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Review

# Volatile compounds in virgin olive oil: occurrence and their relationship with the quality

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**Abstract**

The stimulation of the human sensory receptors by volatile compounds present in virgin olive oils gives rise to the sensory attributes that describe its delicate and fragrant aroma. The composition of the volatile compounds and their biogenesis is briefly illustrated. Analytical methodologies for evaluating the volatile fraction and the sensory properties of virgin olive oils are elucidated. Compounds responsible for typical flavours are examined and the influence of the main factors on the composition of volatile compounds is discussed. The origin of off-flavours are also described and the consequent changes of volatile composition and of sensory characteristics are analysed. The relationships between volatile compounds and sensory attributes are discussed.


*Keywords:* Virgin olive oil; Sensory characteristics; Volatile compounds; Headspace analysis; Flavour; Off-flavour; Solid-phase microextraction; Food analysis

**Contents**



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## 1. Introduction

Virgin olive oil represents the main source of fats in the countries of the Mediterranean basin where the olive oil production is concentrated. However, nowadays, after some studies [1,2] have evidenced the beneficent effects on the health of the so called "Mediterranean diet" founded on the consumption of great amounts of vegetables, cereals, fish and olive oil, the cultivation of the olive tree is spreading in countries where the virgin olive oil consumption is very modest like Australia, Argentina and South Africa [3]. But another reason, owing to the increasing demand for olive oils of high quality, seems to be related to the increased popularity of this commodity, in addition to its healthy properties [4]. It is represented by its peculiar sensory characteristics that, because of use of virgin olive oil as a seasoning of cooked and especially raw foods, has great repercussions on the their acceptability.

A great number of researches proved that some non-volatile compounds are related in an indirect way to the positive effects on the human health [5,6], whereas the sensory attributes are directly ascribable to the strong stimulation of the human sensory receptors by both volatile and some non-volatile compounds [7–11] present in virgin olive oil.

Volatile and non-volatile compounds are retained by virgin olive oils during their mechanical extraction process from olive fruits (*Olea europaea* L.). Non-volatile compounds such as phenolic compounds stimulate the tasting receptors and also the free endings of trigeminal nerve eliciting the former the bitterness perception, the latter pungency, astringency and metallic attributes [12,13]. Instead volatile compounds, stimulating the olfactive receptors, are responsible for the whole aroma of the virgin olive oil.

## 2. Composition and biogenesis of volatile fraction

Many compounds, mainly carbonyl compounds, alcohols, esters and hydrocarbons, were found in the volatile fraction of virgin olive oil [14]. A list of the different compounds until now identified, assigned by GC–MS technique, was recently reported in literature [15].

The $C_6$ and $C_5$ compounds [16–18], especially $C_6$ linear unsaturated and saturated aldheydes represent the most important fraction of volatile compounds of high quality virgin olive oils from a quantitative point of view. But other neo-formation volatile compounds, namely $C_7$–$C_{11}$ monounsaturated aldehydes [19,20], or $C_6$–$C_{10}$ dienals [21], or $C_5$ branched aldehydesand alcohols [22] or some $C_8$ ketones [23], reach high concentrations in the aroma of virgin olive oils affected by organoleptic defects.

$C_6$ and $C_5$ compounds are enzymatically produced from polyunsaturated fatty acids through the so-called lipoxygenase (LOX) pathway and their concentrations depend on the level and the activity of each enzyme involved in this LOX pathway [24–32].

The pathway (Fig. 1) starts with the production of 9- and 13-hydroperoxides of linoleic (LA) and linolenic (LnA) acids mediated by lipoxygenase (LOX). The subsequent cleavage of 13-hydroperoxides is catalysed by very specific hydroperoxide lyases (HPL) and leads to $C_6$ aldehydes, whose unsaturated ones can isomerize from *cis*-3 to the more stable *trans*-2 form. The mediation of alcohol dehydrogenase (ADH) reduces $C_6$ aldehydes to corresponding alcohols, which can produce esters because of the catalytic activity of alcohol acetyl transferases (AAT) [33,34].

But an additional branch of the LOX pathway (Fig. 1) is active when the substrate is LnA. LOX would catalyse, besides the hydroperoxide formation, also its cleavage via an alkoxy radical giving rise to the formation of stabilized 1,3-pentene radicals. These last can dimerize leading to $C_{10}$ hydrocarbons (known as pentene dimers) or couple with a hydroxy radical present in the medium producing $C_5$ alcohols, which can be enzymatically oxidated to corresponding $C_5$ carbonyl compounds [16].

A recent investigation pointed out that olive seeds should contain enzymatic activities metabolising 13-hydroperoxides other than hydroperoxide lyase that are responsible for a decrease in the content of $C_6$ unsaturated aldehydes during the olive oil extraction. Moreover they would contribute with an alcohol dehydrogenase activity more specific for saturated $C_6$ aldehydes and especially with an AAT activity scarcely specific in terms of substrate that would be responsible for all kind of esters [35].

The other accumulation products come from possible fermentations or conversion of some amino acids or from enzymatic activities of moulds or finally from oxidative processes but are generally related to the off-flavour of virgin olive oil. Fig. 2 shows the pathways involved in the aroma production of a virgin olive oil. The size of arrows gives an idea of the importance of each path. The LOX pathway is predominant in oils of high quality, whereas a different relative importance of the pathways, according to the type of the sensory defect, is observed in the disagreeable aromas of defective oils [19,20,22,23].

