Research Article

Received: 28 July 2008 Revised: 18 February 2009 Accepted: 26 February 2009 Published online in Wiley Interscience: 06 April 2009

(www.interscience.wiley.com) DOI 10.1002/jsfa.3588

Cultivar and growing area effects on minor compounds of olive oil from autochthonous and European introduced cultivars in Tunisia

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Abstract

BACKGROUND: Antioxidant profile and volatile compounds were characterized in three virgin olive oils from European countries introduced and cultivated under the same orchard conditions in comparison to some autochthonous cultivars planted in different areas of Tunisia.

RESULTS: Significant differences were observed between the oils. α-Tocopherol content is more important in autochthonous Tunisian cultivars (cvs), higher (400 mg kg^{-1}) than in European cvs. Total phenols showed that *Chétoui* cv. (grown in Zaghouan) had the highest level (446 mg kg^{-1}), followed by *Koroneiki* (403 mg kg^{-1}) and *Chétoui* cvs (grown in Béja) (398 mg kg^{-1}). *Koroneiki* oils had the highest content of (3,4-dihydroxyphenyl)ethanol and (*p*-hydroxyphenyl)ethanol (20.5 and 43.5 mg kg^{-1}, respectively), whereas (3,4-dihydroxyphenyl)ethanol was not detected in *Arbequina, Arbosana* or *Chemlali* cvs (grown in Sahel). *Chétoui* cv. presented the highest content of dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)ethanol (171 mg kg^{-1}), whereas *Chemlali* (Sahel) cv. had the lowest content (29.6 mg kg^{-1}). The volatile compounds showed an increase in C6 compounds and decrease in pentene isomers in olive oils from varieties cultivated in other growing areas.

CONCLUSION: Virgin olive oils studied demonstrate that the differences in phenols, tocopherol levels and volatile profiles may be explained by genetic factors and geographic areas, particularly altitude.

Keywords: autochthonous olive oil; European introduced cultivars; growing area (altitude); α-tocopherol; phenols; volatile compounds

INTRODUCTION

Olive production is an important economic sector throughout the Mediterranean. In some areas such as Tunisia, it is the principal economic activity and the basis for other sectors, upstream and downstream (inputs, processing). For many years, people have known that olives from some cultivars are more suited to the production of table olives, while others are used for oil production, and that the oils obtained from different cultivars have different characteristics.[1]

Olive oil quality depends on market preferences and consumer perceptions of aroma, taste and colour, which may change over time and according to the location of growth.[2] Flavour and aroma of this oil as well as of other vegetable oils vary quite considerably and are derived mainly from a number of volatile constituents that are present at extremely low concentrations.[3–5] The volatile composition of olive oil depends on the level and the activity of enzymes involved in the lipoxygenase pathway.[6,7] The enzymatic levels are genetically determined[8] and a number of factors affect their activities like ripening cycle of the fruit[9–11] and the processing equipment.[4,12–15] The effects of climate and soil type have also been studied.[16] However, the effects of these variables on volatile profiles are ambiguous.

Olive oil is obtained from the fruit of several cultivars of olive tree (*Olea europea* L.), each with particular characteristics. Each one of these cultivars exhibits specific physical and biochemical characteristics, providing oils with different compositions and performances.[17] Virgin olive oil (VOO) is recognized by its oxidative quality and stability properties due to the natural presence of a group of minor components having a marked antioxidant activity, namely phenolic compounds, improperly referred to as polyphenols and tocopherols. Phenolic compounds, characteristic of unrefined olive oil, are also valuable for their functional, biological and nutritional roles,[18] and are responsible for the bitterness and pungency in oils.[19] Therefore, the detailed

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composition of olive oil and its sensorial characteristics, besides being strongly dependent on the nature of the cultivar used for its production, show great importance thanks to their health benefits and effect on oil shelf-life.[17] Taking into account the importance of these compounds in olive oil production and the need to select cultivars with VOO of good quality characteristics, the objective of using new cultivars adapted to our environmental conditions and arid climate was to diversify our olive genetic resources. At present, Tunisian olive growing is dominated by only two main varieties: *Chétoui* and *Chemlali*; however, this latter cultivar contains despite his ordinary organoleptic and taste characteristics, high levels of palmitic and linoleic acids.[20,21]

Since phenolics and volatiles are the compounds mainly responsible for the desirable flavour of extra virgin olive oils (EVOOs) and since, to a large extent, they determine the degree of consumer preference for this highly regarded product, this research assessed the role played by the growing area conditions on the oil quality of some Tunisian autochthonous cultivars (*Oueslati, Chétoui* and *Chemlali*) in comparison to three introduced varieties (*Arbosana, Koroneiki* and *Arbequina*).

MATERIALS AND METHODS

Plant material and growing areas selected

Olive fruits (*Olea europea*) of the varieties *Arbosana, Koroneiki* and *Arbequina* were collected from Tunis, *Chétoui* from Béja and Zaghouan, *Oueslati* from Tunis and Kairouan and *Chemlali* from the Sahel at full maturation from November to January of the 2006–2007 season. The characteristics of production areas of olive varieties studied are reported in Fig. 1. The olives were picked manually, using rakes when the most abundant ripening stage was obtained. After harvesting, the olive fruit samples were immediately transported to the laboratory, where they were transformed into oil within 24 h. Only healthy fruits, without any kind of infection or physical damage, were crushed by continuous processing equipped with a hammer crusher, horizontal malaxator (at a temperature of 30 °C) and a two-phase decanter.

Reagents and standards

(3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cayman Chemical Co. (Ann Arbor, MI, USA), while (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium). The dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), (+)-1-acetoxypinoresinol and (+)-pinoresinol were extracted from VOO using a previously reported procedure.[22] The purity of all the substances obtained from direct extraction was tested by high-performance liquid chromatography (HPLC) and their chemical structures were verified by nuclear magnetic resonance (NMR). Pure analytical standards of volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

Oil sample analysis

Quality indices

The legal quality characteristics of EVOO, i.e., free fatty acids, peroxide value and UV absorption characteristics at 232 and 270 nm, were determined following the analytical methods described in EU official methods.[23]

Free fatty acids, given as percent of oleic acid, were determined by titration of a solution of oil dissolved in ethanol/ether (1 : 1) with 0.1 mol L^{-1} potassium hydroxide ethanolic solution.

Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq kg^{-1}), 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.

