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Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars

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Abstract

Evaluation and identification of wild olive genetic resources allowed us to select new olive varieties and to recognize Tunisian grove richness and diversity. Five new olive cultivars were previously selected among populations of wild olive plants on the basis of agronomic and chemical evaluations. Their virgin olive oils were analysed for their fatty acid composition, quality indices (Free acidity, PV and UV characteristics) and oxidative stability. They were then submitted to solid phase microextraction (SPME) and their volatile compositions were determined.

Forty five compounds were isolated and characterized by GC–MS, representing 85%–98% of the total amount. The presence of some of these compounds in virgin olive oil had not been previously reported. The volatile compounds identified were mainly (E)-3-hexen-1-ol, (E)-2-hexen-1-ol, tricosane and β-selinene. Results demonstrated that the profiles of oleaster oils were distinctly different from those of European and Tunisian oils. All results indicate that there is a wide variability in the chemical and aroma characteristics of the selected oleaster virgin olive oils.

Keywords: Oleasters; Olive oil; Volatile compounds; Solid phase microextraction

1. Introduction

Virgin olive oil has a delicate and unique flavour that distinguishes it from other edible vegetable oils (Boskou, 1996). Its characteristic aroma and, in particular, the oil's green and fruity attributes depend on many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase (LOX) pathway which takes place during the oil extraction process (Angerosa, Mostallino, Basti, & Vito, 2000; Angerosa et al., 2004). Other compounds were derived from autoxidation of fatty acids (Angerosa et al., 2004).

Many analytical procedures have been used to isolate, identify and quantify the volatile components that characterize olive oil aroma (Angerosa, 2002). Among these extraction techniques, solid-phase microextraction (SPME) is a solvent-free sample preparation technique for the extraction of volatile and non-volatile compounds, and is also a simple and fast technique to implement. This method, developed by Arthur and Pawliszyn (1990) for water analyses and then applied to food analysis, has been used recently in food flavour analysis.

Several studies have been published on the analysis of olive oil volatile compounds using SPME, and many components have been identified (Bentivenga, D'Auria,

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De Luca, De Bona, & Mauriello, 2001; Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2004; Vichi et al., 2003).

In previous work, we evaluated the oils of 150 oleasters from six populations throughout Tunisia, and the results obtained showed the performance of five cultivars. The latter have been maintained in the Experimental Station of the Centre of Biotechnology; they have an improved oil composition compared to that of Chemlali. So a further study is required for their complete characterization. Sensory characteristics are used to define virgin olive oil quality.

The aim of this work is to give a contribution to the characterization of these oleasters by analyses of the volatiles obtained from virgin oils.

2. Materials and methods

2.1. Sampling

Six wild olive populations originating from different regions of Tunisia (Mateur, Ichkeul, Enfidha, Grombalia, Sers and Neber) were sampled in 2003 and 2004, totalling 150 trees. All trees were tagged and their exact location was noted. Superior trees were selected on the basis of oil quality, with trees showing high oil content of the fruit (>40% on dry mass basis) and high oleic acid content in the fatty acid profile (>70%) selected for further investigation. After harvesting, fresh olives were washed and deleafed, crushed with a hammer crusher, and the paste was mixed at 25 °C for 30 min, centrifuged without addition of warm water and then transferred into dark glass bottles, and stored in the dark at 4 °C.

2.2. Fatty acid composition

Methyl-esters were prepared from olive oil, after cold saponification, by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium and analysed by gas chromatography (GC) with a Hewlett–Packard (HP 4890 D) chromatograph equipped with a FID detector (Stefanovdaki, Kotsifaski, & Koutsaftakis, 1999).