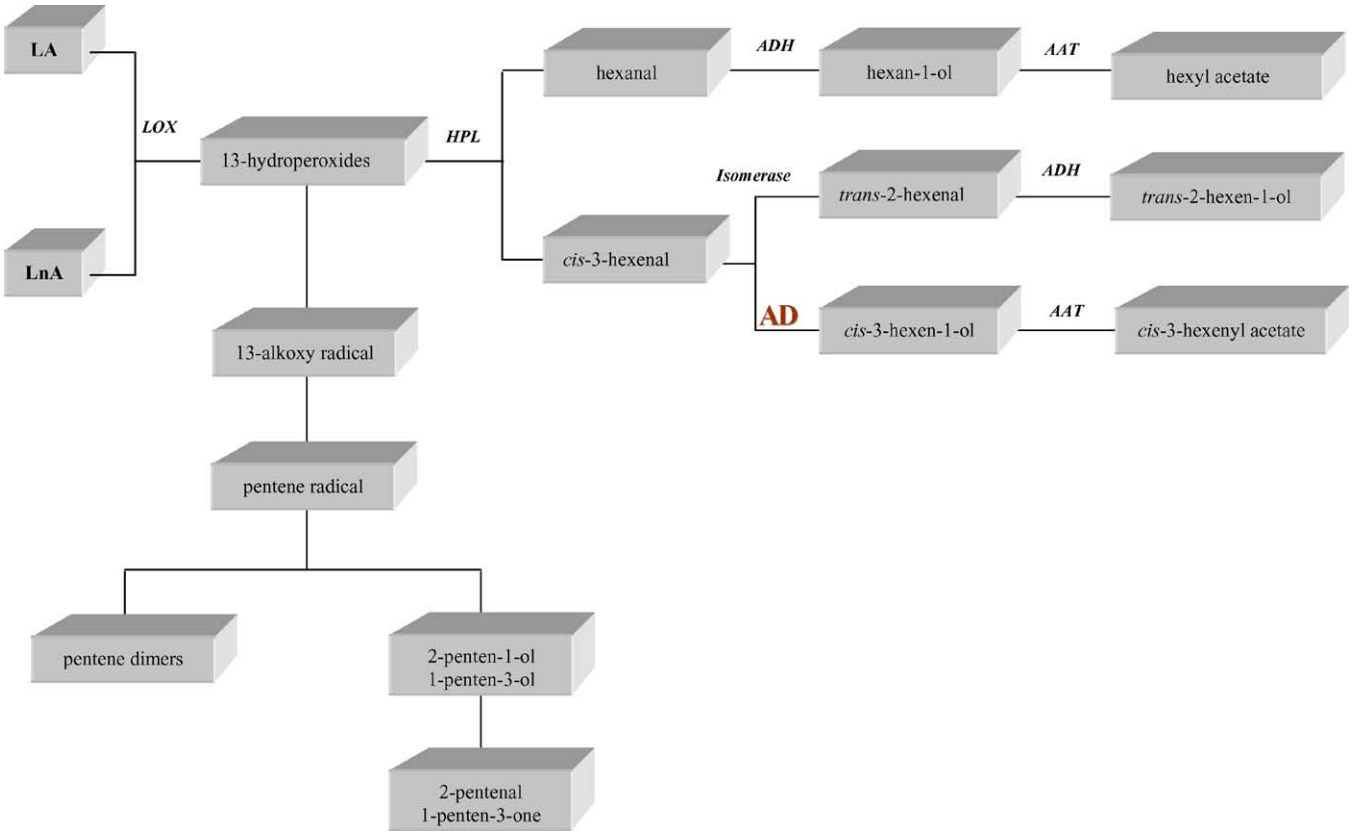


Fig. 1. Lipoxygenase pathways involved in the production of $C_6$ and $C_5$ volatile compounds.

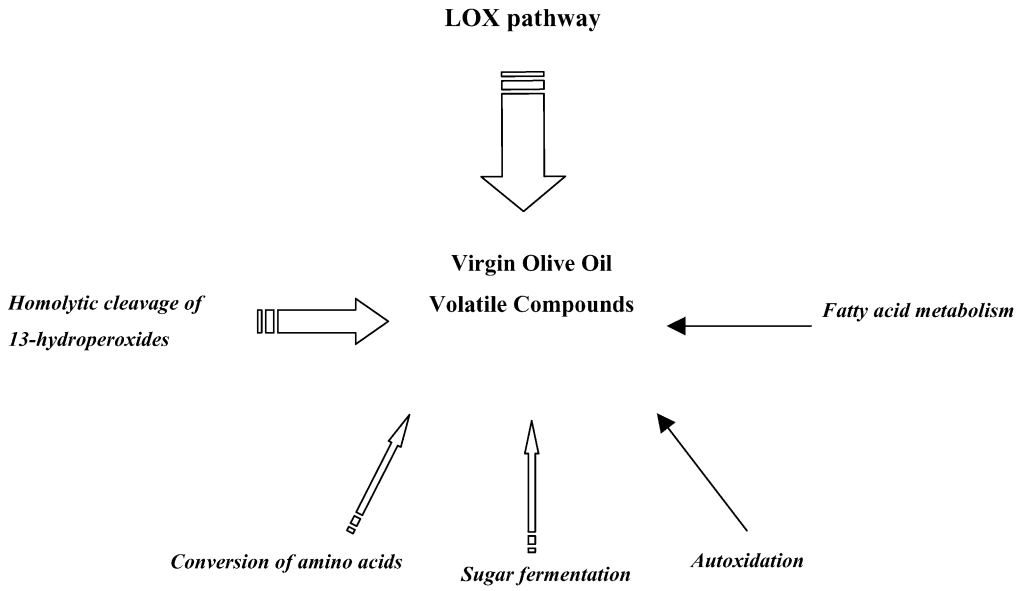


Fig. 2. The enzymatic and chemical paths involved in the production of virgin olive oil volatile compounds.

## 3. Analytical determination of volatile compounds

The most usual methods used for the evaluation of volatile compounds involve techniques with an enrichment step, since, because of the very low concentration of the most part of them, the sensitivity of methodologies like as direct injection or static headspace is very poor so that often does not allow their detection, being their concentrations under gas chromatographic detectability thresholds. This consideration can be applied to the traditional static headspace that utilizes vials of little volume (generally 10 mL) and a quantity of few grams of virgin olive oil. However, Montedoro et al. using a particular static headspace that concentrates, in static conditions, the headspace of 2 L of volume, in a Tenax trap, have obtained interesting results in terms of sampling efficiency of volatile compounds in virgin olive oil [36].

Among techniques with an enrichment step dynamic headspace, involving the stripping of volatile compounds, their trapping on a suitable adsorbent, their subsequent thermic desorption or elution with a solvent, is the most commonly applied.

The technique needs the careful definition of all variables affecting the analysis to have comparable data. The influence of temperature, sample size, absolute amount of gas used for the stripping, geometry of the trap, chemical–physical characteristics of compounds to be stripped on the amount of volatile compounds was the subject of several studies [32,37–43].

In any case in the optimisation of this technique it needs to bear in mind the following points:

- The temperature must be selected within a narrow range since at temperatures less than 20 °C the most part of volatile compounds cannot be stripped in an effective way. The upper limit is established by possible oxidative degradation of the oil matrix.
- The extraction parameters (sample size, flux and stripping time) are established by the extracting conditions from the matrix selected so that the greater number of compounds with a concentration higher than gas chromatographic detectability thresholds is stripped.
- The number, the quality and the quantity of volatile compounds depend on the adsorbent material.
- The desorption had not to produce artefacts.

The most popular adsorbents in determining volatile compounds of virgin olive oils are Tenax [7,9,26,31,36,42–44] and the charcoal [8,19,20,23,29,30,32,37,38,45–47]. Tenax is usually adopted as adsorbent material in purge and trap technique because of its good adsorption capacity of medium and high boiling point compounds, its high thermal stability, its poor affinity against water and last but not least the easiness of cleaning procedures [13]. The sampled compounds are thermally desorbed by heating the adsorbent at 280 °C under the carrier gas flow and sent directly to the GC injector [36,43]. Charcoal, in spite of the negative aspect due to a good affinity against water, has been successfully adopted in the determination of volatile fraction of virgin olive oils since the matrix contains low amount of water and, differently from Tenax, it shows a very strong adsorbent power towards all classes of chemical compounds, including those with low molecular weight [13,15], and therefore its use allows to obtain information about the oil quality and the conditions of technological operations adopted during the oil extraction. The extraction of volatiles from charcoal is generally performed by means of their elution at room temperature with a suitable solvent like diethyl ether. This gives the advantage of both keeping unaltered unsaturated compounds that could undergo molecular changes at the high temperatures (more than 300 °C) needed for their thermal desorption and the preservation of the volatile extract so that the same sample can be analysed more times with the same technique or by using more techniques like as HRGC and GC–MS.

Nowadays other approaches have been developed for the qualitative and quantitative analysis of volatile compounds of virgin olive oils.

Among new methods headspace-solid phase microextraction (HS-SPME) is very suitable and efficient for such analysis, moreover it is not much expensive. It consists of a fiber coated with a stationary phase (adsorbent) where volatile substances are adsorbed, the extent depending on the affinity that these compounds have towards the coating. Commercially are available different fibers for sampling volatiles having different polarity, so it is possible to analyse compounds ranging from apolar to polar. The method consists in exposing, for a determinate period of time, the fiber to the vapour phase (headspace) in equilibrium with the olive oil contained in a thermostated vial, sealed with a perforable septum. The analysis of the sampled compounds is performed by thermal desorption by inserting the fiber directly into the GC injector held at a suitable temperature. All the procedure, from sampling to injection, can be made manually or better using an autosampler.