K232 and K270 extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, in order to study the primary and secondary oxidation of oils, using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Phenol profiles

The phenolic fractions were extracted by liquid–liquid extraction[24] and analysed by HPLC. Before injection, the phenolic extract was solubilized with 1 mL methanol and filtered through a hydrophilic polyvinylidene fluoride (PVDF) syringe filter 0.2 μm. HPLC analysis was conducted as reported by Selvaggini *et al*.,[25] using an Agilent Technologies system model 1100 (Agilent Technologies, Palo Alto, CA, USA), composed of a vacuum degasser, quaternary pump, autosampler, thermostated column compartment, diode array detector (DAD) and fluorescence detector (FLD). The oil extract analysis was performed using C18 columns, Spherisorb ODS-1, 250 × 4.6 mm with a particle size of 5 μm (Phase Separation Ltd, Deeside, UK). The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL min^{-1}, with the following gradient: 95% A/5% B for 2 min, to 75% A/25% B for 8 min, to 60% A/40% B for 10 min, to 50% A/50% B for 16 min, to 0% A/100% B for 14 min, finishing with a plateau with this composition maintained for 10 min. Initial conditions were then reset and equilibration was reached in 13 min. The total run time was 73 min.

α-Tocopherol

α-Tocopherol was evaluated according to Psomiadou and Tsimidou,[26] with the following modifications: 2 g oil were dissolved in 10 mL hexane, filtered through a PVDF syringe filter (0.2 μm) and injected into the HPLC system with a Waters μPorasil column (30 × 3.9 mm × 10 μm (Milford, MA, USA) using the following mobile phase step gradient with a flow rate of 2 mL min^{-1}: 100% *n*-hexane with 0.5% isopropyl alcohol (A)/0% *n*-hexane with 10% isopropyl alcohol (B) for 4 min, to 60% A/40% B for 14 min, to 40% A/60% B for 4 min, to 100% A/0% B for 3 min. This final mix was maintained during a 5 min plateau. The total run time was 30 min and the injection volume was 50 μL. The detector was an FLD operated at an excitation wavelength set at 294 nm and the emission wavelength at 330 nm.

Volatile compounds

The volatile compounds were determined by headspace solid-phase microextraction (SPME)–gas chromatography–mass spectrometry (HS-SPME-GC-MS), as previously reported by Servili *et al*.[27] For the SPME analysis, the oil (3 g) was put into a 10 mL vial and thermostated at 35 °C for 15 min. Then, 65 μm Carbowax/divinylbenzene fibre (Supelco, Inc., Bellefonte, PA, USA) was exposed to the vapour phase for 30 min in order to sample the volatile compounds. Afterwards, the fibre was inserted into the gas chromatograph injector set to splitless mode, using a splitless inlet liner of 0.75 mm i.d. for thermal desorption, where it was left for 5 min. All SPME operations were automated using a Varian 8200 CX AutoSampler (Varian, Walnut Creek, CA, USA).

A Varian 4000 GC equipped with a 1078 split/splitless injector coupled with a Varian Saturn 3 mass spectrometer was used with

a fused-silica capillary column, DB-Wax, 50 m, 0.32 mm i.d., 1 µm film thickness (J&W Scientific, Folson, CA, USA). The column was operated with helium at 35 °C and a pressure of 15 psi at a flow rate of 1.7 mL min^{-1} and a linear velocity of 30.7 cm s^{-1}.

GC oven heating was started at 35 °C. This temperature was maintained for 8 min, then increased to 45 °C at a rate of 1.5 °C min^{-1}, then to 150 °C at a rate of 3 °C min^{-1}, further to 180 °C at a rate of 4 °C min^{-1} and finally increased to 210 °C at a rate of 3.6 °C min^{-1}, where it was held for 14.50 min. The total time of analysis was 80 min. The injector temperature was maintained at 250 °C. The transfer line temperature was fixed at 220 °C. The mass spectrometer was operated in the electron ionization mode at an ionization voltage of 70 eV in the mass range of 10–350 amu at a scan rate of 1 s/scan and a manifold temperature of 180 °C. The volatile compounds were identified by comparison with their mass spectra and retention times against those of reference compounds. When standards were not available, identification of the volatile compounds was obtained by comparing their mass spectral data with those of the Wiley 6 mass spectra library. Integration of all of chromatographic peaks was performed by choosing the three masses, among those specific for each compound, with the highest intensities in order to selectively discriminate them from the nearest neighbours. A quantitative determination of selected volatile compounds was expressed as mg kg^{-1} of oil.

Statistical analysis

All parameters analysed were carried out in triplicate. The results are reported as mean values of three repetitions and standard deviation. Significant differences among varieties studied were determined by analysis of variance which applied a Duncan test with a 95% significant level ($P < 0.05$), using the SPSS program, release 11.0 for Windows. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were carried out using XLStat-Pro 7.5 (2007) for Windows (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

This research was focused on the study of the behaviour of olive oil components of three European olive cultivars (*Arbosana, Koroneiki* and *Arbequina*) grown in Tunis in comparison with some autochthonous olive cultivars: *Chétoui* grown in Béja and Zaghouan, *Oueslati* grown in Tunis and Kairouan and *Chemlali* Sahel grown in different regions in Tunisia (Fig. 1).

Quality indices

The quality indices evaluated in the olive oil samples from the different cultivars studied are shown in Table 1. The quality indices of VOOs revealed differences among varieties. On the one hand, the free fatty acid content of all analysed samples was below 0.8 and fell within the accepted value for EVOOs.[28] On the other hand, peroxide values and UV absorption characteristics (K232 and K270) did not exceed the established limits,[23] except for *Chétoui* Béja, where K232 was higher than the limit of 2.5 established for EVOO. As reported by several authors,[4,14,29] cultivar or origin area had no significant influence on these analytical parameters, which were basically affected by factors causing damage to the fruits, e.g., olive fly attacks or improper systems of harvesting, transport and storage of olives. Consequently, this result could be attributed to the processing technology of oils because the raw material was carefully selected, picked and processed. This sample represented an intermediate level of degradation.