2.3. Free acidity, peroxide value and specific ultraviolet absorbance (K_{232} and K_{270})

Determination of free acidity, peroxide value and ultraviolet absorbance were carried out following the analytical methods described in EEC Regulation (2568/91) and later modifications of the European Union. Free acidity, given as percent of oleic acid, was determined by titration of a solution of oil dissolved in ethanol–ether (1:1) with ethanolic potassium. Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq/kg), was determined as follows: a mixture of oil and chloroform–acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution. K_{232} and K_{270} extinction coefficients were calculated from absorptions at 232 nm and 270 nm, respectively, with a spectrophotometer (BECKMAN, Model 35), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

2.4. Pigment content

Oil (7.5 g) was accurately weighed and dissolved in cyclohexane up to a final volume of 25 ml. Chlorophyll and carotenoid contents were calculated from the absorption spectra of the oils. The absorption at 670 nm is usually considered to be related to the chlorophylls fraction, pheophytin "*a*" being its major component, the dominant pigment in the carotenoids fraction is lutein, and the absorption is measured at 470 nm. Thus, chlorophyll and carotenoid contents were expressed as mg of pheophytin "*a*" and lutein per kg of oil, respectively (Mınguez-Mosquera, Rejano, Gandul, Sanchez, & Garrido, 1991).

2.5. Rancimat assay

Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 743 apparatus (Metrohm Ω), using an oil sample of 3.6 g warmed to 101.6 °C and an air flow of 10 l/h.

2.6. Analysis of volatile compounds

2.6.1. Extraction

Solid phase microextraction was used as a technique for headspace sampling of virgin olive oils. Supelco SPME devices coated with polydimethylsiloxane (PDMS, 100 μm) were used to sample the headspace of 3 ml of virgin olive oil inserted into a 5 ml glass vial and allowed to equilibrate for 30 min. After the equilibration time, the fibre was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the GC and GC–MS system (Campeol, Flamini, Chericoni, Catalano, & Cremonini, 2001).

2.6.2. Identification

GC analyses were accomplished with an HP-5890 series II instrument equipped with a HP-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature programme: 60 °C for 10 min, ramp of 5 °C/min to 220 °C; injector and detector temperatures, 250 °C; carrier gas, nitrogen (2 ml/min); detector FID; split ratio, 1:30; injection, 0.5 μl. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons. The relative proportions

of the constituents were obtained by FID peak area normalization.

GC–EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness = 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas, helium at 1 ml/min; injection, 0.2 μl (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on a comparison of the retention times with those of authentic samples, by means of their LRI relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built from pure substances and components of known oils and literature data. Moreover, the molecular weights of all the identified substances were confirmed by GC–EIMS, using MeOH as CI ionizing gas. All of the reference compounds were obtained from Sigma–Aldrich (Sigma, Aldrich, and Fluka catalogues), with the exception of (E,E)-α-farnesene (TCI America), and (E)-2-decenal which originated from (Lancaster Synthesis Ltd).

2.7. Statistical analysis

The results are reported as the mean values. Data were compared on the basis of standard deviation of the mean values. In addition, Duncan's multiple range tests were used to determine significant differences among data. Statistical analysis was performed using the Statistica 5.0 package (StatSoft '97edition).

3. Results and discussion

3.1. Quality indices

All the oils produced and analysed (Table 1) showed very low values for the regulated physicochemical parameters evaluated (acidity ⩽ 0.8; peroxide index ⩽ 20 meq O_2/kg; $K_{270} \leqslant 0.22$; $K_{232} \leqslant 2.5$), with all of them falling within the "extra virgin" category, as stated by Regulation (IOOC, 2003) (Table 2).

3.2. Fatty acid composition

The distribution of fatty acids, from all tested samples, covered the normal range expected for olive oil (Table 2). Oleic acid is the main monounsaturated fatty acid, representing high concentrations (70.8%–73.9%) according to varieties. Palmitic acid, the major saturated fatty acid, ranged between 9.14% and 15.4%, whereas, linoleic acid was the dominant polyunsaturated fatty acid, ranging from 6.4% to 14.9% (Table 2). Also interesting, for varietal

Table 1
Olive oils examined in the study showing details of the fruit and processing

Geographic origin	Cultivars	Processing date	Maturity index	Malaxation time (min)
Mateur	Mat7	20 November	3.2	60
Ichkeul	I7	20 November	3.4	60
Enfidha	CEG6	25 October	3	60
Enfidha	SB12	5 December	3.2	60
Enfidha	SB6	5 December	3.5	60