The fibers used are: 65 μm Carbowax/divinylbenzene (CW/DVB) [43,48–52], 100 μm polydimethylsiloxane (PDMS) [49,50,53,55,56,58,59], 85 μm polyacrylate (PA) [49,50], 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS) [56], 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) [56] and 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) [49,54,56,57,60–62]. However, as recently reported the better efficiency in sampling the volatile compounds present in virgin olive oils is achieved using CW/DVB and DVB/CAR/PDMS fibers [63].

Even if the number of the compounds that can be sampled with this method is only sligthly less in comparison with dynamic headspace, with SPME is possible to analyse more than 130 chemical compounds, most of them belonging to the following chemical classes: aldehydes, alcohols, esters, hydrocarbons and ketones [51] and that, what is fundamental, SPME is able to evaluate the most volatile compounds

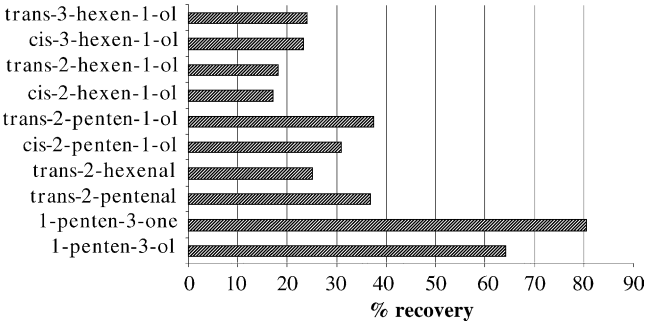


Fig. 3. Recovery (%) of some volatile compounds [8].

related to the flavour and off-flavour of the virgin olive oil [43,48,51,56,60,61,63].

Once volatile substances are desorbed, they are gas chromatographically separated on a capillary column and their quantitation generally is performed by using an internal standard.

However, in this way it is assumed that recoveries of analytes and their gas chromatographic responses are equal to one. Instead it was found that the recovery (DHSA) of the volatile compounds trapped by means of dynamic headspace, more important from a quantitative point of view, is different and depending on the number of carbon atoms and the kind and the position of the functional group (Fig. 3) [8].

A series of calibration straights, or more in general curves, must be carried out to overcome problems related to chromatographic behaviour of each volatile compound and its losses during the sample preparation to obtain their accurate quantitative determination. Both these aspects are brightly resolved by the Stable Isotope Dilution Assay technique in which the addition, as internal standards, of deuterated compounds of the analytes to be determined is involved. The very accurate quantitation of volatile compounds performed with this technique allowed to know which of them mainly contribute to virgin olive oil aroma [64,65].

## 4. The role of volatile compounds in the sensory quality of virgin live oils

The stimulation of olfactory receptors from volatile compounds present in virgin olive oils, transported by the air streams during inhalation and expiration actions, gives rise to perception of their aroma.

It is generally assumed that the intensity of stimuli elicited by volatile substances is related to their amount, therefore the simpler approach to searching of possible relationships between volatile compounds and sensory characteristics of virgin olive oils is to relate the concentrations of volatiles to the intensities of sensory attributes. This allowed evidencing some relationships between some volatile compounds and some sensory notes, especially defective, of virgin olive oils [19,20,23,24,66–69].

But not always the analytical evaluation of the concentration of each volatile compound is the right way to quantify and describe the aroma of virgin olive oils for some main reasons. Indeed, it is not always true that volatile compounds at higher concentrations are the main contributors of the aroma [70] since chemical factors, such as the volatility and the hydrophobic character, and size, shape, conformational structure of the molecules, type and position of functional groups seem to affect the odour intensity more than their concentration [71–73] because of their importance in establishing bonds with olfactory receptor proteins. Moreover each volatile compound contributes to the whole aroma, in addition to its concentration [39,74], according to its own sensory threshold levels [75] and its own odour quality, this last influenced by *cis-trans* isomerism and by the position of the double bond in the molecule [76]. Table 1 summarizes the odour quality found by different authors by means of sniffing tests for some of them [42,51,72,74–78].

Another reason that makes not reliable the analytical evaluation of the aroma is the fact that some compounds present in the oil flavour seem to stimulate at the same time olfactory and gustative receptors, besides the free endings of the trigeminal nerve determining a number of complex interactions [79,80] that give rise to some positive or negative synergisms.

The first point can be surmounted by evaluating the contribution of each volatile compound to the oil flavour by means of the calculation of the ratio of the concentration to its flavour threshold nasally and retronasally evaluated [81,82], known as odour activity value (OAV). Its calculation is very laborious since both quantitative data and sensory threshold have to be determined for the various volatile compounds. The OAV can be obtained more simply by using the Aroma Extract Dilution Analysis (AEDA). In this technique [81,82] the extract of volatile fraction is sequentially diluted according to a given volume ratio R. The diluted fraction is analysed by a reduced number of assessors by means of GC-Olfactometry (GC-O) technique and the procedure is repeated until no sensations are perceived by assessors. The ratio of the concentration of a volatile compound in the initial extract to its concentration in the most dilute extract in which odour was still detected by GC-O represents the flavour dilution factor (FD), that is proportional to OAV of the compound in air and therefore it is possible to calculate its odour activity value. Volatile compounds with higher OAVs mainly contribute to the typical or defective olive oil flavour. The technique, despite the no correction for losses of odorants during the isolation procedure, is the most common methodology for the evaluation of the potency of odorants in food extracts. However this solution is only partial, being the technique dependent by the sensory sensitivity of the assessors [81,82], because of individual differences in sensory thresholds occurring in human normal subjects [83,84].

Table 1
Odour qualities of some volatile compounds found by different authors by means of sniffing technique

