres</th>
<th>Compound name</th>
<th>LRI Experimental [Literature]</th>
<th>SPME Fibres</th>
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<td>E-Hex-3-en-1-ol</td>
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<td></td>
<td>[n.f.</td>
<td></td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>1846</td>
<td>PA</td>
<td>Octanoic acid</td>
<td>2047</td>
<td>PA</td>
</tr>
<tr>
<td></td>
<td>[1822-1883]</td>
<td>CDVB</td>
<td></td>
<td>[2069]</td>
<td>D-C-P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-C-P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylethyl alcohol</td>
<td>1890</td>
<td>PA</td>
<td>Nonanoic acid</td>
<td>2198</td>
<td>PA</td>
</tr>
<tr>
<td></td>
<td>[1859-1919]</td>
<td>CDVB</td>
<td></td>
<td>[n.f.</td>
<td>D-C-P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-C-P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heptanoic acid</td>
<td>1900</td>
<td>PA</td>
<td>4-Ethyl-phenol</td>
<td>2212</td>
<td>D-C-P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.d. denote not determined; n.f. denote not found; LRI denote linear retention indices for DB-Wax column. LRI between brackets represents the data range found in literature: Angerosa, 2002; Contini & Esti 2006; Flath et al., 1973; Kanavouras et al., 2005; Ledauphin et al., 2004; Morales et al., 1994; Morales et al., 1995; Morales et al., 2005; Reiners & Grosch, 1998; Tabanca et al., 2006; Vichi et al., 2003a., 2003b; Vichi et al., 2005; Zunin et al., 2004.

Table 1. Compounds identified in olive oil samples of *Galega Vulgar* by means of HS-SPME-GC/TOFMS. The fibres used are polydimethylsiloxane (PDMS), polyacrylate (PA), carbowax/divinylbenzene (CDVB), and divinylbenzene/carboxene/polidimethylsiloxane (D-C-P). The extraction and analysis procedure for all fibres was: 15 g of olive oil sample in 22 mL vial immersed into a water bath at 38 °C. Extraction time was 30 min. Fibre desorption time was 300 seconds into an injection port heated at 260 °C. Splitless time of 1 min. A GC System 6890N Series from Agilent coupled to a Time of Flight (TOF) mass detector GCT from Micromass using the acquisition software MassLynx 3.5, MassLynx 4.0 and ChromaLynx The system was equipped with a 60 m × 0.32 mm i.d. with 0.5 μm df DB-Wax column or a 30 m × 0.32 mm i.d. with 1 μm df DB-5 column, both purchased from J&W Scientific (Folsom USA). Acquisition was carried out using a mass range of 40-400 u.; transfer line temperature was set at 230 °C; ion source 250 °C. Helium was used as carrier at 100 kPa; Oven temperature was programmed from 50 °C for three minutes and a temperature increase of 2 °C/min up to 210 °C hold for 15 minutes and a rate of 10 °C/min up to 215 °C and hold.
<table>
<thead>
<tr>
<th>Compound name</th>
<th>LRI Experimental [Literature]</th>
<th>m/z -fragmentation pattern</th>
<th>SPME Fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl pentanoate</td>
<td>1050 [1127]</td>
<td>57(66%); 60(36%); 71(5%); 73(31%); 85(100%); 88(87%); 101(30%); 115 (2%) 130 (1%) M⁺</td>
<td>D-C-P</td>
</tr>
<tr>
<td>2-Methyl-heptan-4-one</td>
<td>1063 [n.f.]</td>
<td>41(41%); 43(45%); 55(10%); 57(100%); 69(18%); 71(63%); 85(79%); 95(2%); 100(3%); 113(10%); 128(23%) M⁺</td>
<td>PA D-C-P</td>
</tr>
<tr>
<td>2,6-Dimethyl-oct-2-ene (isomer)</td>
<td>1181 [n.f.]</td>
<td>41(87%); 55(100%); 67(11%); 69(73%); 83(25%); 93(12%); 97(25,74%); 111(16%); 126(9,86%); 130(1%) M⁺</td>
<td>D-C-P</td>
</tr>
<tr>
<td>3-Methyl-pent-3-en-1-ol (isomer)</td>
<td>1306 [n.f.]</td>
<td>41(100%); 42(16%); 55(52%); 56(12%); 67(93%); 69(49%); 70(19%); 82(72%); 83(4%); 100(3%) M⁺</td>
<td>CDVB D-C-P</td>
</tr>
<tr>
<td>2,6-Dimethyl-oct-2,4,6-triene</td>
<td>1318 [n.f.]</td>
<td>77(15%); 79(38%); 91(3%); 93(22%); 95(10%); 105(55%); 121(100%); 122(10%); 136(43%) M⁺</td>
<td>D-C-P</td>
</tr>
<tr>
<td>1-Methoxy-2-(methoxymethyl) benzene</td>
<td>1346 [n.f.]</td>
<td>51(15%); 65(18%); 77(33%); 79(20%); 91(100%); 21(96%); 137(17%); 152(6%) M⁺</td>
<td>D-C-P</td>
</tr>
<tr>
<td>Hex-4-enyl propanoate (isomer)</td>
<td>1350 [n.f.]</td>
<td>41(42%); 55(29%); 57(25%); 67(100%); 82(51%)</td>
<td>PDMS D-C-P</td>
</tr>
<tr>
<td>Decan-2-one</td>
<td>1428 [n.f.]</td>
<td>41(11%); 42(10%); 43(82%); 55(4%); 57(6%); 58(100%); 59(24%); 60 (6%); 71(24%); 85(2%); 98(4%); 113 (2%); 127(2%); 156(2%) M⁺</td>
<td>PA D-C-P</td>
</tr>
<tr>
<td>Nonyl acetate</td>
<td>1526 [n.f.]</td>
<td>43(100%); 56(39%); 61(33%); 70(24%); 83(16%); 98(19%); 126(10%)</td>
<td>PA D-C-P</td>
</tr>
<tr>
<td>Z-Dec-2-enal</td>
<td>1608 [n.f.]</td>
<td>41(64%); 43(55%); 55(100%); 56(98%); 69(71%); 70(94%); 83(57%); 98(34%); 110(5%); 136(2%)</td>
<td>PA D-C-P</td>
</tr>
<tr>
<td>Phenyl acetate</td>
<td>1964 [n.f.]</td>
<td>43(39%); 65(22%); 66(28%); 77(8%); 89(16%); 94(100%); 95(6%); 103(8%); 117(9%); 136(15%) M⁺</td>
<td>D-C-P</td>
</tr>
<tr>
<td>2-Methyl-phenol</td>
<td>2065 [n.f.]</td>
<td>45(7%); 50(5%); 51(9%); 52(4%); 53(8%); 54(4%); 63(3%); 77(24%); 79(19%); 80(8%); 89(4%); 90(8%); 91(3%); 107(100%); 108(98%) M⁺; 109(5%(M+H)⁺</td>
<td>D-C-P</td>
</tr>
<tr>
<td>4-Methyl-biphenyl</td>
<td>2091 [n.f.]</td>
<td>51(6%); 63(5%); 82(10%); 83(12%); 84(11%); 115(10%); 152(21%); 153(17%); 65(32%); 167(71%); 168(100%) M⁺; 169(17%)(M+H)⁺</td>
<td>D-C-P</td>
</tr>
</tbody>
</table>