Table 2
Analytical and compositional characteristics of fresh virgin olive oils from five selected oleasters

Analytical oil characteristics	Population of Mateur Mat7	Population of Ichkeul I7	Population of Enfidha			Chemlali
			CEG6	SB12	SB6	
Palmitic acid, C16:0 (%)	9.14e	12.4c	15.4b	11.1d	12.7c	19.5a
Palmitoleic acid, C16:1 (%)	1.17c	2b	3.1a	1.03c	1.14c	2.26b
Stearic acid, C18:0 (%)	1.42c	3.16a	1.9b	1.9b	3.2a	2.82a
Oleic acid, C18:1 (%)	72.2ab	73.9a	72ab	71.3ab	70.8b	54.8c
Linoleic acid, C18:2 (%)	14.9b	7.41d	6.4d	13.5b	10.6c	18.4a
Linolenic acid, C18:3 (%)	0.57b	0.6b	0.89a	0.37c	0.9a	0.85a
Arachidic acid, C20:0 (%)	0.23c	0.4b	0.26c	0.3c	0.57a	0.52a
Unsaturated fatty acids (%)	89a	83.9c	82.4d	86.2b	83.4cd	76.0e
Monounsaturated fatty acids/polyunsaturated fatty acids ratio	4.72d	9.47b	10.3a	5.2d	6.2c	2.97e
Oleic acid/linoleic acid ratio	4.83d	9.96b	11.2a	5.3d	6.74c	2.99e
Free acidity	0.3b	0.3b	0.5a	0.3b	0.43a	0.48a
$PV_{(meqO_2/kg)}$	2.4c	6.56a	7.66a	5.8b	5.9b	6.7a
K_{232}	0.9c	1.38*a*	1.3b	1.15c	1.12c	1.4a
K_{270}	0.09b	0.11a	0.1b	0.12*a*	0.13a	0.12a
Chlorophylls (mg/kg)	1.9c	2.3bc	4.3*a*	2.8b	4.9a	2.2bc
Carotenoids (mg/kg)	2.40b	1.68c	2.63b	2.24b	4.19a	1.51c
Oxidative stability (h)	48.85b	36.51c	42.1c	70.4a	38.5c	23.8d

Data are expressed by mean values ± SD of four independent experiments. Values followed by same letters are not significantly different (Duncan's test, $P = 0.05$).

characterization are the proportions of some classes of fatty acids. Table 2 shows that the proportion of unsaturated fatty acids also changes according to oleasters. It reaches a maximum value of 89% for Mat7 and a minimal value of 82.4% for CEG6. The other oils have intermediate values. Monounsaturated/polyunsaturated fatty acids ratio (MUFA/PUFA) varies from 4.72 to 10.3 for all studied varieties (Table 2). The oleic/linoleic acid ratio of I7 and CEG6 (9.96 and 11.2, respectively) was relatively higher than the required minimum value of 7 (Kiritsakis, Nauos, Polymenoupoulos, Thomai, & Sfakiotakis, 1998).

3.3. Pigment content

Chlorophylls and carotenoids exert an antioxidant action in the dark but an oxidant one in the light (Gutiérrez, Villafranca, & Castellano, 2002). These substances also have biological and health properties (Boskou, 1996) and occur in the oil at concentrations which usually correlate with those of phenols and volatiles (Ryan, Robards, & Lavee, 1998).

Chlorophylls and carotenoids in Oleaster oils ranged from 1.9 to 4.03 mg/kg and from 1.68 to 4.19 mg/kg, respectively (Table 2). The concentration of pigments correlates well with the stage of maturity of the harvested olives (Boskou, 1996).

