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Comparative study on volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils

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ABSTRACT

The effects of ripening degree of olives on volatile profile of monovarietal virgin olive oils (VOO) from Tunisian and Sicilian cultivars were investigated. Fruits obtained from Tunisia (Chétoui and Chemlali) and Italy (Nocellara del Belice, Biancolilla and Cerasuola) were picked at three different stages of ripeness and then immediately processed. Moreover, the changes in volatile composition were evaluated in Chétoui variety as a function of the irrigation regime versus the rain-fed control. Using headspace–solid-phase microextraction (HS–SPME) technique coupled to GC–MS and GC–FID, the volatile compounds of the monovarietal virgin olive oils were identified and quantitatively analyzed. The proportions of different classes of volatiles of oils showed significant differences throughout the maturity process. The results suggest that adding to the genetic factor; agronomic conditions affect the volatile formation and therefore the organoleptic properties of VOO.

1. Introduction

Virgin olive oil is a valuable vegetable oil, which is often used without any preliminary refining process (Olias, Perez, Rios, & Sanz, 1993). It is extracted from fresh and healthy olive fruits (*Olea europeae* L.) by mechanical or other physical methods (washing, decantation, centrifugation or filtration). These technological procedures, if correctly adopted, are able to preserve the volatile and non-volatile compounds which are mainly responsible for fragrant and delicate flavour of virgin olive oil which is highly prized by consumers. The distinctive aroma of virgin olive oil is attributed to a large number of chemical compounds of different chemical classes, aldehydes, alcohols, esters, hydrocarbons, ketones, furans and, probably, other as yet unidentified volatile compounds (Kalua et al., 2007; Kiritsakis, Nanos, Polymenoupoulos, Thomai, & Sfakiotakis, 1998; Vichi et al., 2003a). The volatile compounds of virgin olive oil do not contribute to its whole aroma with the same importance and the high concentration volatile compounds do not necessarily serve as the major contributors of odour. Several chemical factors such as volatility, hydrophobic character, type and position of functional groups seem to be more related to the odour intensity of a volatile compound than its concentration. Therefore, the influence of different volatile compounds must be evaluated not only on the basis of their concentration, but also on the basis of their odour thresholds, that play a key-role. Indeed, the compound's odour threshold value is its minimum concentration able to give rise to an olfactory response. As an example, hexanal seems to contribute more to green odour than *E*-2-hexenal because of its lower odour threshold (75–300 vs. 420–1125 $\mu g\ kg^{-1}$) (Angerosa, 2002; Aparicio & Luna, 2002; Kalua et al., 2007; Morales, Luna, & Aparicio, 2005; Reiners & Grosch, 1998).

From the biochemical point of view, the volatiles found in virgin olive oil are mainly produced in plant organs by the oxidation of fatty acids though intracellular biogenic pathways (Angerosa & Basti, 2001; Kalua et al., 2007). Some of these volatiles are present in the intact tissue of the fruit and others are formed during disruption of cell structure during virgin olive oil production due to the enzymatic reactions in the presence of oxygen. It is generally agreed that endogenous plant enzymes, through the lipoxygenase pathway (LOX) are responsible for the positive aroma perceptions in olive oil, whereas chemical oxidation and exogenous enzymes, usually from microbial activity, are associated with sensory defects (Angerosa & Basti, 2001; Kalua et al., 2007; Morales, Alonso, Rios, & Aparicio, 1995; Morales, Rios, & Aparicio, 1997). The major volatile compounds responsible for odour notes of virgin olive oils are the C_6 and the C_5 volatile compounds coming from primary or secondary LOX pathway, respectively (Angerosa, 2002; Aparicio & Morales, 1998; Kiritsakis et al., 1998). Moreover, the presence of minor volatile compounds may provide useful quality markers

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and lead to an improved understanding of the formation or degradation of the major volatile compounds (Kalua et al., 2007). It is accepted that the volatile profile of virgin olive oil depends on the level and the activity of enzymes involved in the various pathways (Angerosa, 2002; Angerosa & Basti, 2001; Angerosa, Basti, & Vito, 1999). The enzymatic levels are genetically determined (Campeol, Flamini, Chericoni, Catalano, & Cremonini, 2001) whereas, other factors influenced their activities. In fact, cultivar, geographic region, fruit maturity, processing methods and parameters influence the volatile composition of olive oil (Angerosa, D'Alessandro, Basti, & Vito, 1998; Aparicio & Morales, 1998; Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006; Haddada et al., 2007).

A study of Italian, Spanish and Moroccan extra virgin olive oils (Reiners & Grosch, 1998) confirmed the richness of C_6 volatile compounds in Italian oils but showed that they were poor in fruity esters. Ben Temime et al. (2006) and Haddada et al. (2007) reported that the headspaces of Tunisian virgin olive oils were particularly rich in C_6 aldehydes (hexanal, (*E*)-2-hexenal and (*Z*)-3-hexenal).

Fruits obtained from different cultivars grown under the same environmental conditions produce oils with different volatile compounds, as does fruit of the same cultivar grown in different geographic regions (Angerosa et al., 1999; Baccouri et al., 2007; Ben Temime et al., 2006; Haddada et al., 2007). Moreover, both the processing and the storage of the fruit and the oil contribute greatly to the flavour and overall quality of olive oil (Angerosa, 2002; Venkateshwarlu, Let, Meyer, & Jacobsen, 2004). Analysis of the aroma volatile compounds has been used to evaluate the degree of ripeness of the olive fruit (Aparicio & Morales, 1998). Indeed, an understanding of the stages at which volatile compounds are formed can be used to control the volatile composition of olive oil, allowing the production and consumption of better quality oils. Thus, the aroma compounds increased with the degree of fruit maturity to a certain point (Aparicio & Morales, 1998; Kiritsakis et al., 1998; Ranalli, Tombesi, Ferrante, & De Mattia, 1998). Selection of premium olive fruit at optimum ripeness and optimum processing conditions are factors that can be used to control the process of volatile compound formation. Apart from the condition of the fruit at harvest, differences in post-harvest handling of the fruit and the oil lead to different volatile profiles. Extraction methods and conditions, in particular, the malaxation time and temperature, produce olive oils with different flavours (Angerosa et al., 1998; Ranalli, Contento, Schiavone, & Simone, 2001). A prolonged storage of either the fruit or the oil produces volatile compounds that are responsible for off-flavours (Kiritsakis et al., 1998). The absence of the C_6 aldehydes, alcohols and esters from the lipoxygenase pathway and the presence of many saturated and unsaturated aldehydes from chemical oxidation, including hexanal, characterize this kind of off-flavour of virgin olive oil known by assessors as rancid defect (Angerosa, 2000; Ha, Nihei, & Kubo, 2004). Furthermore, recent researchers reported that the volatile profile of Cornicabra virgin olive oil was influenced by the irrigation and the maturity stage of the olive. The levels of major volatile components decreased in the course of ripening but were higher in irrigated olive oils (Gomez-Rico, Salvador, La Greca, & Fregapane, 2006).