| Compound | Odour quality | References |
|---|---|---|
| Aldehydes | | |
| Acetaldheyde | Pungent, sweet, floral | [51,77] |
| Propanal | Sweet, pungent, floral | [51,77] |
| 2-Methyl-propanal | Cooked, caramel | [51] |
| Hexanal | Green, apple, cut grass | [51,74] |
| Heptanal | Fatty | [64] |
| Octanal | Citrus-like, soapy | [51,64,77] |
| Nonanal | Soapy, citrus-like | [64] |
| Decanal | Soapy, citrus-like | [64] |
| 2-Methyl butanal | Malty | [77] |
| 3-Methyl butanal | Sweet, fruity, malty | [74,77] |
| 2-Methyl-2-butenal | Apple | [51] |
| *trans*-2-Pentenal | Green, apple, floral | [51,74] |
| *cis*-2-Pentenal | Green, pleasant | [74] |
| *trans*-2-Hexenal | Bitter, almonds, green, green apple-like, fatty, bitter almond like, cut grass | [51,64,74,77] |
| *cis*-2-Hexenal | Green, fruity, sweet | [74] |
| *trans*-3-Hexenal | Artichoke, green, floral | [74] |
| *cis*-3-Hexenal | Green leaves, grassy, green, apple-like, leaf-like, cut grass | [51,74,75,77] |
| 2-Octenal | Fruity, soap, fatty | [74,78] |
| *cis*-2-Nonenal | Green, fatty | [77] |
| *trans*-2-Nonenal | Paperlike, fatty, sharp, cut grass | [51,77] |
| 2-Decenal | Fatty | [78] |
| 2,4-Hexadienal | Cut grass | [51] |
| 2,4-Heptadienal | Fatty, nutty | [75] |
| 2,4-Nonadienal | Deep-fried | [77] |
| 2,6-Nonadienal | Cucumber-like | [64] |
| 2,4-Decadienal | Deep-fried | [51,77] |
| Benzaldehyde | Almond | [51] |
| Phenylacetaldheyde | Pungent, phenolic | [51] |
| Ketones | | |
| Pentan-3-one | Sweet | [74] |
| 1-Penten-3-one | Sweet, strawberry, sharp, Pungent, green, metallic | [51,71,74,77] |
| 1-Octen-3-one | Mushroom-like | [51,77] |
| Alcohols | | |
| Ethanol | Alcoholic, ripe apple, floral | [51,77] |
| Pentan-1-ol | Pungent | [74] |
| Hexan-1-ol | Fruity, aromatic, soft, cut grass | [39,51,74] |
| 2-Methyl-propan-1-ol | Ethyl acetate-like | [74] |
| 2-Methylbutan-1-ol | Fish oil | [74] |
| *cis*-2-Penten-1-ol | Banana | [74] |
| *trans*-3-Hexen-1-ol | Fruity, fatty, pungent, cut grass | [51,76] |
| *cis*-3-Hexen-1-ol | Banana, leaf-like, green-fruity, pungent | [74,76,77] |
| *trans*-2-Hexen-1-ol | Green, grassy, fruity, fatty, pungent | [74,76] |
| *cis*-2-Hexen-1-ol | Green fruit, green-fruity | [74,76] |
| 1-Penten-3-ol | Wet earth | [74] |
| Esters | | |
| Methyl acetate | Ester | [51] |
| Butyl acetate | Green, pungent, sweet | [74] |
| Ethyl acetate | Sweet, aromatic | [74] |
| Ethyl propanoate | Sweet, strawberry, apple | [74] |
| Ethyl butyrate | Cheesy, fruity | [77] |
| Ethyl isobutyrate | Fruity | [77] |
| Ethyl 2-methylbutyrate | Fruity | [77] |
| Ethyl 3-methylbutyrate | Fruity | [74] |
| *cis*-3-Hexenyl acetate | Green-banana, fruity, Green, green leaves, floral, ester | [39,51,64,74] |
| Hexyl acetate | Sweet, fruity, floral | [51,74] |
| 3-Methylbutyl acetate | Banana | [74] |
| Methyl 2-methylbutyrate | Fruity | [77] |
| Methyl decanoate | Fresh | [74] |
| Methyl nonanoate | Fruity, sweet, floral | [39,74] |
| Acids | | |
| Acetic acid | Pungent, like acetic acid | [51,74,77] |
| Propanoic acid | Aromatic, pungent | [74] |
| Butanoic acid | Buttery, rancid | [78] |
| Pentanoic acid | Sweaty, pungent, putrid | [51,78] |
| Hexanoic acid | Sweaty, pungent | [74] |
| 3-Methylbutyric | Sweaty | [77,78] |
| 2-Methylbutyric | Sweaty | [77,78] |
| Others | | |
| Methylbenzene | Glue, solvent-like | [74] |
| Ethylbenzene | Strong | [74] |
| Ethylfuran | Sweet, rancid | [39,74] |
| Dimethyl sulfide | Organic, wet earth | [51] |
| Dipropyl disulfide | Cooked meat | [51] |
| Cyclopropane | Musk | [51] |

A variation of the dilution technique is represented by the methodology developed by Acree et al. [85]. They by using a video terminal in addition to a HRGC apparatus, calculate "charm" values. They construct a charm response chromatograph plotting the dilution value on the *y*-axis versus retention index on the *x*-axis. The resulting peak areas are relative measures of the odour intensities eluting from the gas chromatograph in a particular region.

On the contrary, analytical tools are not able to evaluate the importance of interactions and synergisms arising from the involvement of more than one kind of receptor.

Nevertheless, the application of statistical procedures to amounts of volatile compounds and to intensities of different sensory notes evaluated by means of the official methodology evidenced other relationships. A Linear Regression Analysis, highlighting some correlations between volatile compounds arising from the LOX pathway and the nuances of "green" sensory attributes [86], gave evidence that hexanal concentration affects the most part of "green" attributes, whereas the inter- and intra-relationships occurring between the sensory

notes and the concentrations of volatile compounds were evidenced by Morales et al. [7] by means of the application of Multidimensional Scaling (MDS) technique. Moreover, accurate predictions of overall gradings given by tasters according to the official sensory analysis to virgin olive oils of different quality and origin sprang from the application of an Artificial Neural Network (ANN) to the amount of all volatile compounds extracted with a dynamic headspace technique [87]. The same results were achieved by Servili et al. [51,88] who applied Principal Component Analysis (PCA) and Partial Least Square (PLS) to concentrations of volatile compounds and to sensory attributes evaluated by means of free-choice profiling analysis.

The construction, by means of statistical procedures, of a sensory wheel [9,89] gave as a result the clustering of sensory notes with similar semantic description into a number of sectors generally identified by a given sensory property. The attribution of the sensory wheel coordinates to each volatile compound allowed Aparicio and Morales to obtain information on the sensory characteristics of different volatile compounds on the basis of the sensory properties of the sectors of the wheel where each of them fell. “Green” sector included *cis*-3-hexen-1-ol, *cis*-3-hexenal and *cis*-3-hexenyl acetate; alcohols such as *trans*-2-hexen-1-ol and hexan-1-ol belonged to the undesirable sector, whereas a few compounds fell in the “bitter-pungent” sector, evidencing their synergic effect on these attributes, confirmed by results of others with other statistical techniques [51], putting in evidence also a great importance of *trans*-2-hexenal in describing the “green” sensory note.

A recent approach that takes into account of possible synergisms among the different volatile compounds that form the virgin olive oil aroma is represented by the application of electronic nose (EN) to its headspace. ENs consist of an array of sensors, a electronic circuitry, a sampling system and a data analysis software. The sensor array is formed by some conducting polymers, which can change their conductance when exposed to gases and are be able to recognize a food fingerprint. The response intensity of each sensor is related to its sensitivity to food product, but it can be suitably modified by acting properly on the variable to be optimised: obviously the optimum is represented by the balance of the response intensities of all sensors. The electronic olfactometry, an artificial approach to human olfaction, combined with pattern recognition techniques, in addition to the advantage of providing results which derive from the global information coming from the whole volatile fraction, allows to obtain the rapid, and effective classification of odours by means of a very simple sample preparation that does not require the previous separation of compounds present in the volatile fraction of a food, their identification and quantitation, with a reduction in the time and cost of analysis for sample. In spite of they are still in a developmental phase, ENs have already provided interesting results on the evaluation of food quality and authenticity, on the quality control of oils especially for the monitoring of rancidity, and the discrimination of quality, olive variety and geographical origin [90–97].

## 5. Effect of agronomic and technological aspects on the volatile fraction

A number of investigations were aimed to find some correlations that can explain the presence of positive or negative sensory notes that are perceived by tasters during the virgin olive oil tasting.

The most important positive attribute is represented by fruity sensory note, the sensation reminiscent of healthy fruits harvested at the right ripening degree. To the aroma of high quality oil, in addition to fruity, generally contribute the “green” sensation reminiscent of just cut grass, leaf, tomato, artichoke, walnut husk, apple or other fruits. The flavour of these oils is accompanied by more or less intense notes of bitterness and pungency, ascribable to secoiridoid compounds.

The determination of volatile compounds highlights that $C_6$ and $C_5$ compounds mainly form the volatile fraction. The concentration of each of them, responsible for the different nuances of the positive attributes [86], is dependent on the level and the activity of enzymes involved in the LOX pathway. The enzymatic levels are genetically determined, whereas a number of factors affect their activities.

### 5.1. Agronomic and climatic aspects

Following will be briefly examined the different agronomic and climatic effects that influence virgin olive oil volatile compounds.