3.4. Oxidative stability

The oxidative stability of the olive oils studied varied according to cultivars. It ranged between 36.5 h and 70.4 h (Table 2). The five oleasters exhibited higher oil stability than did Chemlali, the most important variety cultivated in Tunisia (23.8 h). Virgin olive oil provides a rich source of natural antioxidants. These include carotenoids, and phenolic compounds which may act, by different mechanisms, to confer an effective defence system against free radical attack. Some authors have estimated their contribution to oil stability, that of phenolic compounds being around 30%, fatty acids 27% and carotenoids 6% (Aparicio, Roda, Albi, & Gutiérrez, 1999).

3.5. Volatile compounds

Flavour is an important quality criterion for virgin olive oils. The identification of the compounds causing the flavour or off-flavour is therefore the key for quality control. Virgin olive oil has a delicate and unique flavour (Angerosa et al., 2000; Ridol, Terenziani, Patumi, & Fontanazza, 2002).

Headspace-solid phase microextraction was used to characterize the volatile compounds present in virgin olive oils from some selected oleasters. Forty five volatile compounds were isolated and characterized by GC–MS analysis (Table 3).

In the five oleaster oils, the isolated and identified compounds are mainly alcohols, with 16.0% to 83.3% of the total peak area percentage, such as hexanol, (E)-2-hexen-1-ol and (E)-3-hexen-1-ol. Furthermore, the percentage of aldehydes differed according to the cultivar. It ranged from 0.9% (SB12) to 22.42% (Mat7). For the oleasters involved in this study, several terpenic hydrocarbons (mono- and sesquiterpenes) were often detected in the main aroma fractions of the oils tested and the sum of their areas accounted for 4.5% (SB6) to 57.3% (I7) (Fig. 1).

The major constituents of the volatile fraction of I7 oils were β-selinene (25.2%), 1,8-cineole (12.2%), *n*-hexanol (8.95%), *ortho*-guaiacol (8.74%), phenyl ethyl alcohol (5.89%), α-pinene (5.07%) and heptanal (4.35%) (Table 3).

The volatile fraction of Mat7 oil was characterized by the dominance of three compounds: (E)-2-hexen-1-ol (40.8%), (E)-2-hexenal (10.56%) and 1,8-cineole (7.75%) (Table 3). Trans-2-hexenal was the most important positive contributor of lawn perception. The other compounds identified were mainly (E,E)-2,4-octadienal (4.42%), nonanal (3.82%), mesitylene (3.13%) and β-caryophyllene (2.73%) (Table 3).

The main constituents that characterize the volatile fraction of SB12 oil were tricosane (44.8%), 1,8-cineole (13.6%), (E)-2-hexen-1-ol (11.9%) and (E)-2-decenal (4.84%). SB6 is distinguishable from the others by its higher content of (E)-3-hexen-1-ol (83.3%).

Finally, (E)-2-hexen-1-ol (28.8%) and β-selinene (24.8%) were the two compounds that qualitatively characterized the volatile fraction of CEG6 (Table 3).

Comparing the five oleasters collected at the same stage of ripeness, we can observe that there were some differences in the constituents of the volatile fraction. Only in oleaster I7 were *n*-hexanol, heptanol, myrcene, ethyl hexanoate, 1-undecene, *meta*-cresol and *ortho*-guaiacol present. 6-Methyl-5-hepten-2-one and liguloxide were present only in CEG6, whereas tricosane was detected only in SB12 (Table 3).

The volatile compounds identified were mainly (E)-3-hexen-1-ol, (E)-2-hexen-1-ol, tricosane and β-selinene in contrast to Tunisian monovarietal virgin olive oils (Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006) and European oils (Cavalli et al., 2004). The presence of tricosane in virgin olive oil aroma has not been previously reported in the literature.

The low level of esters in the five oleasters also indicates a lower content of alcohol acetyl transferase (AAT) in these olive oils (Cavalli et al., 2004). Moreover, the high level of (E)-2-hexen-1-ol and (E)-3-hexen-1-ol in SB6 and Mat7 oils shows the pre-eminence of the (E)-hex-2-enal/(E)-hex-2-enol pathway compared to the hexanal/hexanol pathway in all the oleasters considered.

The most notable feature of the data was the distinction from European and Tunisian oils in which E-hexenal was the dominant volatile in the profile of all published oils (Cavalli et al., 2004; Ridol et al., 2002; Vichi et al., 2003).