A very recent paper (Tena, Lazzez, Aparicio-Ruiz, & Garcia-Gonzalez, 2007) is focused on the characterization of several Tunisian oils at four stages of ripeness by means of volatile compounds analyzed by SPME–GC and metal oxide sensors (MOSs); authors highlighted the importance of these informations on a future traceability of Tunisian virgin olive oils produced in costal and inland farms of different geographical areas. Nevertheless, up to now, there is no detailed information available on the influence of irrigation on the volatile composition of Tunisian VOO. Concerning this, a correct evaluation of the effects of sustainable irrigation conditions on Chétoui VOO aroma is a very important aspect, especially for this variety cultivated in the Tunisian north, where the water resources allowed the use of the irrigation treatments, while, this agronomic practice is not foreseeable for the Chemlali trees cultivated in central and southern areas of the country, due to very limited water resources available in these regions.

To analyze volatile fraction of virgin olive oil, solid-phase microextraction (SPME) has been introduced as a pre-concentration method prior to GC analysis in alternative to the dynamic headspace. SPME is an analytical technique where the analytes are either adsorbed or absorbed onto the fibre from the sample matrix and subsequently desorbed into an analytical instrument. SPME is simple, rapid, cheap and solvent-less. This technique has gained popularity in the analysis of volatile compounds since its development in the early 1990s (Arthur & Pawliszyn, 1990).

This paper intends to first characterize and compare the volatile profiles of different monovarietal oils largely produced in the north, the centre and the south regions of Tunisia (cvv. Chétoui and Chemlali) and in Sicily (Nocellara del Belice, Biancolilla and Cerasuola); secondly, determine the effects of the olive ripening degree on the volatile composition of the studied virgin olive oils; finally, evidence the influence of the irrigation regime on the formation of volatiles of Chétoui virgin olive oil. Concerning the last purpose, in this paper the behaviour of only *cv*. Chétoui has been studied so the results obtained should be considered as preliminary. A study in depth on higher number of samples will be indispensable to confirm the trend of volatile compounds observed.

2. Materials and methods

2.1. Oil samples

Monovarietal virgin olive oils, properly obtained from healthy fruits without any kind of infection or physical damage, were collected in triplicate, at three different stages; the beginning of the harvest period when fruits were green colored (unripe) in the middle of the harvesting (medium ripe) and at the end when fruits were colored in black both on the skin and on the pulp (over-ripe) (FLAIR, 1991). Five varieties were studied: (Chétoui and Chemlali, Tunisia; Nocellara del Belice, Biancolilla and Cerasuola, Sicily-Italy). Moreover, the olives *cv*. Chétoui were tested in a rain-fed control and an irrigation regime. In the last condition, the water requirements were calculated using a methodology based on the crop evapotranspiration (ET_c) which proposed by the United Nations Food and Agriculture Organization (Doorenbos & Pruitt, 1977).

After harvesting, the olives from Tunisian cultivars were washed, deleafed and then transported to a laboratory (Abencor system, MC" Ingenierias y sistemas, Sevilla, Spain) where they were immediately transformed. This extraction technology reproduced at laboratory scale the industrial process and following the same phases: milling, beating, centrifuging and decanting. Monovarietal olives oils obtained from Sicilian varieties (Nocellara del Belice, Biancolilla and Cerasuola) were processed in an industrial plant. All the oil samples were processed at the same conditions: crushed with a hammer crusher, then the paste was mixed at 28 °C for 40 min and treated with a 3-phases decanter system. All the tested oils were not filtered and stored at 9 °C in darkness using amber glass bottles without headspace prior to analysis.

2.2. Volatile compounds analyses

2.2.1. Standards

All standards 95–99% pure (4-methyl-2-pentanone, hexanal, *E*-2-hexenal, *Z*-3-hexen-1-ol, *E*-2-hexen-1-ol, 1-hexanol, hexylacetate, *Z*-2-pentenol, 3-pentanone, *E*-2-pentenal, 1-penten-3-ol, 1-penten-3-one, farnesene, limonene, β-ocimene, α-pinene, α-cop-

aene, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid and octanoic acid) were purchased from Fluka (Buchs, Switzerland).

2.2.2. SPME sampling conditions

The oil sample was spiked with 4-methyl-2-pentanone (internal standard) to a concentration of 5 μg g^{-1} and 1.5 g of the sample was weighed into 10 mL vial fitted with a silicone septum. The vial was immersed in a water bath at 40 °C and the oil was maintained under magnetic stirring. After 2 min of sample conditioning a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA) was exposed to the sample headspace for 30 min and immediately desorbed for 3 min at 250 °C in the gas chromatograph.

2.2.3. GC–FID and GC–MS analysis

Volatile compounds were identified and quantified by gas chromatography coupled with quadrupolar mass-selective spectrometry, using an Agilent 6890N Network gas chromatograph and an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a ZB-WAX column 30 m × 0.25 mm ID, 1.00 μ film thickness (Phenomenex, Torrance, CA, USA). Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C min^{-1}. The FID temperature was set at 250 °C and the ion source and the transfer line were at 180 and 230 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 15–250 amu mass range, 2 scan s^{-1}. The identification of the volatile compounds was first carried out by mass spectrometry and later checked with standards (see Section 2.2.1). Moreover, the volatile identification was obtained by a comparison of their mass spectral data with the information from the National Institute of Standards and Technology (NIST) library (2005 version) and MS literature data (Jennigs & Shibamoto, 1980; Joulain & Konig, 1998; Joulain, Konig, & Hochmuth, 2001). The volatiles were also identified using the relative retention times of the standards with respect to the internal standard. The quantification by HS–SPME/GC–FID was carried out using conditions identical to those used for the identification by GC–mass spectrometry (MS) then, relative amounts of volatile compounds were expressed respect to internal standard as micrograms per gram of oil or milligrams per kilogram of oil.

2.2.4. Statistical analysis

The results reported in this study are mean values of at least three repetitions ($n = 3$) unless otherwise stated. Chemical data were analyzed by the SPSS r.11.0.0 statistical software (SPSS Inc., Chicago, IL, USA). The significance of differences at a 5% level between averages was determined by one-way ANOVA using Tukey's test.