Table 2. New tentatively identified compound in olive oil samples of *Galela vulgar* by means of HS-SPME-GC/TOFMS. Extraction and analytical conditions according to described in table 1. m/z fragmentation patterns are presented; n.f. denote not found; LRI denotes linear retention indices as in table 1. LRI between brackets represents the data range found in literature, according to table 1.
Co-elutions are often impossible to detect and identify with some GC/MS instruments, in spite of the use of selective single ion monitoring mode (SIM), or complex deconvolution processes. The development of new analytical techniques, that maximize analyte separation, has always been a target. Multidimensional chromatography and comprehensive two-dimensional chromatography (David & Sandra, 1987; Bertsch, 1999) are an example of such achievements. The high complexity of the chromatograms points out new ways of chromatography, such as multidimensional-gas chromatography systems (MD-GC), where the analytes are submitted to two or more independent separation steps, in order to achieve separation. In spite of its efficiency, MD-GC is a time consuming technique, with long analysis times, which does not fit with the demands of routine analysis. Additionally, it is technically difficult to carry out sequential transfers in a narrow window of retention times, since co-elutions are foreseen (Poole, 2003). Nevertheless, MD-GC is a precious tool in peak identification for olive oil analysis when co-elutions occur (Reiners & Grosch, 1998). In 1991, comprehensive two-dimensional gas chromatography (GC × GC) was introduced by Liu & Phillips. The GC × GC system consists of two columns with different selectivities; the first and second dimension columns are serially connected through a suitable interface, usually a thermal modulator (Phillips & Beens, 1999; Marriott & Shellie, 2002). When performing GC × GC technique the entire sample, separated on the first column, is transferred to the second one, resulting in an enhanced chromatographic resolution into two independent dimensions, where the analytes are separated by two independent mechanisms (orthogonal separation) (Venkatramani et al., 1996; Phillips & Beens, 1999; Marriott & Shellie, 2002; Dallüge et al., 2003). The modulated zones of a peak are thermally focused before the separation on the second column, in a mass conservative process; the resulting segments (peaks), of the modulation, are much narrower with higher S/N ratios, than in conventional GC (Lee et al., 2001; Dallüge et al., 2002), improving the detection of trace analytes and the chromatographic resolution. Fast acquisition TOF spectrometers are the suitable detectors for this technique and have considerably enlarged the application of GC × GC. Few applications are still reported for olive oil analysis, nevertheless, they already showed its potential. GC × GC techniques allowed identification of olive oil key flavour compounds, present in very low concentrations (Adahchour et al. 2005); it has also been used as a flexible technique for the screening of flavours and other classes of (semi-)polar compounds, using the conventional orthogonal approach and the reverse, non-orthogonal approach in order to obtain ordered structures that can simplify the identification task (Adahchour et al. 2004); finally this separation technique can allow easy fingerprint analysis of several olive oil matrices directly, or using image processing statistics (Vaz-Freire et al., 2009).