For the oleasters involved in this study, mono and sesquiterpene hydrocarbons have been detected in the main aroma fractions of the oils tested. The role of these compo-

Table 3
Volatile compounds extracted by SPME in the five oleaster virgin olive oils

Volatile compounds[a]	LRI[b]	I7	Mat7	SB6	SB12	CEG6
Hexanol	848	8.95				
(E)-3-hexen-1-ol	852			83.3		
(E)-2-hexenal	856		10.6			
(E)-2-hexen-1-ol	862		40.8		11.9	28.8
Heptanal	901	4.35	2.38			
Tricyclene	931	1.11		0.2		1.8
α-Pinene	942	5.07		2.19		3.32
Camphene	959			0.03		
Heptanol	974	0.76				
Sabinene	979	1.38		0.09		
β-Pinene	984	0.59			0.42	
6-Methyl-5-hepten-2-one	988					3.83
Myrcene	992	0.91				
2,3-Dehydro-1,8-cineole	994	0.75				1.9
Mesitylene	996		3.13			0.92
Ethyl hexanoate	999	1.19				
(E)-3-hexen-1-ol-acetate	1005					0.08
3-Methyl-4-penten-1-ol acetate	1005		2.65			0.75
o-Methyl anisole	1013					0.13
p-Cymene	1030	3.03	0.91		1.61	1.13
Limonene	1035	3.77	1.7		2.02	3.26
1,8-Cineole	1039	12.2	7.75	tr[c]	13.6	7.45
(E)-ocimene	1052	1.1	1.3		1.09	
γ-Terpinene	1064	0.62			0.45	
1-Undecene	1075	0.74	tr			
meta-Cresol	1077	0.97				
ortho-Guaiacol	1089	8.74				
Linalool	1101				0.5	
Nonanal	1104	1.93	3.82		0.9	
(E,E)-2,4-octadienal	1115		4.42			
Phenyl ethyl alcohol	1117	5.89			1.67	
cis-*p*-Menth-2-en-1-ol	1129				1.23	
Camphor	1154		0.45		0.48	
α-Terpineol	1191				0.74	
Decanal	1205		0.74	10.1		2.38
(E)-2-decenal	1263		0.5			
α-Copaene	1377	tr		1.17	4.84	
Longifolene	1387		0.56			
β-Caryophyllene	1420	0.98	2.73		0.87	
β-Selinene	1487	25.2	2.46		2.03	24.8
(E,E)-α-farnesene	1506	0.67				2.3
Kessane[d]	1530		0.61	0.82		
Liguloxide	1533					2.93
Tricosane	2300				44.8	

[a] Percentages obtained by FID peak-area normalization (HP-5 column).
[b] Linear retention indices (DB-5 column).
[c] tr ⩽ 0.1%.
[d] Tentative identification (no reference compound available).

nents in the definition of flavour is not clear. In fact, in the literature, only very few papers (Bentivenga et al., 2001; Vichi et al., 2003) report the presence of these compounds, which could play a very important role in the fragrance of this valuable food.

Published work has focussed on the C5, C6 compound arising from the LOX pathway (Angerosa, 2002; Cavalli et al., 2004). In the present work, this has been extended to less volatile compounds with retention indices exceeding 1400. The focus on C5, C6 compounds has also been criticised by Reiners and Grosch (1998) who found that "heavier" volatiles, up to C10 (e.g., deca-2,4dienal), are significant components of the volatile aroma fraction.