3. Results and discussion

The characteristic and the unique flavour of virgin olive oil, in particular, its green and fruity attributes depend on many volatile compounds (Angerosa, 2000; Angerosa, 2002). Concentration and odour threshold of volatile compounds, whether major or minor, are crucial to virgin olive oil quality. Consequently, the identification and the quantification of the compounds causing the flavour or off-flavour is considered the key for quality control. The analysis of volatile fractions from oils obtained from five varieties of *Olea europaea* L. (Chétoui and Chemlali, Tunisia; Nocellara del Belice, Biancolilla and Cerasuola; Italy) picked at three different stages of ripeness, by headspace–solid-phase microextraction, separation and identification of their components by GC–MS, showed that the virgin olive oil aroma consisted of a complex mixture of more than 49 compounds, representing 97.8–99.9% of the total GC area (data not shown). Table 1 shows basic information on the chemical compounds of the tested virgin olive oils. The table indicates interesting quantitative differences. The total volatile content (TVs) of all the analyzed samples ranged from 10.0 to 55.7 mg kg^{-1}. Only Chétoui VOO obtained in the rain fed conditions had values below 16.0 mg kg^{-1}, the Chétoui subjected of the irrigation regime and the Chemlali VOO being between 34.4 and 46.6 mg kg^{-1}. The trend of the three Sicilian cultivars was toward the highest volatile concentration that ranged from 37.6 to 55.7 mg kg^{-1}.

Table 1
Total content of volatiles

	TVs	THs	TALCs	TACs	TKs	TALDs	TESTs	TFs	TC_6	TC_5
CT RF I	$14.41^{a,y}$	$1.80^{c,z}$	$5.21^{a,x}$	$1.00^{ab,z}$	$3.53^{a,x}$	$2.54^{a,z}$	$0.17^{ab,yz}$	$0.16^{a,v}$	$4.82^{a,z}$	$1.93^{a,z}$
CT RF II	$10.00^{b,y}$	$3.00^{b,y}$	$2.12^{b,x}$	$0.64^{ab,z}$	$2.21^{b,y}$	$1.85^{b,z}$	$0.20^{ab,yz}$	$tr^{bc,x}$	$2.06^{b,z}$	$1.52^{a,z}$
CT RF III	$15.96^{a,y}$	$9.17^{a,x}$	$1.30^{c,y}$	$0.56^{ab,z}$	$2.13^{b,y}$	$2.70^{a,z}$	$0.10^{ab,z}$	$0.01^{bc,w}$	$2.41^{b,z}$	$1.23^{ab,z}$
CT IR I	$44.11^{a,w}$	$4.16^{c,y}$	$11.58^{a,w}$	$3.65^{ab,y}$	$11.92^{a,v}$	$8.80^{b,y}$	$3.89^{a,v}$	$0.06^{a,w}$	$14.10^{a,y}$	$5.85^{a,w}$
CT IR II	$43.12^{a,w}$	$9.90^{b,x}$	$9.43^{b,w}$	$4.48^{a,y}$	$7.40^{b,vw}$	$11.49^{a,y}$	$0.29^{b,yz}$	$0.12^{a,w}$	$11.18^{b,y}$	$4.37^{b,x}$
CT IR III	$39.69^{b,x}$	$12.73^{a,w}$	$5.39^{c,x}$	$4.02^{a,y}$	$8.18^{b,vw}$	$9.12^{b,y}$	$0.09^{c,z}$	$0.10^{a,w}$	$4.07^{c,z}$	$5.63^{a,w}$
CM I	$34.35^{c,x}$	$5.40^{c,y}$	$3.59^{b,x}$	$1.29^{ab,z}$	$3.89^{b,x}$	$19.50^{a,w}$	$0.48^{ab,yz}$	$0.19^{a,v}$	$19.08^{a,xy}$	$2.75^{a,y}$
CM II	$39.98^{b,x}$	$9.08^{b,x}$	$3.45^{b,x}$	$1.51^{ab,z}$	$7.14^{a,vw}$	$18.37^{a,w}$	$0.38^{ab,yz}$	$0.04^{b,wx}$	$17.90^{b,y}$	$3.07^{a,y}$
CM III	$46.61^{a,vw}$	$19.43^{a,v}$	$5.03^{a,x}$	$1.72^{ab,z}$	$4.04^{b,w}$	$15.99^{b,x}$	$0.35^{ab,yz}$	$0.05^{b,wx}$	$16.75^{b,y}$	$2.56^{b,y}$
CE I	$55.22^{a,v}$	$4.49^{b,y}$	$15.53^{a,v}$	$10.14^{b,w}$	$5.08^{a,w}$	$17.89^{b,w}$	$1.98^{b,x}$	$0.10^{a,w}$	$27.33^{a,w}$	$4.66^{a,x}$
CE II	$47.44^{b,vw}$	$3.88^{b,y}$	$9.86^{b,w}$	$7.24^{c,x}$	$3.51^{b,x}$	$21.47^{a,vw}$	$1.39^{b,x}$	$0.09^{a,w}$	$26.47^{a,w}$	$3.76^{b,y}$
CE III	$52.11^{ab,v}$	$8.20^{a,x}$	$8.46^{b,w}$	$13.63^{a,v}$	$4.81^{ab,w}$	$14.20^{c,x}$	$2.71^{a,w}$	$0.09^{a,w}$	$17.35^{b,y}$	$3.51^{b,y}$
BA I	$37.59^{b,x}$	$3.95^{b,y}$	$9.74^{a,w}$	$5.15^{b,xy}$	$4.47^{ab,w}$	$13.46^{c,x}$	$0.73^{b,y}$	$0.08^{b,w}$	$15.38^{c,y}$	$5.03^{a,w}$
BA II	$51.77^{a,v}$	$4.92^{b,y}$	$10.48^{a,w}$	$5.95^{b,xy}$	$5.06^{ab,w}$	$24.40^{a,v}$	$0.78^{b,y}$	$0.20^{a,v}$	$27.59^{a,w}$	$5.67^{a,w}$
BA III	$53.12^{a,v}$	$9.35^{a,x}$	$7.43^{b,wx}$	$9.58^{a,w}$	$4.20^{ab,w}$	$19.00^{b,vw}$	$3.50^{a,v}$	$0.08^{b,w}$	$22.66^{b,x}$	$2.82^{b,y}$
NB I	$50.61^{b,v}$	$7.75^{a,xy}$	$13.38^{b,v}$	$4.55^{b,xy}$	$5.31^{a,w}$	$18.09^{b,w}$	$1.46^{ab,x}$	$0.07^{a,w}$	$22.73^{c,x}$	$8.79^{a,v}$
NB II	$55.69^{a,v}$	$4.96^{b,y}$	$13.69^{b,v}$	$5.81^{b,xy}$	$4.00^{b,w}$	$25.94^{a,v}$	$1.21^{ab,x}$	$0.08^{a,w}$	$31.19^{a,v}$	$6.45^{b,w}$
NB III	$50.47^{b,v}$	$4.37^{b,y}$	$15.07^{a,v}$	$7.28^{a,x}$	$3.64^{bc,x}$	$18.13^{b,w}$	$1.89^{a,x}$	$0.10^{a,w}$	$26.61^{b,w}$	$4.08^{c,x}$

Total concentrations of hydrocarbons, alcohols, acids, ketones, aldehydes, esters and furans and C_6 and C_5 compounds (mg of internal standard kg^{-1} oil) in the studied virgin olive oils.