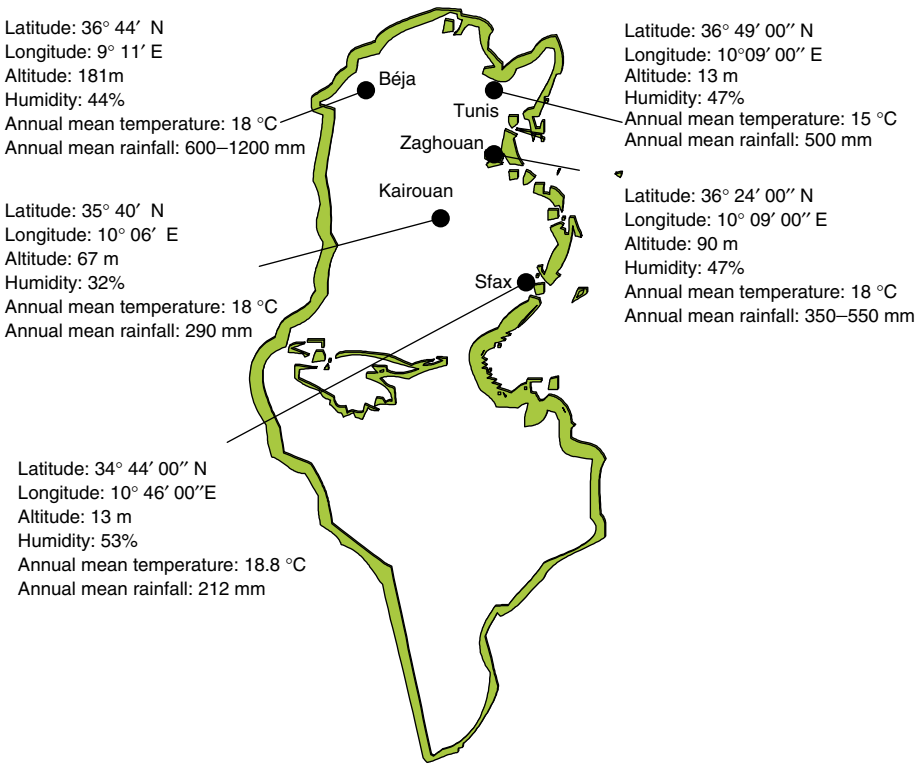

Figure 1. Description of the growing areas of the different cultivars studied.

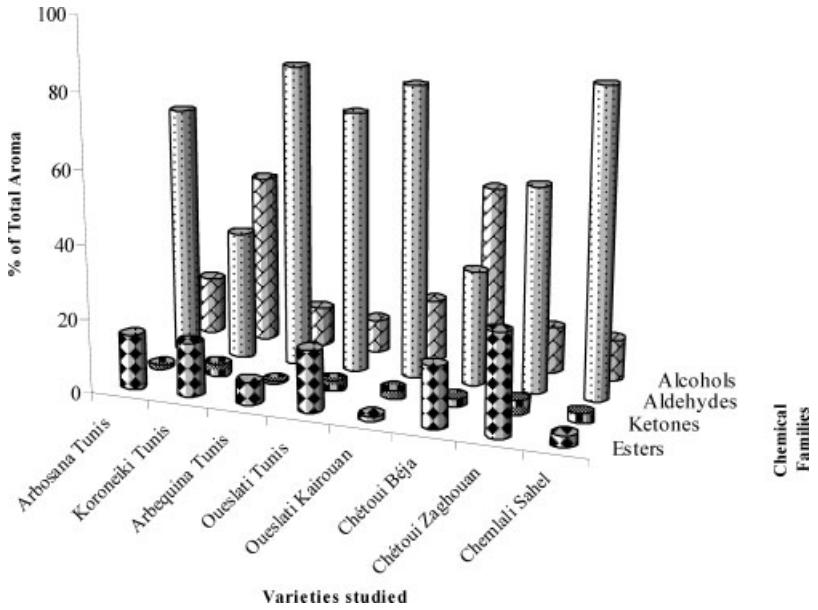

Figure 2. Chemical families of volatile compounds present in the analysed headspaces of virgin olive oils obtained from different cultivars studied (results expressed as percent of total aroma).

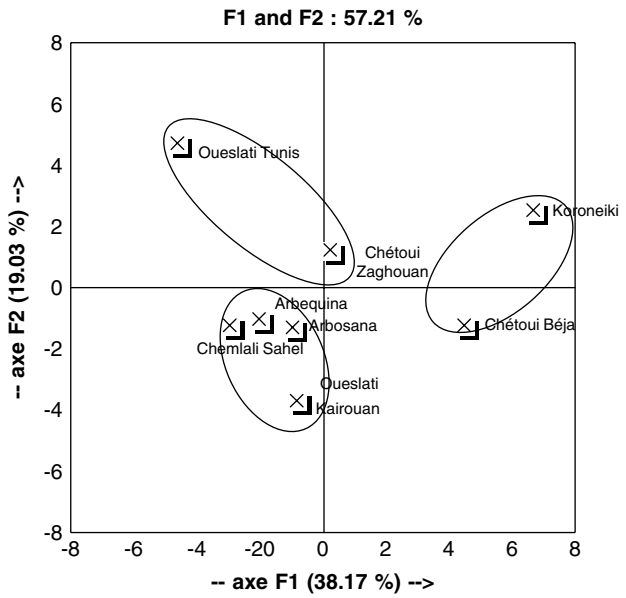

Figure 3. Scores plot of principal component analysis applied to the dataset of phenolic fraction and volatile compounds of virgin olive oils obtained from different cultivars studied.

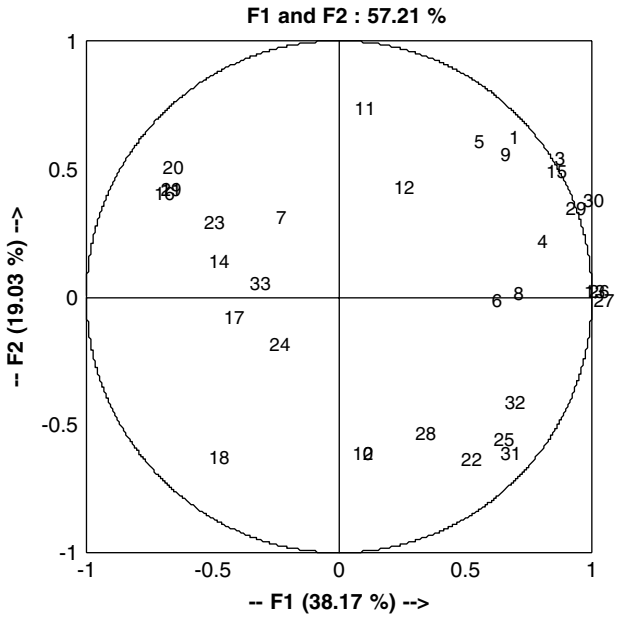

Figure 4. Loading plot of principal component analysis applied to the dataset of phenolic fraction and volatile compounds of virgin olive oils obtained from different cultivars studied (see Table 4 for list of variables).