#### 5.1.1. Fruit soundless

An essential point to obtain high quality olive oil is the processing of healthy fruits. Unfortunately when *Bactrocera oleae* attacks fruits damages cause a considerable increase of carbonyl compounds and alcohols is related to the stage of the development of the olive fly and the intensity of attack [66,67].

#### 5.1.2. Cultivar

The influence of the cultivar [25,51] can be evidenced by the different amounts of $C_6$ compounds arising from the enzy-

Table 2
Composition of $C_6$ compounds (ppm) of oils from three different cultivars [25]

| Compound | Gentile di Chieti | Leccino | Koroneiki |
|---|---|---|---|
| Hexanal | 1.4 | 2.0 | 0.9 |
| Hexan-1-ol | 0.1 | 2.2 | 0.1 |
| Hexyl acetate | 0.2 | 0.0 | 0.4 |
| *trans*-2-Hexenal | 7.0 | 53.0 | 4.9 |
| *trans*-2-Hexen-1-ol | 0.1 | 6.3 | 0.3 |
| *cis*-3-Hexen-1-ol | 1.1 | 0.5 | 1.0 |
| *cis*-3-Hexenyl acetate | 0.8 | 0.0 | 1.6 |

Table 3
Concentration of *trans*-2-hexenal (expressed as ppm) and percent distribution of metabolites from LnA in oils from fruits of the main Spanish, Greek and Italian cutivars

| Cultivar | *trans*-2-Hexenal (ppm) | *trans*-2-Hexenal (%) | *trans*-2-Hexen-1-ol (%) | *cis*-3-Hexen-1-ol (%) | *cis*-3-Hexyl acetate (%) |
|---|---|---|---|---|---|
| Mastoidis | 17.1 | ■■■ | | | |
| Canino | 30.3 | ■■■ | | | |
| Picual | 23.2 | ■■■ | | ● | |
| Leccino | 47.3 | ■■ | ●● | | |
| Dritta | 11.4 | ■■ | ●● | | |
| Bosana | 12.1 | ■■ | ●● | | ● |
| Carolea | 7.4 | ■■ | | ●● | |
| Provenzale | 5.7 | ■■ | | ● | ● |
| Nocellara del Belice | 6.8 | ■■ | | ●●● | ● |
| Gentile di Chieti | 6.5 | ■■ | | ●●● | |
| Koroneiki | 4.6 | ■ | | ●●● | ●●●● |
| Moraiolo | 1.8 | ■ | ● | ●●●●●●●● | ● |

● > 5% (each symbol more increase of 5 units the percentage value); ■ < 50%; ■■ > 50%; ■■■ > 90%. Unpublished results; the evaluation of volatile compound was performed as reported previously by Angerosa et al. [16].

matic oxidation of linolenic acid of oils obtained in the same operative conditions of the extraction process from fruits of different cultivars harvested at the same ripening stage (Table 2). The minor dependence of the amount of volatile compounds from the climatic variables and where fruits are grown [25] underlines that cultivar is the dominant factor in the formation of the oil aroma. This feature, together with the different concentration of *trans*-2-hexenal, represents an effective tool [25] to differentiate monovarietal oils from different cultivars (Table 3).

However, when fruits from two different varieties are simultaneously processed, their enzymatic stores interact and some synergisms occur [98]. The volatile profile changes from the qualitative and, more often, quantitative point of view in relation to the percentage of fruits of each cultivar and does not reflect the volatile composition of any of the considered cultivars nor of the blend of oils of the same varieties at the same percentage. A greater accumulation of volatile compounds (Fig. 4) is observed at a given percentage of fruits, that is different according to cultivars used [98].

### 5.1.3. Ripeness

Several researches [28,47,99,100] proved that during the olive ripeness the amount of volatile compounds, especially of *trans*-2-hexenal, increases until a maximum concentration occurring when fruits turn their skin colour from yellow-green to purple (Fig. 5).

Beyond this time the concentration of volatile compounds decreases because of a lower activity of enzymes involved in their production with a weakening of the intensity of some “green” sensory notes. This trend was not found by Aparicio and Morales [27] who described a steady decrease of the concentration of the volatile compounds, including *trans*-2-hexenal, from the unripe to the over-ripe stages, except for oil from Coratina fruits that, on the contrary, confirmed the behaviour observed by the other researchers [28,47,99,100].

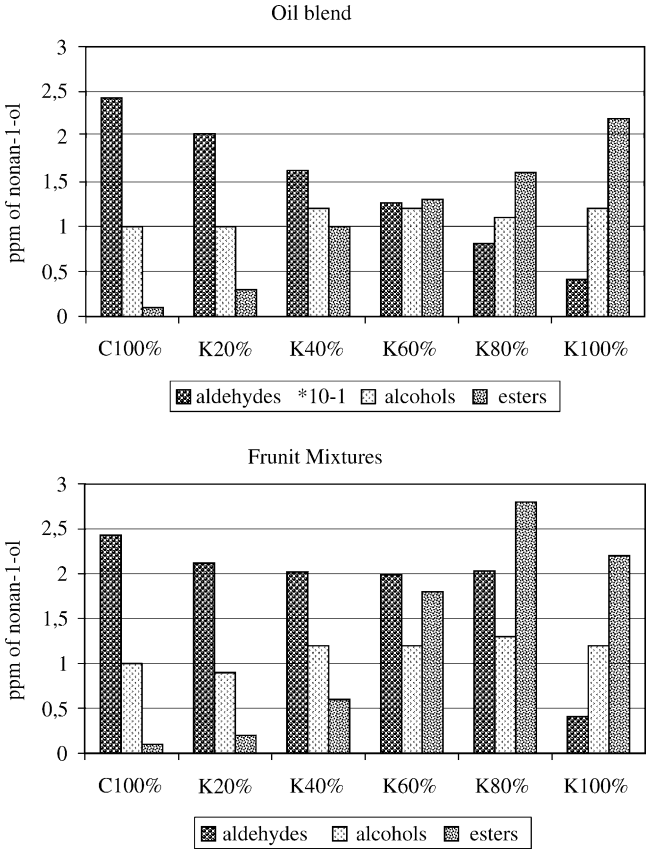


Fig. 4. Changes in the concentration of $C_6$ aldehydes, alcohols and esters in samples from both the blend of monovarietal oils and from the processing of mixtures of olive fruits. C = Coratina cv; K = Koroneiki cv. [98].

### 5.1.4. Climatic conditions

The genetic effect related to the cultivar is one of the most important aspects of volatile composition of olive oil. However, climatic and agronomic conditions of olive growing can affect volatile composition of olive oils obtained by the same cultivar. In this ambit the relationships between the water availability during fruit ripening and the volatile composition

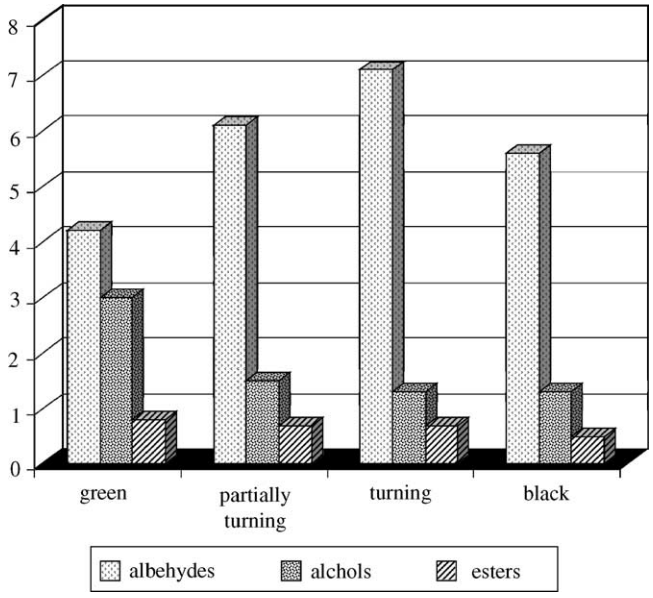


Fig. 5. Evolution of amounts of $C_6$ aldehydes, alcohols and esters of oils obtained from fruits (cv Gentile di Chieti) at different ripening degrees [100].

has been studied [91,101]. Results showed that, in the climatic conditions of central Italy, the rainfall effect is pre-eminent with respect to temperature and that some compounds, such as hexanal and isobutyl acetate, were negatively correlated to rainfall.