NB: Nocellara del Belice; BA: Biancolilla; CE: Cerasuola; CT RF: Chétoui rain-fed; CT IR: Chétoui irrigated; CM: Chemlali; I, II, III: stages of olive ripening.

TVs: total volatiles; THs: total hydrocarbons; TALCs: total alcohols; TACs: total acids, TKs: total ketones; TALDs: total aldehydes; TESTs: total esters; TFs: total furans; TC_6: total C_6 compounds; TC_5: total C_5 compounds.

$^{a-c}$Different letters in the same column concerning the same cultivar indicate significantly different values ($p < 0.05$).

$^{v-z}$Different letters in the same column concerning all studied samples indicate significantly different values ($p < 0.05$).

Table 2
Amounts of C_6 volatile compounds (mg of internal standard kg^{-1} oil) valued in tested virgin olive oils with regard to fruit ripening stage

	HEX	*E*-2-HEX	C_6ALD	*Z*-3-HEX AC	HEX-1-OL	*Z*-3-HEX-1-OL	*E*-2-HEX-1-OL	C_6ALCs	%C_6ALCs/ΣC_6	%C_6ALDs/ΣC_6
CT RF I	$0.45^{a,x}$	$0.19^{bc,z}$	$0.63^{b,z}$	$0.17^{a,y}$	$0.60^{a,x}$	$2.69^{a,w}$	$0.61^{a,z}$	$4.01^{a,x}$	83.29	13.16
CT RF II	$0.37^{a,x}$	$0.30^{bc,z}$	$0.66^{b,z}$	$0.20^{a,xy}$	$0.10^{b,y}$	$0.44^{b,y}$	$0.65^{a,z}$	$1.2^{b,y}$	58.36	32.16
CT RF III	$0.45^{a,x}$	$1.30^{a,z}$	$1.75^{a,z}$	$0.10^{b,y}$	$0.05^{c,z}$	$0.10^{c,z}$	$0.41^{ab,z}$	$0.56^{c,z}$	23.23	72.59
CT IR I	$1.64^{a,v}$	$2.62^{ab,z}$	$4.26^{a,yz}$	$1.89^{a,w}$	$1.30^{a,wx}$	$4.64^{a,v}$	$1.90^{a,x}$	$7.95^{a,w}$	56.39	30.21
CT IR II	$1.50^{a,v}$	$3.63^{a,z}$	$5.14^{a,yz}$	$0.29^{b,xy}$	$1.05^{ab,wx}$	$3.13^{b,v}$	$1.53^{a,\ xy}$	$5.75^{b,wx}$	51.44	45.94
CT IR III	$0.33^{b,x}$	$0.87^{c,z}$	$1.20^{b,z}$	$0.09^{c,z}$	$0.78^{b,x}$	$0.96^{c,xy}$	$0.97^{b,y}$	$2.78^{c,x}$	68.31	29.42
CM I	$1.44^{a,v}$	$15.80^{a,w}$	$17.24^{a,vw}$	$0.02^{a,z}$	$0.34^{b,y}$	$0.41^{a,y}$	$1.05^{a,y}$	$1.82^{b,y}$	9.51	90.38
CM II	$1.56^{a,v}$	$14.78^{b,wx}$	$16.35^{a,w}$	$0.03^{a,z}$	$0.29^{b,y}$	$0.06^{c,z}$	$1.20^{a,y}$	$1.56^{b,y}$	8.66	91.34
CM III	$1.68^{a,v}$	$11.52^{c,x}$	$13.20^{b,x}$	$tr^{b,z}$	$1.98^{a,w}$	$0.28^{b,y}$	$1.22^{a,y}$	$3.52^{a,x}$	21.01	78.79
CE I	$1.29^{a,vw}$	$11.22^{b,x}$	$12.52^{b,x}$	$1.24^{a,w}$	$3.80^{a,v}$	$2.74^{a,w}$	$6.92^{a,v}$	$13.57^{a,v}$	49.66	45.79
CE II	$1.60^{a,v}$	$16.39^{a,w}$	$17.99^{a,vw}$	$1.22^{a,w}$	$1.16^{c,wx}$	$2.18^{a,w}$	$4.15^{b,\ w}$	$7.55^{b,w}$	28.53	67.98
CE III	$0.92^{b,w}$	$8.78^{c,y}$	$9.70^{c,y}$	$0.92^{b,x}$	$2.35^{b,w}$	$1.74^{b,x}$	$2.24^{c,x}$	$6.44^{c,w}$	37.10	55.90
BA I	$1.12^{a,vw}$	$7.18^{c,y}$	$8.30^{c,y}$	$0.56^{b,xy}$	$1.53^{b,w}$	$3.79^{a,v}$	$1.08^{b,y}$	$6.52^{a,w}$	42.38	54.00
BA II	$1.88^{a,v}$	$18.72^{a,v}$	$20.60^{a,v}$	$0.34^{b,xy}$	$1.61^{b,w}$	$2.60^{b,w}$	$2.27^{a,x}$	$6.66^{a,w}$	24.12	74.65
BA III	$1.18^{a,vw}$	$12.65^{b,x}$	$13.83^{b,x}$	$2.86^{a,v}$	$2.38^{a,w}$	$3.49^{a,v}$	$tr^{c,z}$	$5.97^{ab,w}$	26.35	61.03
NB I	$1.72^{a,v}$	$11.91^{b,x}$	$13.63^{b,x}$	$0.83^{b,x}$	$0.85^{b,x}$	$4.73^{a,v}$	$1.88^{c,x}$	$7.65^{c,w}$	33.61	59.95
NB II	$1.28^{ab,vw}$	$17.32^{a,v}$	$18.60^{a,vw}$	$1.10^{a,w}$	$2.97^{a,vw}$	$3.49^{b,v}$	$5.26^{b,v}$	$11.77^{b,v}$	37.72	59.63
NB III	$1.09^{ab,vw}$	$10.88^{b,x}$	$11.98^{c,x}$	$1.46^{a,w}$	$2.58^{a,vw}$	$4.18^{a,v}$	$6.69^{a,v}$	$13.55^{a,v}$	50.87	45.00

HEX: hexanal; *E*-2-HEX: *E*-2-hexenal; C_6ALD: total aldehydes C_6; *Z*-3-HEX AC: *Z*-3-hexenyl acetate; HEX-1-OL: hexen-1-ol; Z-3-HEX-OL: *Z*-3-hexen-1-ol; *E*-2-HEX-1-OL: *E*-2-hexen-1-ol; C_6ALCs: total C_6 alcohols; %C_6ALCs/ΣC_6: % total C_6 alcohols respect to sum of C_6; %C_6ALDs/ΣC_6: % total C_6 aldehydes respect to sum of C_6; tr: traces (<0.01 mg kg^{-1}).
$^{a-c}$Different letters in the same column concerning the same cultivar indicate significantly different values ($p < 0.05$).
$^{v-z}$Different letters in the same column concerning all studied samples indicate significantly different values ($p < 0.05$).