Antioxidant compounds

Tocopherols and polyphenols are recognized as antioxidants and their presence in olive oils has been related to their general quality, improving stability, nutritional value and sensorial properties.[4,5] Fat-soluble α-tocopherol, the analogue having the highest biological activity, is the predominant representative of vitamin E in VOO. It is the main chain-breaking antioxidant of the oil.[30,31] Mean values for α-tocopherol content of the cultivars studied varied between 240 and 480 mg kg^{-1} (Table 2). Oils from *Oueslati* Kairouan cv. have higher α-tocopherol content (480 mg kg^{-1}) than other cultivars. Comparison between the different cultivars studied showed that the oils from autochthonous cultivars were richer in tocopherol than those from introduced varieties. Differences in α-tocopherol contents were also observed in oils from the same cultivar planted in different areas; hence *Oueslati* Kairouan presented a higher level than *Oueslati* Tunis (about twofold). The same result was observed for the two *Chétoui* cultivars planted in the north of Tunisia, in Béja and Tunis (Table 2). This result could be attributed to the large difference in their location and consequently in the geographic altitude of these two zones. Tocopherol content increased with the increase in the altitude, whereas water availability (Fig. 1) did not show an influence on this antioxidant. The amount of phenolic compounds in VOO

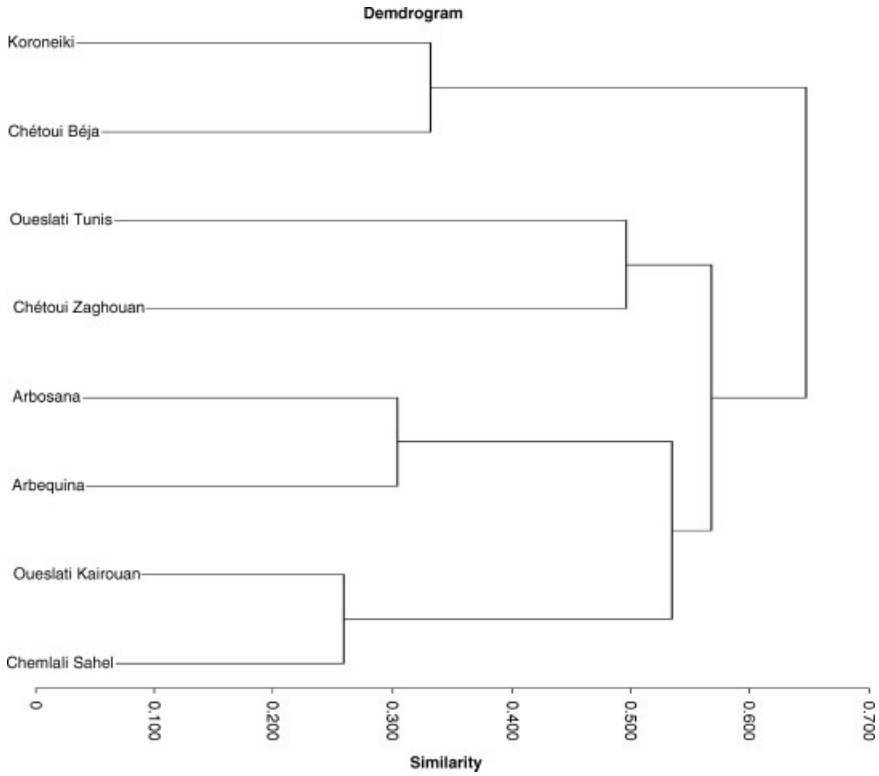

Figure 5. Dendogram showing clustering of the phenolic fraction and volatile compounds of virgin olive oils obtained from the different cultivars studied.

is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation and, to a certain extent, are responsible for its sharp bitter taste.[32] Comparison of the total phenol content evaluated by HPLC in oils produced from the different cultivars studied revealed that the oils produced from *Chétoui* Zaghouan cv. showed the highest level (446 mg kg^{-1}), followed by *Koroneiki* cv. (403 mg kg^{-1}) and *Chétoui* Béja cv. (398 mg kg^{-1}). Concerning the phenolic profile and in order to simplify the result, only the most important phenol compounds (3,4-DHPEA, *p*-HPEA, 3,4-DHPEA-EDA, *p*-HPEA-EDA, (+)-1-acetoxypinoresinol, (+)-1-pinoresinol and 3,4 DHPEA-EA) were studied by HPLC (Table 2). 3,4-DHPEA and *p*-HPEA are the main phenolic alcohols in VOO. Our results showed that the most abundant phenolic alcohols were revealed in *Koroneiki* oils (20.5 and 43.5 mg kg^{-1} 3,4-DHPEA and *p*-HPEA, respectively), whereas 3,4-DHPEA was not detected in oils from *Arbequina, Arbosana* and *Chemlali* Sahel (Table 2). This result confirms our previous studies[33] indicating that the concentration of phenolic acids generally is low in fresh VOO but increases during oil storage due to the hydrolysis of VOO secoiridoids, which contain 3,4-DHPEA and *p*-HPEA in their molecular structure.[34] The prevalent phenols of VOO, however, are the secoiridoids, which are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure (Table 2). The major secoiridoids of VOO are 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA. Comparing studied samples, *Chétoui* cvs present the highest content of 3,4-DHPEA-EDA (178 and 164 mg kg^{-1} for Zaghouan and Béja cvs, respectively), whereas *Chemlali* Sahel has the lowest content (29.6 mg kg^{-1}). Approximately the same result was observed for *p*-HPEA-EDA and 3,4-DHPEA-EA.[35] Lignans, especially (+)-1-acetoxypinoresinol and (+)-pinoresinol, are also found as prevalent phenolic compounds in VOO. *Chétoui* Zaghouan had the lowest and the highest contents of (+)-1-acetoxypinoresinol and (+)-pinoresinol (7 and 28.6 mg kg^{-1}, respectively). In contrast, *Oueslati* Tunis had a higher content of (+)-1-acetoxypinoresinol (24 mg kg^{-1}) and the lowest content of (+)-pinoresinol (7 mg kg^{-1}). Phenol concentrations in the oils were markedly different between introduced and autochthonous cultivars; autochthonous varieties exhibited the highest contents of phenols except for oils obtained from *Koroneiki* cv. (403 mg kg^{-1}), which showed the most stable antioxidant contents in comparison to *Arbosana* and *Arbequina* when planted in Tunisia.