These results were recently confirmed in Leccino cultivar grown in Tuscany under two different hydric conditions (Table 4) [102].

In conclusion we can consider that several agronomic and climatic parameters can affect the volatile composition of olive oils. For this reason volatile compounds can be considered as markers, with sensory impact, that can be used to differentiate them.

### 5.1.5. Origin area

Also the geographic origin of oils plays a fundamental role in defining the volatile compounds profile of virgin olive oils. Early studies conducted by Montedoro et al. [103], have shown the ability of volatiles, sampled with the static headspace, in discriminating different oils coming from different Italian regions. Successive studies, obtained using HS-SPME–GS/MS, confirmed the relationships between the volatile composition of virgin olive oil and their origin area. In fact, the score plot of principal component analysis (PCA) applied to Mediterranean and Australian virgin olive oils, reported in Fig. 6, shows a good discrimination of oil according to the origin area, when volatile compounds are used as analytical parameters to build the multivariate statistical model were well discriminated using volatile compounds as markers.

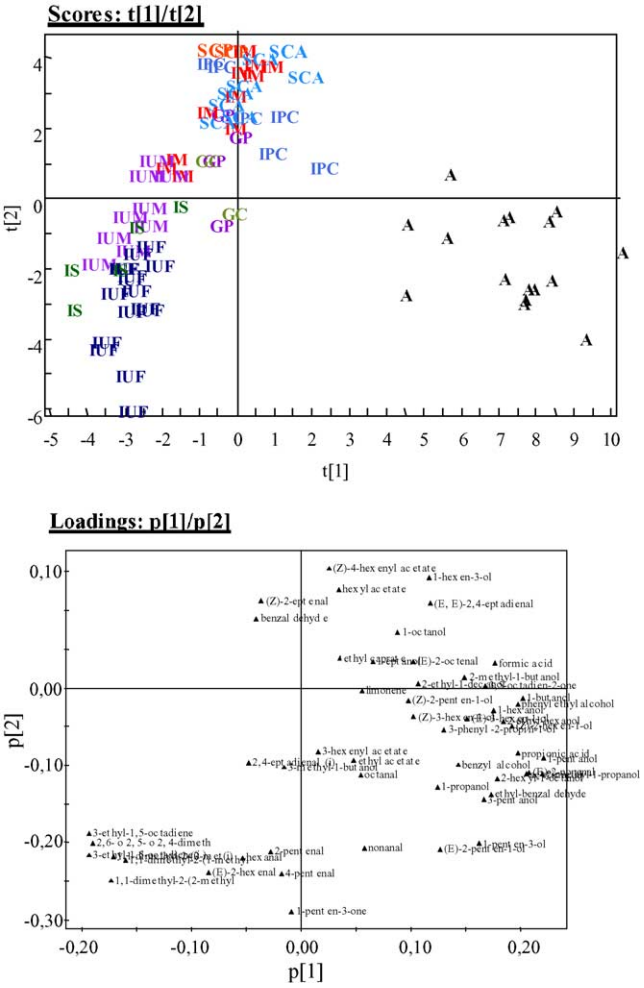


Fig. 6. Score-plot and loading-plot of the first two components of the PCA model obtained with 79 virgin olive oil samples from different origin areas. Abbreviation used: the first character denotes the country (I = Italy; G = Greece; S = Spain; A = Australia), the second, where present, denotes the region (U = Umbria; M = Molise; A = Apulia; S = Sardinia; C = Crete; P = Peloponnesus; C= Catalonia) and the third, where present, defines the cultivars (M = Moraiolo; F = Frantoio; C = Coratina; A= Arbequina; P= Picual). Unpublished results; the evaluation of volatile compound was performed as reported previously by Servili et al. [51].

## 5.2. Technological aspects

The different technological operations have repercussion on the volatile composition. The influence of each single operation will be separately analysed.

### 5.2.1. Harvesting methods

Nowadays olive harvesting is mechanically performed and always less frequently olives are picked by hand from the tree. However, in some areas of olive production olives are gathered from the ground by using brushes and aspirators at regular intervals of time until the end of the spring [104]. A considerable increase of volatile alcohols and carbonyl compounds having unpleasant aroma and the appearance of a typical defect, reminiscent of “mouldy” and “earthy” tastes

Table 4
Concentrations of volatile compounds in virgin olive oils from Leccino olives trees grown under irrigated or rainfed conditions at Bibbona (Italy)

| | Green skin | | | | Black skin | | | |
|---|---|---|---|---|---|---|---|---|
| | Irrigated | | Rainfed | | Irrigated | | Rainfed | |
| | Mean | C.V. | Mean | C.V. | Mean | C.V. | Mean | C.V. |
| l-Propanol | 1026 | 3.3 | 1206 | 11.0 | 1283 | 8.8 | 1866 | 15 |
| Hexanal | 86539 | 5.8 | 67461 | 2.8 | 51501 | 27 | 62315 | 12 |
| 3-Pentanol | 2228 | 8.1 | 2113 | 8.7 | 2075 | 13 | 1942 | 9 |
| 1-Butanol | 1945 | 9.8 | 5616 | 5.3 | 3328 | 11 | 2193 | 30 |
| 2-Pentenal | 7215 | 15.2 | 4116 | 13.2 | 4438 | 30 | 3636 | 18 |
| l-Penten-3-ol | 91407 | 16.3 | 63087 | 19.5 | 81484 | 20 | 97994 | 17 |
| (*Z*)-2-Hexenal | 22134 | 9.0 | 22635 | 4.5 | 26898 | 12 | 25238 | 7 |
| (*E*)-2-Hexenal | 4121439 | 4.6 | 3550408 | 10.0 | 4038044 | 5 | 4015511 | 3 |
| Hexyl acetate | 1329 | 17.9 | 1724 | 9.5 | 727 | 5 | 1490 | 4 |
| 3-Hexenyl acetate | 22218 | 1.5 | 2500 | 10 | 133841 | 8 | 24063 | 14 |
| (*E*)-2-Penten-l-ol | 8624 | 6.5 | 6208 | 0.1 | 8094 | 1 | 8358 | 29 |
| (*Z*)-4-Hexenyl acetate | 5273 | 2.0 | 4145 | 31.5 | 5675 | 13 | 4552 | 0.7 |
| (*Z*)-2-Penten-l-ol | 24403 | 7.8 | 25436 | 6.6 | 26418 | 17 | 27989 | 22 |
| 6-Methyl-5-hepten-2-one | 116 | 141.4 | 7165 | 4.1 | 0 | 0 | 348 | 11 |
| 1-Hexanol | 22346 | 13.1 | 535 | 15.1 | 33195 | 30 | 24121 | 15 |
| (*E*)-3-Hexen-l-ol | 317 | 1.1 | 2599 | 18.0 | 901 | 46 | 623 | 13 |
| (*Z*)-3-Hexen-l-ol | 29451 | 10.7 | 16387 | 26.9 | 39279 | 9 | 34736 | 17 |
| (*E*)-2-Hexen-l-oI | 34582 | 8.0 | 35581 | 12.5 | 44914 | 5 | 44249 | 6 |
| (*Z*)-2-Hexen-l-ol | 68786 | 0.4 | 20364 | 2.2 | 206088 | 11 | 196386 | 4 |
| l-Hexen-3-ol | 927 | 6.7 | 184 | 10.2 | 2190 | 18 | 1858 | 32 |

Fruits were harvested at green skin or black skin ripening stage. Values indicate means and coefficients of variation (C.V.) of area counts [102].

at the same time [67], can be considered as a consequence of the prolonging of contact time of fruits with the ground.