On the other hand, Chétoui oils obtained under the irrigation conditions displayed higher whole aroma concentration than non-irrigated ones. Hence, these different amounts of total volatiles should be related to the agronomic conditions (the ripening stage and the irrigation regime).

Furthermore, in the headspace of all oil samples, the isolated and identified compounds were mainly aldehydes (ranged from 1.9 to 25.9 mg kg^{-1}), hydrocarbons (1.8–19.4 mg kg^{-1}), alcohols (1.3–15.5 mg kg^{-1}), acids (0.6–13.6 mg kg^{-1}), ketones (2.1–11.9 mg kg^{-1}), esters (<4 mg kg^{-1}) and furans (less than 1 mg kg^{-1}) (Table 1).

3.1. Volatile C_6 compounds from the lipoxygenase pathway

Because of the importance of the green perception in the virgin olive oil flavour, the C_6 compounds – also called "green volatiles" (Aparicio & Morales, 1998) – were quantified in all the analyzed varieties (Table 1). These compounds are derived from the cascade of enzymatic reactions starting with the formation, by lipoxygenase (LOX) action, of 13-hydroperoxides from linoleic and linolenic acid (Angerosa et al., 1998; Olias et al., 1993; Williams, Salas, Sanchez, & Harwood, 2000) and the different accumulations of metabolites from the lipoxygenase cascade were highly dependent on the levels of enzymes involved, the extraction conditions, the storage time and the degree of ripening of olives (Angerosa et al., 1998; Aparicio & Morales, 1998; Gomez-Rico et al., 2006). In addition, climatic and environmental growth conditions may also influence the production of volatiles (Aparicio & Morales, 1998; Ben Temime et al., 2006; Montedoro, Bertuccioli, & Anichini, 1978; Servili, Baldioli, Beglimini, Selvaggini, & Montedoro, 2000; Vichi et al., 2003a).

Tables 1 and 2 report the concentrations of C_6 volatile compounds from the lipoxygenase (LOX) pathway, expressed as milligrams of internal standard (4-methyl-2-pentanone) per kilogram of oil, in some Tunisian and Sicilian virgin olive oils taken throughout the course of fruit ripening.

All the analyzed samples evidenced high amounts of C_6 volatile compounds. The range of concentrations varied from 2.1 to 31.2 mg kg^{-1}, although most varieties (Chemlali and Sicilian varieties) had concentrations within the range of 15.4–31.2 mg kg^{-1} (Table 1), C_6 aldehydes being the major contributor. Chétoui VOO registered the lowest content of C_6 aldehydes (0.7–5.1 mg kg^{-1}) (Table 2). The overall amounts of C_6 aldehydes were clearly higher than the sum of C_6 alcohols in Chemlali and Sicilian samples, as reported for virgin olive oil analyzed by others (Angerosa et al., 1999). Whereas, in Chétoui oils the sum of C_6 alcohols was generally higher than that of C_6 aldehydes. These results may be explained by differential activity of the enzyme alcohol dehydrogenase (ADH) which reduces the C_6 aldehydic compounds in the corresponding alcohols (Angerosa & Basti, 2001).

It is also noteworthy that the content of *E*-2-hexenal, which gives the typical "green note" to extra virgin olive oil, is by far the major C_6 aldehyde compound in all studied oils in almost the stages of olive ripening (Table 2). In Chétoui VOO obtained in the rain-fed conditions, the levels of *E*-2-hexenal increased progressively from 0.2 to 1.3 mg kg^{-1}. Nevertheless, in the tested Sicilian samples, it increased until reaching a maximum of 16.4, 18.7 and 17.3 mg kg^{-1} in Cerasuola, Biancolilla and Nocellara del Belice, respectively, at the second stage of olive ripening and then decreased. Other researches (Angerosa, Di Giacinto, & D'Alessandro, 1997; Montedoro et al., 1978) have shown that during olive ripening the amount of C_6 aldehydes, especially *E*-2-hexenal, increased to a maximum, which occurred when fruit skin colour turned from yellow–green to purple. Beyond this time, the concentration of these volatile compounds decreased because of a lower activity of enzymes involved in their production with a weakening of the intensity of some "green" sensory notes (Angerosa & Basti, 2001).

In Chemlali oils, the levels of these C_6 aldehydes decreased progressively from 15.8 down to 11.5 mg kg^{-1} as ripening progressed. A similar behaviour was observed in Chétoui samples obtained in the irrigation regime (Table 2). This trend of to C_6 compounds decreased in the late stage of olive maturity as reported by several authors (Aparicio & Morales, 1998; Gomez-Rico et al., 2006; Tena et al., 2007) who described a steady decrease of the concentration of the volatile compounds, including *E*-2-hexenal, from the unripe to the over-ripe stages.

In terms of C_6 alcohols, Sicilian VOO had higher values (6–13.6 mg kg^{-1}) than Tunisian samples, which ranged from 0.6 to 8 mg kg^{-1}. These compounds have less sensory significance than aldehydes because of their higher odour threshold values and their sensory descriptions being associated with fruity, soft green and

aromatic sensory notes (Luna, Morales, & Aparicio, 2006). For instance, among volatile alcohols, the odour threshold values estimated by different authors (Kalua et al., 2007; Morales et al., 2005) range from 5000 to 8000 and from 1100 to 6000 μg kg^{-1} for *E*-2-hexen-1-ol and *Z*-3-hexen-1-ol, respectively; instead, the odour threshold value for hexan-1-ol is about 400 μg kg^{-1}. Considering the concentrations of this last alcohol in the headspace of tested VOO, its contribution to the sweet, fruity and tomato odour notes is more significative in Sicilian samples than in Tunisian ones. The chemical compositions of all analyzed virgin olive oil headspaces evidenced that the hexan-1-ol, the *Z*-3-hexen-1-ol and the *E*-2-hexen-1-ol were the most C_6 alcohols (Table 2). With respect to the evolution of C_6 alcohols, a slight decrease in *Z*-3-hexen-1-ol content was observed in all tested VOO from unripe to over-ripe. The *E*-2-hexen-1-ol in Chétoui olive oils (obtained in rain-fed and irrigation regime) showed also a slight decrease during the olive maturation process. In Biancolilla samples, this compound was not detected at the last ripening stage. In Chemlali oils, the amount of this volatile remained unchanged throughout the maturity process. Nevertheless, in Nocellara del Belice VOO, this trend was not observed; in fact, the content on *E*-2-hexen-1-ol increased considerably from 1.9 to 6.7 mg kg^{-1}. The changes in hexan-1-ol amounts in the course of fruit ripening were similar in Biancolilla, Nocellara del Belice and Chemlali VOO: an increase during ripening, however, in Chétoui and Cerasuola samples a slight decrease was observed (Table 2). Gomez-Rico et al. (2006) had already observed an increase in some C_6 alcohol contents in Cornicabra olive oil. Nevertheless, other researchers in other VOO varieties (Picual and Coratina) have not evidenced the moderate increase in the hexan-1-ol content (Angerosa & Basti, 2001; Aparicio & Morales, 1998) probably due to the different activities of alcohol dehydrogenase (ADH) which is genetically determined in each cultivar (Angerosa et al., 1999). Regardless of these observations, it has been reported (Aparicio & Morales, 1998) that hexan-1-ol does not contribute to ripeness characterisation.