As for α-tocopherol, differences in phenolic content were also observed in the same variety cultivated in different areas, where *Oueslati* cvs showed big differences in contrast to *Chétoui* varieties. The two *Chétoui* cvs presented nearly the same results (398 and 446 mg kg^{-1} for *Chétoui* Béja and *Chétoui* Zaghouan, respectively), whereas *Oueslati* exhibited high variation (148 and 292.6 mg kg^{-1} for *Oueslati* cultivated in different areas in Kairouan and Tunis, respectively).

Among European varieties, *Koroneiki* cv. showed a higher level of total phenols than *Arbosana* and *Arbequina* cvs. Similarities in phenolic levels were observed between *Koroneiki* and *Chétoui* Zaghouan cv. (403 and 446 mg kg^{-1}, respectively). Another similarity was also observed between *Arbosana* and *Chemlali* Sahel cvs, where their phenol contents were moderate (133.6 and 136 mg kg^{-1}, respectively). However, *Arbequina* cv. grown in Tunis had the lowest value for phenolic compounds (95.5 mg kg^{-1}). Comparing some cultivars grown in different areas, level and composition of phenolic compounds were different between *Oueslati* and *Chétoui* cvs. Total phenolic compound level of *Chétoui* Zaghouan was higher than those of *Chétoui* Béja; this variability is observed more for *Oueslati* when cultivated in Tunis and Kairouan (origin location). This difference may be due to the geographic

Table 1. Quality indices of virgin olive oils from introduced and autochthonous varieties cultivated in different areas

	Introduced cultivars and growing areas			Autochtonous cultivars and growing areas				
	Arbosana	Koroneiki	Arbequina	Oueslati		Chétoui		Chemlali
Quality index	Tunis	Tunis	Tunis	Tunis	Kairouan	Béja	Zaghouan	Sahel
Free fatty acids (% oleic acid)	0.40 ± 0.03e	0.60 ± 0.03b	0.32 ± 0.02e	0.69 ± 0.04a	0.47 ± 0.04d	0.54 ± 0.04c	0.35 ± 0.04de	0.37 ± 0.03de
Peroxide index (meq kg^{-1})	12.58 ± 0.10c	11.15 ± 0.10d	10.96 ± 0.31de	18.00 ± 0.06a	10.74 ± 0.17e	16.44 ± 0.05b	10.06 ± 0.06f	12.60 ± 0.18c
K232	2.12 ± 0.01c	1.67 ± 0.08d	2.46 ± 0.06b	1.75 ± 0.04d	2.07 ± 0.07c	2.88 ± 0.26a	2.08 ± 0.06c	2.17 ± 0.06c
K270	0.11 ± 0.02bc	0.11 ± 0.03bc	0.08 ± 0.01c	0.11 ± 0.03bc	0.13 ± 0.03ab	0.15 ± 0.03ab	0.18 ± 0.04a	0.13 ± 0.01ab

Values are the means of the three different VOO samples ($n = 3$) ± standard deviations. Different letters indicate significant differences ($P < 0.05$) between cultivars.

Table 2. Antioxidants (mg kg^{-1} of oil) evaluated in virgin olive oils from introduced and autochthonous varieties cultivated in different areas

	Introduced cultivars and growing areas			Autochtonous cultivars and growing areas				
	Arbosana	Koroneiki	Arbequina	Oueslati		Chétoui		Chemlali
Antioxidant	Tunis	Tunis	Tunis	Tunis	Kairouan	Béja	Zaghouan	Sahel
α-Tocopherol	326.7 ± 0.2e	240.1 ± 0.5h	246.9 ± 0.3g	279.8 ± 0.6f	480.1 ± 1.5a	449.7 ± 1.5b	405.4 ± 1.6c	397.4 ± 1.2d
Total phenols	133.6 ± 0.3g	403.2 ± 1.1b	95.50 ± 0.5h	292.6 ± 1.4d	148.1 ± 1.1e	398.3 ± 0.8c	446.1 ± 0.9a	135.9 ± 0.00f
3,4-DHPEA	0.1 ± 0.00g	20.50 ± 0.04a	nd h	4.90 ± 0.02c	1.3 ± 0.01e	6.4 ± 0.04b	4.2 ± 0.03d	0.70 ± 0.00f
p-HPEA	11.40 ± 0.00c	43.50 ± 0.08a	10.40 ± 0.00d	3.10 ± 0.00h	7.70 ± 0.00f	8.90 ± 0.02e	4.70 ± 0.02g	12.70 ± 0.06b
3,4-DHPEA-EDA	44.20 ± 0.35e	128.10 ± 0.35d	16.1 ± 0.25h	135.80 ± 1.10c	37.4 ± 0.73f	163.70 ± 0.48b	178.30 ± 0.03a	29.60 ± 0.46g
p-HPEA-EDA	9.30 ± 0.36g	41.20 ± 0.04c	18.20 ± 0.07e	13.50 ± 0.34f	28.70 ± 0.18d	51.20 ± 0.32b	62.20 ± 0.38a	41.30 ± 0.33c
(+)-1-Acetoxypinoresinol	25.30 ± 0.15b	20.30 ± 0.13d	26.10 ± 0.05a	24.40 ± 0.18c	14.20 ± 0.15e	7.20 ± 0.01h	9.50 ± 0.03g	11.60 ± 0.11f
(+)-1-Pinoresinol	9.50 ± 0.13e	13.30 ± 0.15c	10.00 ± 0.08d	7.30 ± 0.05g	7.40 ± 0.05g	28.60 ± 0.00a	22.30 ± 0.15b	8.50 ± 0.12f
3,4-DHPEA-EA	33.80 ± 0.18f	136.20 ± 0.60b	14.70 ± 0.26h	103.70 ± 0.98d	51.50 ± 0.45e	132.30 ± 0.18c	164.90 ± 1.93a	31.50 ± 0.06g

nd not detectable.
Values are the means of the three different VOO samples ($n = 3$) ± standard deviations. Different letters indicate significant differences ($P < 0.05$) between cultivars.