5.3 Future perspectives for olive oil volatile analysis: Identification tools and fingerprinting

A limitation of electron ionization (EI) in MS analysis is due to the fact that, too often, the molecular ions do not survive fragmentation and, consequently, are not "seen". One way to overcome this problem is to use a complementary technique, that provides "soft" ionization of the molecules, allowing molecular ions detection. Chemical ionization (CI) performs this task (McMaster and McMaster, 1998; Herbert and Johnstone, 2003). The mass spectra obtained by CI are simpler than EI, though most of the interpretable structural information is missing. However the compound's molecular ions appears as a high intensity fragment
and sometimes is the major fragment of the spectra. Thus, molecular weight determination of an analyte becomes possible. Other soft ionization techniques are field ionization (FI) and field desorption (FD). Both produce abundant molecular ions with minimal fragmentation (Herbert and Johnstone, 2003). FI and FD are applicable to volatile and thermally stable samples (Niessen, 2001; Dass, 2007). If high resolution mass analysers are coupled with these ionization techniques, high capability of identification can be achieved. Together with GC × GC a potentially new tool in olive oil compound identification is reachable and desirable.

The application of a multimolecular marker approach to fingerprint allows, in an easy way, the identification of certain sample characteristics. Chromatographic profiles can be processed as continuous and non-specific signals through multivariate analysis techniques. This allow to select and identify the most discriminant volatile marker compounds (Pizarro et al., 2011). The quantity and variety of information, provided by two-dimensional-GC (2D-GC) systems, promoted the increasingly application of chemometrics in order to achieve data interpretation in a usefull and, potentially, easy way. Linear discriminant analysis (LDA) and artificial neural networks (ANN), among other statistical classification methods, can be applied in order to control economic fraud. These applications have been carefully reviewed recently (Cajka et al., 2010). Together with 2D-GC systems the advantage is clear, since, instead of a time consuming trial to determine which variables should be considered for the statistical classification method, the selection may now become as simple as inspecting the 2D contour plots obtained (Cardeal et al 2008, de Koning et al., 2008). Also the use of statistical image treatment, of 2D-GC generated contour plots, can be applied for fingerprint recognitions, precluding the alignment of the contour plots obtained, which already allowed the identification of varieties as well as extraction technologies used to produce high quality Portuguese olive oils (Vaz Freire et al., 2009).

6. Conclusion

A final word should also be addressed to spectral libraries. Commercial spectral libraries are becoming increasingly more complete and specific, making GC/MS one of the most used techniques for routine identifications. However, several compounds are not yet described in library databases and, in spite of better algorithmic calculations, databases are only reliable for target analysis, or when the compounds under study are known, and already characterized with a known mass spectra. Additionally, the full separation of peaks to ensure clean mass spectra, in order to achieve a reliable peak analyte confirmation, is still a necessary goal.

Until now most of the analytical systems used to analyse olive oil volatile compounds are performed in 1D-GC systems with polar or apolar column phases. Since olive oil volatile fraction is very complex, frequent co-elutions occur. Mass spectra obtained are, consequently, not pure, which should preclude the possibility to compare the spectra obtained with the, claimed pure, spectra in the databases. However, tentative identifications are reported in the literature, and it is not rare that some inconsistencies occur, even when linear retention indices LRIs are presented. Because of their nature, the LRIs obtained in apolar columns are more reliable. Nevertheless, a better separation is obtained in 1D-GC systems when polar stationary phases are used, because of the wide chemical variety
comprised in the volatile fraction of olive oils. Unfortunately, these columns present a high variability, at least, among different purchasers, which do not facilitate LRIs comparison with literature data. Multidimensional techniques, hyphenated with mass-spectrometry, are now fulfilling this gap also in the separation of optical active compounds, when chiral column phases are used. Clean mass spectra together with compound LRIs in polar, apolar and chiral column phases represents an improved tool in compound identification and thus in olive oil matrices characterization. LRIs considering probability regions in the 2D resulting plot of a GC × GC experiment (with different column set combinations, e.g. polar × apolar, polar × chiral, etc.), can enable comparing standard compounds with the sample compounds retention indices and thus a more reliable peak identification can be achieved, if mass spectrometric data are simultaneously recorded. In the future, for 2D systems, more comprehensive mass spectral libraries should include retention index probability regions for different column sets in order to allow correlation of the results obtained in the used systems with spectral matches and literature LRIs.

7. Acknowledgment

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Olive Oil Composition: Volatile Compounds


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