Esters, compounds associated with fruity sensory notes (Aparicio & Luna, 2002; Luna et al., 2006) as hexyl acetate and *Z*-3-hexenyl acetate, were present in aroma of all studied VOO, but there are minor components compared with aldehydes or alcohols (Table 2). In particular, the amounts of hexyl acetate (not reported in Table 2) resulted lower than *Z*-3-hexenyl acetate and below it odour threshold (1040 μg kg^{-1} according to Aparicio & Luna, 2002). The *Z*-3-hexenyl acetate ranged from 0.3 to 2.9 mg kg^{-1} in Sicilian samples. Chemlali VOO had very low values (<0.04 mg kg^{-1}) indicating that there was little activity of the alcohol acyl transferase (AAT) (Angerosa et al., 1999). Chétoui oils obtained in the rain-fed conditions had content around 0.2 mg kg^{-1}. However, under the irrigation regime the amount of this compound reached 1.9 mg kg^{-1}. A slight decrease in the values of this ester was evidenced in Tunisian and Cerasuola VOO from unripe to over-ripe stages. Aparicio and Morales (1998) reported similar trends in Arbequina, Picual, Coratina and Koroneiki varieties. While, in Biancolilla and Nocellara del Belice samples, an increase was obtained at the last stage of ripeness.

The odour threshold of *Z*-3-hexenyl acetate, measured by different authors (Reiners & Grosch, 1998; Aparicio & Luna, 2002) as 200–750 μg kg^{-1} indicates that this ester is linked to the green and banana pleasant notes especially in Sicilian VOO rather than Tunisian samples.

Table 1 shows that the total concentration of volatile compounds was significantly affected by the irrigation regime. Indeed, oils obtained from irrigated trees had higher levels of total volatile compounds than non-irrigated ones. Furthermore, the Chétoui VOO produced in the first period was particularly rich in alcohols and *Z*-3-hexen-1-ol is the most abundant. In the rain-fed regime the C_6 alcohols percentages accounted for 83.3% of the whole C_6 fractions, the percentage of these compounds fall drastically down to 23.2% throughout the picking period (Table 2). Whereas, on oils obtained from irrigated trees the amounts of C_6 alcohols increased from 56.4 to 68.3% (Table 2). Moreover, the variation of percentages of C_6 aldehydes throughout the maturity process expressed deep differences according the agronomical practices. In fact, in Chétoui oils obtained in the rain-fed conditions, the amounts of these volatiles increased substantially from 13.2 to 72.6%. However, in the irrigation regime, the C_6 aldehyde percentages increased from 30.2 to 45.9% and then decreased down to 29.4%. Work by Gomez-Rico et al. (2006) demonstrated that the C_6 volatile compounds practically *E*-2-hexenal, *Z*-3-hexen-1-ol and hexan-1-ol were affected by the irrigation in the sense that the increase in the water applied to the Cornicabra olive trees led to an increase in these volatiles, mainly in oils from fruits with ripeness index of >2.5–3.

3.2. Volatile C_5 compounds

A secondary metabolic pathway of LOX is active on the linolenic acid substrate, leading to the production of C_5 volatile compounds, which are also present in the VOO aroma (Angerosa, 2000). Considerable amounts of various classes of C_5 volatile compounds were found in all the oils examined in the present study (Table 1). Pentene dimers, pentenols and C_5 carbonyl compounds were proposed by Angerosa et al. (1998) to be products of the bioformation of olive oil aroma. They are thought to derive from the hydroxylation or dimerization of pentene radicals originated by β-scission of alkoxy radicals formed from 13-hydroperoxides by an enzyme-mediated mechanism (Angerosa et al., 1998). Reasonable amounts of C_5 ketones (1-penten-3-one and 3-pentanone), C_5 aldehydes (pentanal and *E*-2-pentenal), C_5 alcohols (1-penten-3-ol, 1-pentanol and *Z*-2-pentenol) and 3-ethyl-1,5-octadiene pentene dimer were found in the analyzed VOO. Pentanal was the main C_5 aldehyde quantified in all the studied samples. Quite low amounts of *E*-2-pentenal were found in the headspaces of oils analyzed in the present work (Table 3). Sicilian VOO had higher level of pentanal (fluctuant from 1 to 4.3 mg kg^{-1}) whereas, in Tunisian samples the contents of this volatile ranged between 0.31 and 1.26 mg kg^{-1} over the course of fruit maturation (Table 3). The amounts of C_5 aldehydes and alcohols decreased during the maturity process in almost all studied VOO from the first harvest time to the last one. Kalua et al. (2007) reported that C_5 aldehydes and alcohols contribute to the positive attributes of olive oil, providing pungent sensations and correlated with bitterness. In particular, odour notes of green apple and fruity-pungent were recognized for *E*-2-pentenal and 1-pentanol, respectively. However, considering their odour thresholds (300 and 470 μg kg^{-1}) (Aparicio & Luna, 2002; Morales et al., 2005) and concentrations found in analyzed samples, these volatiles do not seem significatively contribute to the aroma of Sicilian and Tunisian VOO. Generally, the 1-penten-3-one levels did not exceed 1 mg kg^{-1} in all analyzed VOO (Table 3). This volatile have been mostly associated with fruity, sweet and pleasant attribute as tomato and strawberry (Angerosa, 2000; Aparicio & Luna, 2002; Luna et al., 2006; Morales et al., 1995). Other authors proposed this ketone as a useful marker of metallic off-flavour (Venkateshwarlu et al., 2004). On the other side, the 1-penten-3-one is characterized by a very low odour threshold (0.7–50 μg kg^{-1}) so its contribution to whole aroma can be considered important. The pentanal percentage in Chétoui VOO subjected of the irrigation regime showed during the olive maturation a decreasing trend such like the same cultivar obtained in the rain-fed but starting to higher percentage. The 1-penten-3-one fraction increased from the first olive harvest to the last one in the rain-fed Chétoui oils, while, in Chétoui samples obtained under the irrigation, the levels of this volatile decreased drastically (Table 3). Nevertheless,

Table 3
Amounts of C_5 and some minor volatile compounds (mg of internal standard kg^{-1} oil) identified in tested oils with regard to fruit ripening stage