Table 3. Volatile compounds (mg kg^{-1}) evaluated by HS-SPME-GC-MS in virgin olive oils from introduced and autochthonous varieties cultivated in different areas

	Introduced cultivars and growing areas			Autochtonous cultivars and growing areas				
	Arbosana	Koroneiki	Arbequina	Oueslati		Chétoui		Chemlali
Volatile compounds	Tunis	Tunis	Tunis	Tunis	Kairouan	Béja	Zaghouan	Sahel
6-Methyl-5-hepten-2-one	0.01 ± 0.00a	0.05 ± 0.00a	0.01 ± 0.00a	0.03 ± 0.00c	0.02 ± 0.00a	0.05 ± 0.00a	0.03 ± 0.00a	0.02 ± 0.00a
(*E*)-2-Heptenal	0.29 ± 0.02e	nd f	0.39 ± 0.01d	1.35 ± 0.06a	0.56 ± 0.01c	0.30 ± 0.00e	0.70 ± 0.01b	0.72 ± 0.05b
(*E*,*E*)-2,4-Hexadienal	0.35 ± 0.03b	0.17 ± 0.03e	0.23 ± 0.01c	0.51 ± 0.01a	0.23 ± 0.04c	0.18 ± 0.00e	0.34 ± 0.01b	0.34 ± 0.03b
(*E*,*E*)-2,4-Hexadienal	0.21 ± 0.02b	0.14 ± 0.02c	0.16 ± 0.02c	0.27 ± 0.00a	0.17 ± 0.03c	0.14 ± 0.00c	0.20 ± 0.00b	0.23 ± 0.01b
2-Methyl-1-propanol	0.02 ± 0.01de	0.03 ± 0.00cd	0.04 ± 0.00bcd	nd e	0.05 ± 0.02bc	0.08 ± 0.03a	0.03 ± 0.00cd	0.06 ± 0.02ab
1-Butanol	0.02 ± 0.01b	0.02 ± 0.01b	0.02 ± 0.00b	0.04 ± 0.02a	0.02 ± 0.00b	0.02 ± 0.01b	0.02 ± 0.00b	0.04 ± 0.01a
2-Methyl-1-butanol/3-Methyl-1-butanol	0.02 ± 0.00e	0.02 ± 0.00a	0.02 ± 0.00a	0.01 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.00a
Benzyl alcohol	0.11 ± 0.00e	0.13 ± 0.00c	0.08 ± 0.00g	0.08 ± 0.00g	0.16 ± 0.00a	0.10 ± 0.00f	0.12 ± 0.00d	0.14 ± 0.01b
Phenylethyl alcohol	0.34 ± 0.01a	0.28 ± 0.00c	0.19 ± 0.00f	0.16 ± 0.01g	0.26 ± 0.00d	0.30 ± 0.01b	0.28 ± 0.02c	0.24 ± 0.01e
Phenol	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a	0.05 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.00a
3-Pentanone	0.21 ± 0.01c	0.40 ± 0.01a	0.08 ± 0.01f	0.09 ± 0.01c	0.23 ± 0.00b	0.24 ± 0.01b	0.17 ± 0.01d	0.11 ± 0.00e
1-Penten-3-one	0.05 ± 0.00f	nd g	0.07 ± 0.00e	0.33 ± 0.01c	0.29 ± 0.01d	0.01 ± 0.00g	0.47 ± 0.01a	0.39 ± 0.02b
(*E*)-2-Pentenal	0.20 ± 0.02e	0.08 ± 0.00f	0.18 ± 0.00e	0.26 ± 0.03d	0.34 ± 0.03c	0.08 ± 0.01f	0.52 ± 0.01a	0.40 ± 0.04b
1-Penten-3-ol	0.12 ± 0.01f	0.16 ± 0.00e	0.09 ± 0.00g	0.24 ± 0.00d	0.39 ± 0.01b	0.15 ± 0.01e	0.32 ± 0.00c	0.41 ± 0.01a
1-Pentanol	0.02 ± 0.00a	0.05 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a	0.03 ± 0.00a	0.06 ± 0.00a	0.03 ± 0.00a	0.02 ± 0.00a
∑C5 compounds	0.60 ± 0.04f	0.69 ± 0.01e	0.44 ± 0.01h	0.94 ± 0.05d	1.28 ± 0.01c	0.54 ± 0.01g	1.51 ± 0.01a	1.33 ± 0.05b
Ethyl acetate	0.24 ± 0.00a	nd e	0.12 ± 0.01d	nd e	0.18 ± 0.01b	0.12 ± 0.00d	0.14 ± 0.00c	n.d.e
Hexanal	0.79 ± 0.03cd	0.03 ± 0.00f	1.38 ± 0.03b	2.46 ± 0.01a	0.80 ± 0.03cd	0.61 ± 0.09e	0.71 ± 0.06de	0.92 ± 0.05c
1-Hexanol	0.57 ± 0.03d	1.44 ± 0.02a	0.57 ± 0.01d	0.19 ± 0.01g	0.60 ± 0.01c	1.01 ± 0.01b	0.39 ± 0.00e	0.35 ± 0.02f
Hexyl acetate	0.54 ± 0.01c	0.54 ± 0.01c	0.42 ± 0.00d	0.83 ± 0.01b	0.04 ± 0.00f	0.43 ± 0.02d	1.12 ± 0.01a	0.23 ± 0.00e
∑C6 compounds from LA	1.90 ± 0.01e	2.10 ± 0.16cd	2.37 ± 0.02b	3.48 ± 0.01a	1.44 ± 0.04f	2.05 ± 0.06d	2.22 ± 0.05c	1.50 ± 0.07g
(*E*)-2-Hexenal	13.53 ± 0.37b	4.00 ± 0.21e	15.08 ± 0.62a	5.74 ± 0.15d	14.68 ± 0.41a	2.61 ± 0.03f	6.80 ± 0.09c	13.54 ± 0.50b
(*E*)-2-Hexen-1-ol	1.91 ± 0.79ab	1.40 ± 0.76abc	0.95 ± 0.03bcd	0.25 ± 0.03d	1.75 ± 0.51ab	2.11 ± 0.86a	0.43 ± 0.00cd	0.70 ± 0.30cd
(*E*)-3-Hexen-1-ol	0.01 ± 0.00a	0.14 ± 0.00a	nd a	nd a	nd a	0.03 ± 0.00a	nd a	nd a
(*Z*)-3-Hexen-1-ol	0.43 ± 0.01c	2.30 ± 0.00a	0.28 ± 0.00f	0.33 ± 0.01e	0.15 ± 0.00g	1.07 ± 0.01b	0.41 ± 0.01d	0.14 ± 0.00g
(*Z*)-3-Hexenyl-acetate	2.61 ± 0.06b	1.37 ± 0.04e	0.77 ± 0.01f	1.67 ± 0.02e	0.06 ± 0.00h	1.55 ± 0.03d	3.33 ± 0.02a	0.34 ± 0.00g
∑C6 compounds from LnA	18.49 ± 1.23a	9.21 ± 0.93e	17.08 ± 0.64b	8.00 ± 0.17f	16.64 ± 0.10b	7.37 ± 0.91f	10.97 ± 0.12d	14.72 ± 0.20c
Total LOX products	20.93 ± 1.22a	11.31 ± 0.79d	19.45 ± 0.66a	11.47 ± 0.16d	18.08 ± 0.06b	9.42 ± 0.97e	13.19 ± 0.17c	16.22 ± 0.13b
% Branch LA/∑C6	9.34 ± 0.61e	18.68 ± 2.68c	12.19 ± 0.31d	30.34 ± 0.51a	7.95 ± 0.25e	21.87 ± 1.62b	16.82 ± 0.16c	9.25 ± 0.50e
% Branch LnA/∑C6	90.66 ± 0.61a	81.32 ± 2.68c	87.81 ± 0.31b	69.65 ± 0.51e	92.05 ± 0.25a	78.13 ± 1.62d	83.18 ± 0.16c	90.75 ± 0.50a
(*E*)-2-Hexen-1ol/(*E*)-2-Hexenal ratio	0.14 ± 0.05bc	0.34 ± 0.17b	0.06 ± 0.00c	0.04 ± 0.00c	0.12 ± 0.03bc	0.81 ± 0.32a	0.06 ± 0.00c	0.05 ± 0.02c
1-Hexanol/Hexanal ratio	0.72 ± 0.06c	48.56 ± 1.19a	0.41 ± 0.02c	0.08 ± 0.00c	0.75 ± 0.01c	1.68 ± 0.27b	0.55 ± 0.05c	0.38 ± 0.00c