### 5.2.2. Olive fruit storage

The storage of olives in unsuitable conditions, into sacks or in piles, has heavy negative repercussions on the sensory quality of resulting oils. The production of different metabolites, according to the type of microorganisms from environment [22] whose development is promoted by the temperature reached in the pile and the humidity degree, gives rise to different sensory defects, better evidenced by the weakening of positive perceptions related to the decrease in concentrations of compounds from LOX cascade. First *Clostridia* and *Pseudomonas* genera develop producing (Fig. 7) branched aldehydes, branched alcohols and their corresponding acids [22,66,105,106] of which concentrations in a few days their overstep the threshold levels for the perception of "fusty" defect.

However sometimes, especially if the temperature is relatively high, it can occur an important growth of yeasts with production of considerable amounts of ethanol and ethyl acetate and the onset of the "winey" defect. The possible presence of *Acetobacter* is responsible for the "vinegary" defect because of the production of acetic acid [22].

On the other hand if the fruit storage lasts several days it can be a development of moulds, generally belonging to *Penicillium* and *Aspergillus* genera [107], whose enzymes interfere with those of the olive fruit involved in the LOX pathway [108–110] causing, according to the importance of the mould invasion, in addition to the complete rotting of fruits, both a reduced production of $C_6$ compounds and the formation of $C_8$ compounds [23], common metabolites of the LOX pathway of moulds.

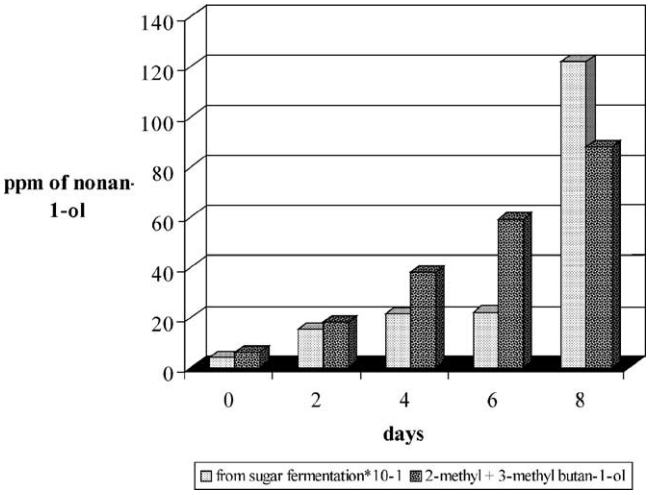


Fig. 7. Production of metabolites from sugar fermentation and from degradation of some amino acids during the conservation of olives in jute sacks [22].

Storage temperatures at about 5 °C in air considerably could reduce the fungal growth, so that olives could be stored for at least 30 days without great changes in the sensory quality of the resulting oil [111].

### 5.2.3. Washing operation

Washing operation is always recommended by technologists and is particularly important when olives, after several

rainy days, can be spattered with mud or can keep some earth [112].

So far however hot-water treatments of olive fruits, before the processing, change the volatile aroma profile of virgin olive oils. Generally the main changes concern the decrease in content of $C_6$ aldehydes and $C_5$ compounds, probably for a partial deactivation of the lipoxygenase/hydroperoxide lyase enzyme system, whereas $C_6$ alcohols and esters keep constant their concentration suggesting that the activities of ADH and AAT are slithly affected by heat treatments [113].

### 5.2.4. Crushing

As it was already remembered, almost all volatile compounds of a good quality olive oil give rise at the moment of tissue disruption of the olive pulp, therefore the effectiveness of crushing plays an important role in their production. The use of an hammer mill crushers [29], which determining a more violent grinding of pulp tissues causes an increase of the olive paste temperature and the reduction of hydroperoxide lyase activity [114,115], has as a consequence the production of oils characterized by a lower amount of volatile compounds, especially of *trans*-2-hexenal, hexanal and *cis*-3-hexen-1-ol, compared with the concentration of the same compounds in oils obtained with the same processing diagram except for the crushing performed by means of a stone mill (Fig. 8) [29].

In the last 5 years a new generation of continuous crushers have been done. In this context the use of new crushers such as the blade crusher improves the concentration of volatile compounds, especially of hexanal, *trans*-2-hexenal and $C_6$ esters, with a consequent increase of the intensity of grass and floral sensory notes [116].

### 5.2.5. Malaxation

Time and temperature of malaxation affect the volatile profile and therefore the sensory characteristics of the resulting oils.

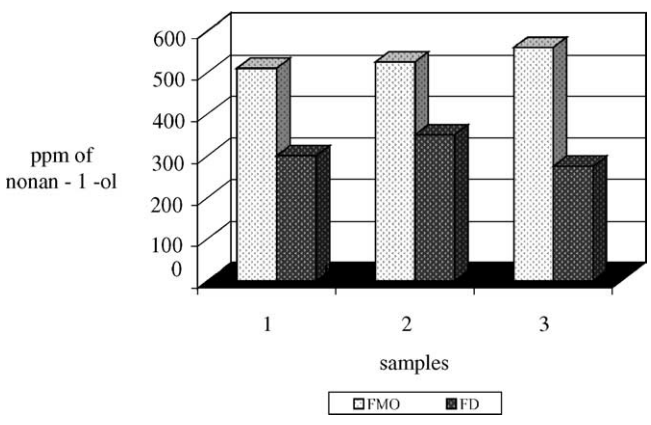


Fig. 8. Total amount of volatile compounds of oils obtained with the same processing diagram by using a stone mill (FMO) and a hammer crusher (FD) [29].

The increase of alcohols and of $C_6$ and $C_5$ carbonyl compounds, especially of hexanal, which, due to its low odour threshold [79], represents an important contributor to the olive oil flavour, is the main effect of the malaxation time, whereas high temperatures of malaxation [31,32] promote a fall of esters and *cis*-3-hexen-1-ol and an accumulation of hexan-1-ol and *trans*-2-hexen-1-ol, both considered by some authors as eliciting odour not completely agreeable. In addition high temperatures in the malaxation step make active the amino acid conversion pathway with production of considerable amounts of 2-methyl-butanal and 3-methyl-butanal, but without accumulation of corresponding alcohols [32], correlated with "fusty" defect [22].

The sensory analysis highlights a weakening of typical "green" attributes with the prolonging of malaxation time and of all sensory notes with high temperatures during the malaxation [32]. Furthermore, in both conditions there is a decrease of bitterness and pungency due to the losses of phenols [117–119].