	PENT	*E*-2-PENT	C_5ALD	1-P-3-ONE	3-P-ONE	C_5Ks	1-P-3-OL	1-P-OL	*Z*-2-P-OL	C_5ALCs	PDIMs	TOL	AC
CT RF I	$0.88^{a,y}$	$0.04^{a,x}$	$0.92^{a,y}$	$0.12^{b,x}$	$0.21^{a,vw}$	$0.33^{a,x}$	$0.26^{a,y}$	$0.11^{a,xy}$	$0.31^{a,vw}$	$0.68^{a,y}$	$0.29^{a,\ x}$	$0.44^{c,y}$	$0.39^{a,z}$
CT RF II	$0.40^{b,z}$	$0.07^{a,x}$	$0.46^{b,z}$	$0.42^{a,w}$	$0.06^{b,x}$	$0.48^{a,x}$	$0.25^{a,y}$	$0.10^{a,xy}$	$0.22^{ab,x}$	$0.58^{a,y}$	$0.29^{a,\ x}$	$1.73^{b,y}$	$0.36^{a,z}$
CT RF III	$0.31^{b,z}$	$0.05^{a,x}$	$0.36^{b,z}$	$0.41^{a,w}$	$0.05^{b,x}$	$0.46^{a,x}$	$0.19^{a,z}$	$0.05^{a,xy}$	$0.16^{b,x}$	$0.40^{a,y}$	$0.16^{b,y}$	$8.20^{a,wx}$	$0.23^{a,z}$
CT IR I	$1.22^{a,x}$	$0.33^{a,v}$	$1.56^{a,x}$	$1.32^{ab,v}$	$0.23^{a,vw}$	$1.55^{b,w}$	$1.13^{a,x}$	$0.73^{a,w}$	$0.88^{a,v}$	$2.74^{a,w}$	$0.35^{a,x}$	$0.71^{c,y}$	$1.90^{a,y}$
CT IR II	$1.26^{a,x}$	$0.16^{b,w}$	$1.41^{a,x}$	$0.50^{b,w}$	$0.30^{a,v}$	$0.80^{c,x}$	$1.11^{a,x}$	$0.48^{a,w}$	$0.57^{ab,v}$	$2.16^{a,w}$	$0.25^{a,x}$	$6.98^{b,wx}$	$1.68^{a,y}$
CT IR III	$1.08^{a,xy}$	$0.07^{bc,x}$	$1.15^{ab,\ y}$	$2.45^{a,v}$	$0.28^{a,v}$	$2.74^{a,v}$	$0.91^{a,\ x}$	$0.65^{a,w}$	$0.19^{b,x}$	$1.55^{b,wx}$	$tr^{b,\ z}$	$10.52^{a,w}$	$0.84^{b,yz}$
CM I	$0.75^{ab,yz}$	$0.12^{a,w}$	$0.87^{ab,y}$	$0.53^{ab,w}$	$0.07^{b,x}$	$0.60^{ab,x}$	$0.48^{a,\ y}$	$0.16^{b,xy}$	$0.64^{a,v}$	$1.28^{a,wx}$	$0.53^{a,x}$	$0.51^{c,y}$	$0.44^{a,z}$
CM II	$0.51^{ab,yz}$	$0.14^{a,w}$	$0.65^{ab,y}$	$0.93^{ab,vw}$	$0.14^{a,x}$	$1.07^{a,w}$	$0.32^{a,\ y}$	$0.61^{a,w}$	$0.42^{ab,v}$	$1.35^{a,wx}$	$0.52^{a,x}$	$5.66^{b,wx}$	$0.46^{a,z}$
CM III	$0.92^{a,xy}$	$0.13^{a,w}$	$1.05^{a,y}$	$0.40^{ab,w}$	$0.14^{a,x}$	$0.54^{ab,x}$	$0.19^{b,\ z}$	$0.46^{a,w}$	$0.32^{ab,vw}$	$0.97^{b,x}$	$0.22^{b,x}$	$13.29^{a,v}$	$0.57^{a,z}$
CE I	$2.69^{a,w}$	$0.19^{a,w}$	$2.87^{a,w}$	$0.10^{ab,x}$	$0.19^{b,w}$	$0.29^{ab,x}$	$0.35^{ab,y}$	$0.78^{b,w}$	$0.37^{ab,v}$	$1.50^{a,wx}$	$0.71^{a,\ w}$	$0.06^{b,yz}$	$7.98^{bc,w}$
CE II	$0.99^{c,xy}$	$0.26^{a,w}$	$1.25^{b,x}$	$0.30^{ab,w}$	$0.37^{a,v}$	$0.68^{ab,x}$	$0.64^{ab,y}$	$0.78^{b,w}$	$0.41^{ab,v}$	$1.83^{a,wx}$	$0.60^{a,\ w}$	$tr^{c,z}$	$5.63^{bc,w}$
CE III	$1.25^{b,x}$	$0.14^{a,w}$	$1.39^{b,x}$	$0.16^{ab,x}$	$0.36^{a,v}$	$0.52^{ab,x}$	$0.24^{ab,y}$	$1.06^{a,v}$	$0.30^{ab,vw}$	$1.60^{a,wx}$	$0.28^{b,\ x}$	$2.60^{a,y}$	$11.63^{a,v}$
BA I	$1.44^{a,x}$	$0.16^{a,w}$	$1.69^{a,x}$	$0.37^{a,w}$	$0.26^{a,vw}$	$0.62^{ab,x}$	$2.06^{a,w}$	$0.33^{ab,x}$	$0.41^{ab,v}$	$2.80^{a,w}$	$0.74^{a,\ w}$	$0.14^{a,y}$	$2.75^{c,x}$
BA II	$1.33^{a,x}$	$0.25^{a,w}$	$1.49^{a,x}$	$0.53^{a,w}$	$0.19^{b,w}$	$0.72^{ab,x}$	$2.12^{a,w}$	$0.74^{ab,w}$	$0.52^{ab,v}$	$3.37^{a,v}$	$0.79^{a,w}$	$0.08^{ab,yz}$	$4.28^{b,w}$
BA III	$1.21^{a,x}$	$0.13^{a,w}$	$1.34^{a,x}$	$0.26^{a,w}$	$0.25^{a,vw}$	$0.50^{ab,x}$	$0.41^{b,y}$	$0.18^{b,xy}$	$0.39^{ab,v}$	$0.98^{b,x}$	$0.57^{b,\ x}$	$0.14^{a,y}$	$5.02^{a,w}$
NB I	$1.91^{c,x}$	$0.36^{a,v}$	$2.27^{b,w}$	$0.97^{a,vw}$	$0.21^{a,vw}$	$1.17^{a,w}$	$4.55^{a,v}$	$0.24^{ab,x}$	$0.55^{a,v}$	$5.35^{a,v}$	$1.21^{a,v}$	$0.13^{a,y}$	$2.57^{c,x}$
NB II	$4.31^{a,v}$	$0.19^{ab,w}$	$4.49^{a,v}$	$0.22^{b,x}$	$0.19^{a,w}$	$0.41^{b,x}$	$0.52^{b,y}$	$0.65^{a,w}$	$0.38^{ab,v}$	$1.54^{b,wx}$	$0.81^{b,w}$	$0.08^{ab,yz}$	$3.81^{b,x}$
NB III	$2.35^{b,w}$	$0.23^{ab,w}$	$2.58^{b,w}$	$0.09^{c,y}$	$0.19^{a,w}$	$0.28^{b,x}$	$0.33^{b,y}$	$0.46^{a,w}$	$0.43^{ab,v}$	$1.22^{b,wx}$	$0.74^{b,\ w}$	$0.07^{ab,yz}$	$5.96^{a,w}$