nd not detectable.
Values are the means of the three different VOO samples ($n = 3$) ± standard deviations. Different letters indicate significant differences ($P < 0.05$) between cultivars.

conditions, particularly the high altitude in Kairouan and Béja (Fig. 1), which influences the quality of the olive oil as well as the levels and composition of phenolic compounds. Consequently, and from the results presented for autochthonous varieties, *Chétoui* cv. is more stable than *Oueslati* under varying geographic conditions. Comparison of introduced and autochthonous cvs proved the richness of the Tunisian varieties in phenolic content in VOO except for *Koroneiki* cv. (Table 2).

Table 4. List of variables used for the multivariate statistical analysis

Stability parameters	Ketones
1 Total phenols	13 3-Pentanone
2 α-Tocopherol	14 1-Penten-3-one
3 3,4-DHPEA	15 6-Methyl-5-hepten-2-one
4 *p*-HPEA	
5 3,4-DHPEA-EDA	
6 *p*-HPEA-EDA	
7 (+)-1-Acetoxypinoresinol	
8 (+)-1-Pinoresinol	
9 3,4-DHPEA-EA	
Esters	**Alcohols**
10 Ethyl acetate	22 2-Methyl-1-propanol
11 Hexyl acetate	23 1-Butanol
12 (*Z*)-3-Hexenyl-acetate	24 1-Penten-3-ol
	25 2-Methyl-1-butanol/3-Methyl-1-butanol
	26 1-Pentanol
	27 1-Hexanol
	28 Benzyl alcohol
	29 (*E*)-3-Hexen-1-ol
	30 (*Z*)-3-Hexen-1-ol
	31 (*E*)-2-Hexen-1-ol
	32 Phenylethyl alcohol
	33 Phenol
Aldehydes	
16 Hexanal	
17 (*E*)-2-Pentenal	
18 (*E*)-2-Hexenal	
19 (*E*)-2-Heptenal	
20 (*E*,*E*)-2,4-Hexadienal	
21 (*E*,*E*)-2,4-Hexadienal	

Volatile compounds

The fragrance and unique flavour of EVOO represent some of the most important qualitative aspects of this vegetable oil, and play a major role in consumer approval. Although a full description of the organoleptic characteristics of the oil is only obtainable through sensory analysis, qualitative and quantitative determination of the volatile compounds can provide very useful information on product quality.[36] In order to evaluate matrix volatile composition, HS-SPME was used. Twenty-four volatile compounds (Table 3) were isolated and quantified by GC-MS analysis. Figure 2 shows the influence of cultivar on the percentage of the four groups (esters, ketones, aldehydes and alcohols) of volatiles obtained from oils of different cultivars harvested at the same degree of ripeness and processed under the same operating conditions. These compounds represent around 90% of the total aroma matrix.

From a quantitative point of view, we can see in Fig. 2 that aldehydes and alcohols are the main chemical families present in the analysed headspaces oils. These results can be explained by differential activity of the enzyme alcohol dehydrogenase (ADH), which catalyses the reversible reduction of aliphatic aldehydes to alcohols. ADH is widespread in the plant kingdom and is responsible for the formation of volatile alcohols that contribute to the aroma of vegetable products.[37] In addition, results showed that aldehydes are the most abundant volatiles in oils studied, except for *Koroneiki* and *Chétoui* Béja cvs having the highest concentration of alcohols (46% and 49% of the total aroma, respectively). The increase of alcohols is the main effect of the malaxation time and is considered by some authors as eliciting odour not completely agreeable,[6] and is a typical defect.[38] Total aroma compounds ranged between a minimum of 12.7 mg kg^{-1} for oils produced from *Chétoui* Béja to a maximum of 22.7 mg kg^{-1} for oil produced from *Arbosana* cv. Differences between cultivars are much stronger; hence while highest levels of esters were observed in oils from *Chétoui* Zaghouan (28% of the total aroma), *Chemlali* Sahel olive oil had the highest level of aldehydes (83% of the total aroma).