The LOX is not the only oxidoreductase active in the olive pastes during malaxation. In fact, peroxidase and polyphenoloxidase are activated during crushing and oxidize phenolic compounds during malaxation reducing their concentrations in the pastes and in the oils. For this reason during the last 10 years several works were performed to control selectively endogenous oxidoreductases in the pastes during this technological process. In this ambit the use of inert gas to remove oxygen in the headspace of malaxer was studied [120–123]. The results show that the use of $N_2$ during malaxation not only reduces the oxidative degradation of phenolic antioxidants but, at the same time, hardly modifies the volatile composition of oil (Table 5). To optimize and increase volatile and phenolic composition the Time of Exposure of Olive Pastes to Air Contact (TEOPAC) was consequently studied as processing parameter to control the oxygen concentration in the pastes during malaxation [125].

### 5.2.6. Separation system

The final volatile profile is also influenced by the system used for extracting oil. The most widespread systems for the separation of the oil from the olive pastes are the centrifugation and pressing methods. The losses in volatile compounds depend on the importance of the interactions between on one hand oil and solids and on the other oil and vegetation waters that are reduced to a minimum when the traditional pressing plant is adopted. But it must be underlined that for obtaining high quality olive oils pressing plant needs to work fruits of the same good quality and in a continuous way to prevent possible fermentations and/or degradation phenomena of residues of pulp and of vegetation waters on the filtering diaphragms, which could give rise to the defect named "pressing mats" [124].

The addition of warm water to dilute the olive pastes to be extracted by means of the tri-phase centrifugal decanters can explains the decrease in $C_6$ alcohols, hexan-1-ol and *trans*-2-hexen-1-ol with respect to oils separated by pression

Table 5
Effect of the TEOPAC on the main volatile compounds of virgin olive oil [122]

| | Air exposure time | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0′ | 10′ | 20′ | 30′ | 40′ | 50′ | 60′ |
| Pigmentation index: 2.2 | | | | | | | |
| Volatile compounds (μg/kg) | | | | | | | |
| Hexanal | 512 ± 26 | 490 ± 135 | 469 ± 10 | 560 ± 27 | 348 ± 59 | 420 ± 35 | 494 ± 151 |
| 1-Penten-3-ol | 640 ± 22 | 891 ± 51 | 782 ± 16 | 781 ± 43 | 711 ± 111 | 810 ± 21 | 1027 ± 95 |
| (*E*) 2-Hexenal | 12754 ± 586 | 18044 ± 1256 | 12254 ± 780 | 12124 ± 541 | 17146 ± 511 | 14035 ± 7 | 17465 ± 266 |
| 1-Hexanol | 144 ± 18 | 252 ± 9 | 170 ± 19 | 117 ± 1 | 104 ± 1 | 108 ± 17 | 251 ± 10 |
| (*Z*) 3-Hexen-1-ol | 1454 ± 43 | 1448 ± 74 | 1469 ± 86 | 1429 ± 48 | 1199 ± 77 | 1337 ± 20 | 1505 ± 3 |
| (*Z*) Hexen-1-ol | | | | | | | |
| | 321 ± 71 | | | | | | |
| | 471 ± 14 | | | | | | |
| | 333 ± 6 | | | | | | |
| | 380 ± 11 | | | | | | |
| | 406 ± 13 | | | | | | |
| | 394 ± 22 | | | | | | |
| | 424 ± 20 | | | | | | |
| Pigmentation index: 2.6 | | | | | | | |
| Volatile compounds (μg/kg) | | | | | | | |
| Hexanal | 287 ± 9 | 552 ± 52 | 492 ± 24 | 611 ± 13 | 549 ± 67 | 482 ± 10 | 490 ± 32 |
| 1-Penten-3-ol | 494 ± 52 | 786 ± 51 | 816 ± 21 | 769 ± 13 | 771 ± 46 | 780 ± 8 | 892 ± 115 |
| (*E*) 2-Hexenal | 22918 ± 80 | 28060 ± 4698 | 27977 ± 276 | 27050 ± 329 | 29663 ± 168 | 24393 ± 756 | 40994 ± 2288 |
| 1-Hexanol | 289 ± 2 | 345 ± 28 | 386 ± 3 | 429 ± 13 | 437 ± 2 | 358 ± 15 | 385 ± 5 |
| (*Z*) 3-Hexen-1-ol | 1095 ± 39 | 1826 ± 213 | 1903 ± 3 | 1801 ± 24 | 1660 ± 6 | 1542 ± 134 | 1376 ± 13 |
| (*Z*) Hexen-1-ol | | | | | | | |
| | 707 ± 58 | | | | | | |
| | 519 ± 56 | | | | | | |
| | 600 ± 20 | | | | | | |
| | 770 ± 30 | | | | | | |
| | 711 ± 27 | | | | | | |
| | 635 ± 57 | | | | | | |
| | 864 ± 19 | | | | | | |
| Pigmentation index: 2.9 | | | | | | | |
| Volatile compounds (μg/kg) | | | | | | | |
| Hexanal | 258 ± 3 | 467 ± 0 | 409 ± 18 | 314 ± 45 | 365 ± 39 | 354 ± 59 | 352 ± 28 |
| 1-Penten-3-ol | 275 ± 37 | 496 ± 46 | 552 ± 97 | 452 ± 118 | 506 ± 1 | 606 ± 14 | 538 ± 15 |
| (*E*) 2-Hexenal | 4872 ± 163 | 8532 ± 230 | 12194 ± 658 | 9309 ± 293 | 8771 ± 316 | 11011 ± 332 | 8028 ± 518 |
| 1-Hexanol | 441 ± 9 | 412 ± 12 | 417 ± 22 | 308 ± 4 | 507 ± 17 | 672 ± 51 | 699 ± 19 |
| (*Z*) 3-Hexen-1-ol | 412 ± 21 | 384 ± 9 | 381 ± 18 | 307 ± 3 | 402 ± 21 | 539 ± 21 | 575 ± 19 |
| (*Z*) Hexen-1-ol | 334 ± 9 | 365 ± 9 | 406 ± 20 | 340 ± 2 | 389 ± 41 | 499 ± 15 | 615 ± 61 |

[30,125]. The loss in volatile compounds and, especially in phenolic substances [11,117,125], has been surmounted by the adoption of decanters able to separate the oily phase from the malaxed pastes without requiring any addition of warm water [125–127].

### 5.2.7. Oil storage

The olive oil profile changes during its storage because of the simultaneous drastic reduction of compounds from LOX pathway and the neo-formation of some volatile compounds [20,128,129] responsible for some common defects known as “rancid”, “cucumber” and “muddy sediment” attributes. The newly formed volatile compounds arise from the fragmentation of odourless and tasteless hydroperoxides [128]. They are radically produced from the oxidation process of lipids, promoted by several factors like as light, temperature, metals, pigments, unsaturated fatty acid composition, quantity and kind of natural antioxidants and amount of sterols [13,129].

The most important contributors, because of their low odour thresholds [130], are unsaturated aldehydes whose concentration increases with prolonging the storage time, but

other chemical species belonging to saturated aldehydes, ketones, acids, alcohols, hydrocarbons, lactones, furans and esters [129] contribute to the complete definition of the typical undesirable oil aroma.

Among saturated aldehydes nonanal and above all hexanal [131] increase in parallel to the oxidation process, but this last cannot be considered an useful marker of oxidation since it is also present in the aroma of high quality virgin olive oils [15,39]. Morales, Rios and Aparicio suggested that the ratio hexanal/nonanal can describe the evolution of oxidation [131], whereas Solinas and co-workers [19,20] found *trans*-2-heptenal to be correlated with the rancidity perception.

Finally, the presence of the sediment consequent to unfiltered olive oil decantation during its storage can determine, under suitable conditions of temperature, the production of unpleasant compounds responsible for the typical "muddy sediment" defect due to the fermentation that produce compounds, likely of butyric kind. In fact, a large numbers of butyrates and 2-ethyl butyrates is found in these oils.

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