PENT: pentanal; *E*-2-PENT: *E*-2-pentenal; C_5ALD: total C_5 aldehydes; 1-P-3-ONE: 1-penten-3-one; 3-P-ONE: 3-pentanone; C_5Ks: total C_5 ketons; 1-P-3-OL: 1-pentan-3-ol; 1-P-OL: 1-pentanol; *Z*-2-P-OL: *Z*-2-pentenol; C_5ALCs: total C_5 alcohols; P DIMs: penten dimers; TOL: toluene; AC: acetic acid; tr: traces (<0.01 mg kg^{-1}).
$^{a-c}$Different letters in the same column concerning the same cultivar indicate significantly different values ($p < 0.05$).
$^{v-z}$Different letters in the same column concerning all studied samples indicate significantly different values ($p < 0.05$).

Gomez-Rico et al. (2006) studies had not evidenced an effect of the irrigation strategies on the C_5 volatile Compounds in Cornicabra VOO.

3.3. Minor volatile compounds

The hydrocarbons of olive oils have been studied by different authors as possible markers to distinguish virgin olive oil from different olive varieties or different geographical origins (Aparicio & Luna, 2002; Ben Temime et al., 2006; Haddada et al., 2007, Zunin, Boggia, Salvadeo, & Evangelisti, 2005). The hydrocarbons octane, 2,4-dimethyl-heptane, 4-methyl-octane, toluene, 4,8-dimetil-nona-1,7-diene, 11-tricosene, methylcycloctane, 1-nonene, 2-octanone, 1,4-hexadiene and limonene, a terpenoid hydrocarbon, have been detected in the aroma fractions of the tested oils. The contents of limonene did not exceed 0.8 mg kg^{-1}. This component could play a very important role in the fragrance of this precious food (Vichi et al., 2003a; Zunin et al., 2005). Their response to the maturation process and the irrigation was quite low (data not shown).

The toluene, a volatile aromatic hydrocarbon, was found in the headspaces of VOO tested in the present study (Table 3). The origin of this compound in VOO is largely unknown. Low amounts of this volatile were tentatively detected in the Sicilian samples, while in the Tunisian VOO, their levels were high (0.4–10.5 mg kg^{-1} and 0.5–13.3 mg kg^{-1} in Chétoui and Chemlali samples, respectively). A clear increase in the toluene content was observed for the Tunisian samples throughout the olive maturation. Some studies of the presence of this aromatic hydrocarbon in virgin olive oil have been carried out by other authors, revealing that they might arise from both exogenous contamination and endogenous pathways (Biedermann, Grob, & Morchio, 1995).

Other minor volatile compounds were observed in tested VOO. Among them, the hydrocarbons octane and the aldehydes heptanal, (*E*)-2-heptenal, (*E*)-2-octanal, octanal and nonanal are due to autoxidation reactions (Morales et al., 1997) that inevitably start after the virgin olive oil has been extracted (Morales et al., 2005). reported that almost all of these volatiles are responsible for virgin olive oil off-flavors that have undergone oxidation. However, in the analyzed samples, the amounts of compounds formed from oxidation reactions were low (data not shown). Moreover, carboxylic acids such as acetic, propanoic, butanoic, pentanoic, hexanoic, heptanoic and octanoic were also found in all the tested VOO. Velasco and Dobarganes (2002) work showed that a high oxygen concentration, from the storage of olive oil in contact with the air or frequent opening of oil containers, leads to a rate of formation of hydroperoxides that is higher than their decomposition rate. This leads to the production of carboxylic acids. Kalua et al. (2007) reported that carboxylic acids with two or three carbon atoms are associated with microbial fermentation and other fruit handling defects, whereas the higher carboxylic acids are linked to oxidative rancidity. Generally, these compounds are linked to sour and pungent sensations synonymous with sensory defects in olive oil. In all the tested samples, the most representative carboxylic acid is acetic acid ranged between 2.6 and 11.6 mg kg^{-1} in Sicilian VOO, while, their content in Tunisian ones did not exceed 2 mg kg^{-1} (Table 3). In almost studied oils, these levels increased considerably throughout the maturity process. Other studies reported that the presence of acetic acid in the headspaces of olive oil might be the result of the fermentation process in the olives. This compound generates the off-flavour: winey-vinegary in VOO (Angerosa, 2000; Morales et al., 2005). Quite low levels of 2-penthyl-furan (less than 1 mg kg^{-1}) were found in the volatile composition of all examined samples (data not shown). This is the first time that such volatile is tentatively detected in Tunisian VOO. The presence of similar compound in the headspace of fresh virgin oil was reported by Morales et al. (1995), whereas, Vichi et al. (2003b) reported that 2-penthyl-furan might be useful in distinguishing oxidation at the late stages. Indeed, their presence may be due to the degradation reactions of linoleate and linolenate hydroperoxides respectively, although the mechanisms of formation remain unclear (Frankel, 1980).

4. Conclusions

The application of SPME to the analysis of virgin olive oil headspace allowed the detection of significant differences in the proportions of volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils tested in their original cultivation area in relation to olive ripening stages and some agronomic practices such as irrigation.

As Tunisian and Sicilian oils were tested in their original growing area, additional works will be addressed in these local cultivars obtained in different growing districts in order to separate the genetic effect from the environmental factor in the volatile production.

Even if the data on the influence of the irrigation regime on volatile profiles of Chétoui virgin olive oils should be considered preliminary, due to the limited number of samples analyzed, the trend of the major compounds were according to those evidenced by other authors on different olive varieties. In general, Chétoui virgin olive oils were characterized by the lowest content of C_6 aldehydes as *E*-2-hexenal and esters, compounds give the typical notes associated with "green" and "fruity", respect to Chemlali and Sicilian samples. However, Chétoui oils obtained under the irrigation conditions displayed higher whole aroma concentration than non-irrigated ones and the amounts of some single volatile compounds reached so, through agronomical choices, could be possible an improvement of Chétoui aroma.

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