Among the C6 compounds, (E)-2-hexenal distinguished the sample from Mat7, whereas the corresponding alcohol, (E)-2-hexen-1-ol, characterized Mat7, SB12 and CEG6. The overall amounts of C6 alcohols were higher than the sum of C6 aldehydes and C6 esters in the five oleasters (Fig. 2). These results may be explained by differential activity of the enzyme alcohol dehydrogenase (ADH), which reduces the C6 aldehydic compounds in the corresponding alcohols. Thus, hexanol, (E)-3-hexen-1-ol,

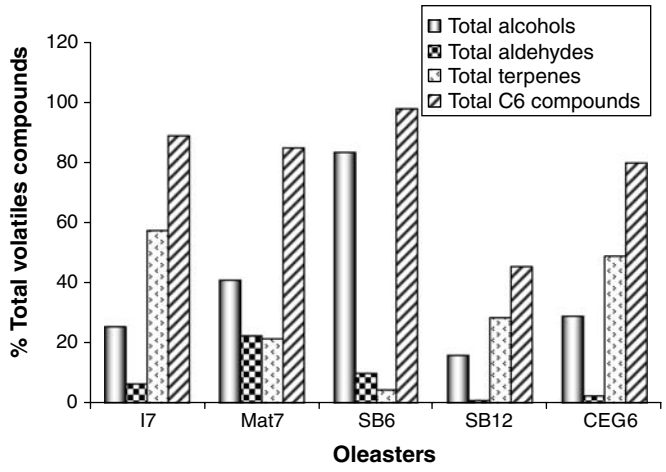

Fig. 1. Distribution of aldehydes, alcohols and terpenic hydrocarbons in relation to total volatile compounds.

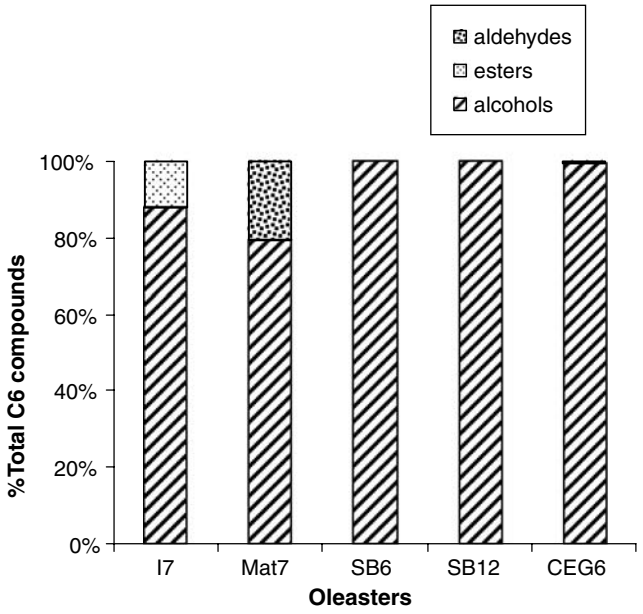

Fig. 2. Distribution of C6 aldehydes, alcohols and esters in relation to total C6 compounds.

(E)-2-hexenal and (E)-2-hexen-1-ol showed strong dependence on cultivar effect, suggesting the exclusive effect of the cultivar on the concentrations of the volatile compounds.

A comparison with literature data on the chemical composition of olive oils is difficult because of the great variability of the volatile compositions. Some differences can be found in the fatty acid content of varietal virgin olive oils (Table 2) but they do not vary so much as to be determinants of the profile of volatiles (Table 3). In fact, it has been reported that the concentrations of volatile compounds depend on the enzymatic activity though external parameters (e.g., climate, soil, harvesting and extraction conditions) and may alter the inherent olive oil sensory profile (Kanavouras, Kiritsakis, & Hernandez, 2005; Morales, Luna, & Aparicio, 2005; Vichi et al., 2003).

4. Conclusion

In conclusion, the results confirm that there is a great variation in all of the measured characters among the oleaster populations in Tunisia. The five oleasters prove to be interesting and distinctly present an improved olive oil quality compared to that of the Chemlali variety.

Analysis of the five oleaster virgin olive oils by SPME enabled us to identify 45 compounds, representing 85% to 98% of the chemical composition. The genetic effect related to the cultivar is one of the most important aspects of volatile composition of olive oil. All these results indicate that olive oil aroma compounds accumulate differently according to the cultivar. In fact, accumulation of these metabolites has a close dependence on the enzymatic store, which is genetically determined, according to results of other researches (Angerosa, Basti, & Vito, 1999).

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