Concentrations of C6 and C5 volatile compounds from the lipoxygenase (LOX) cascade, which is the most important pathway for the formation of the olive aroma, are reported in Table 3. These compounds, responsible for the positive green sensory notes in VOO, are enzymatically produced from polyunsaturated fatty acids through the LOX pathway,[6] which occurs during crushing of olive fruit and olive paste malaxation and are incorporated into the oily phase.[39] Fig. 2 shows that the amount of these different metabolites changes in relation to the cultivar. In all the VOO samples analysed, especially C6 linear unsaturated and saturated aldehydes represent the most important fraction of the volatile compounds, where content was highly variable between the varieties studied. This result is in agreement with those of Angerosa *et al.*[6] In particular, (*E*)-2-hexenal, the most prominent compound evaluated by SPME and which is responsible for bitter, green, green apple-like, fatty, bitter almond-like and cut grass sensory notes,[40] presents levels ranging from 15 to 2.6 mg kg^{-1}. These levels showed the dominance of this component due to its low odour threshold, which contributed to the olive oil flavour when VOO is considered of high quality.[6] *Arbequina* cv. has the highest amount of (*E*)-2 hexenal (15 mg kg^{-1}) but *Koroneiki* and *Chétoui* Béja cvs contained very low amounts of this compound (4 and 2.6 mg kg^{-1}, respectively). Consequently, aldehydes are more predominant in *Arbequina, Oueslati* Kairouan and *Chemlali* Sahel cv. (>79% of the total aroma). Analysis of aroma matrix show similarities between *Koroneiki* and *Chétoui* Béja cvs and the *Arbosana, Arbequina, Oueslati* Kairouan and *Chemlali* Sahel cvs. These results agree with previous findings[41] suggesting that monovarietal VOO could be distinguished by (*E*)-2-hexenal. Moreover, the amount of hexanal with apple and green fruity attributes[42] was lower and varied between 2.5 mg kg^{-1} for *Oueslati* Tunis VOO and 0.03 mg kg^{-1} for *Koroneiki* VOO samples. We observed variability for the same cultivar planted in different areas (Table 3). These differences are more pronounced for *Oueslati* oils when produced in Tunis compared to their area of origin (Kairouan): in fact, the (*E*)-2-hexenal level is threefold higher in oils from their area of origin than in oils cultivated in Tunis. However, this last oil contained threefold of the hexanal level in oils from area of origin (Table 3). Furthermore, differences of the

(*E*)-2-hexen-1-ol/(*E*)-2-hexenal and 1-hexanol/hexanal ratios were observed in *Oueslati* and *Chétoui* oils according to cultivation site. These differences may be due to the great variability of the climatic and altitude conditions of the areas (Fig. 1); other climatic variables such as soil characteristics of olive grove zones and soil salinity influence the chemical composition and sensory profiles of VOO.[7] Nevertheless, the (*E*)-2-hexen-1-ol/(*E*)-2-hexenal and 1-hexanol/hexanal ratios were similar in oils on one hand from *Arbequina* and *Chemlali* Sahel cvs and from *Arbosana* and *Oueslati* (Kairouan) cvs on the other (Table 3). Accumulation in the oil of the amount of C6 compounds coming only from α-linoleic acid is practically different according to the cultivars regardless of the climatic variables and the area where olives are grown. Statistically, significant differences were likewise found in C6 alcohol content (related to fruity, green, grassy and sweet sensory notes) between the different cultivars studied. (*E*)-3-hexen-1-ol was the minor C6 alcohol found in all eight cultivars and was present as a trace of the total volatiles in the *Arbosana, Koroneiki* and *Chétoui* Béja VOO, but it was not detected in the other samples. 1-Hexanol, (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol values were lower in the cultivars studied and each C6 alcohol ranged from 0.2 to 1.4, 0.3 to 2 and 0.1 to 2.2 mg kg^{-1}, respectively.

In addition, the *Koroneiki* and *Chétoui* Béja VOO samples had higher (*Z*)-3-hexen-1-ol concentrations (1–2 mg kg^{-1}), whereas the highest (*E*)-2-hexen-1-ol values were found in the *Chétoui* Béja and *Arbosana* varieties (2 mg kg^{-1}). Alcohols produced by the action of ADH can form volatile esters. C6 esters such as hexylacetate (related to sweet, floral and fruity notes) and (*Z*)-3-hexenylacetate (green banana, fruity green, green leaves and floral notes) were also present in all VOO samples except for *Oueslati* Kairouan oils. This indicated the presence of an alcohol acyl transferase (AAT) activity in the majority of the cultivars catalysing the production of these acetate esters through acetyl-CoA derivatives.[2,39] The maximum activity for AAT in olives is found with hexanol and *cis*-3-hexenol; *trans*-2-hexenol is a poorer substrate.[43] It appears that the amount of volatile compounds is influenced by enzyme activity, as previously reported in the literature.[7] Concerning autochthonous varieties, (*Z*)-3-hexenylacetate was more important in *Chétoui* cv. than *Chemlali* cv., this indicated then a higher activity of AAT in *Chétoui* cv., which confirms the findings of Dhifi *et al*.[44]

Moreover, the high level of (*E*)-hex-2-enal in olive 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 studied varieties (Table 3). This result shows that the amounts of compounds arising from linolenic acid (LnA) are always greater (Table 3) than those of compounds from linoleic acid (LA); this is in agreement with previous work.[45] An additional branch of the LOX pathway is active when the substrate is LnA, leading to the production of pentene isomers (C5 volatile compounds), which are also present in the VOO aroma.[6] For these compounds we observed trace levels of 1-penten-3-one and 1-penten-3-ol in all VOO cultivars. Consequently, the genetic effect related to cultivar is one of the most important factors of the volatile composition of olive oil. However, the altitude of olive growing can affect volatile composition of olive oils obtained by the same cultivars.[6] Therefore, Tunisian cultivars showed a variation in the same variety planted in different locations, which is more obvious for *Oueslati* cv. (15 and 21 mg kg^{-1} for Tunis and Kairouan, respectively).

Chemometric analysis

PCA was used in exploratory analysis to analyse the influence of geographic area on the components of the different cultivars studied. PCA was applied to the dataset of phenolic and volatile compounds of VOO from the different cultivars studied and two factors were selected justifying 57% of total variance (F1: 38%; F2: 19%). Regarding these factors, cultivars could be discriminated on the PCA plane. Figure 3 shows three distinctive groups. The first group is composed of four cultivars (*Arbosana, Arbequina, Oueslati* Kairouan and *Chemlali* Sahel cvs). The second group was characterized by *Koroneiki* and *Chétoui* Béja cvs. The last group is composed of two autochthonous samples (*Chétoui* Zaghouan and *Oueslati* Tunis). F1 was dominated by the following variables: α-tocopherol; 3,4-DHPEA; 3,4-DHPEA-EDA; (+)-1-pinoresinol; 3-pentanone; 6-methyl-5-hepten-2-one; hexanal; 2-methyl-1-butanol/3-methyl-1butanol; 1-pentanol; 1-hexanol; benzyl alcohol; (*Z*)-3-hexen-1-ol; (*E*)-3-hexen-1-ol; (*E*)-2-hexen-1-ol; and phenylethyl alcohol (Fig. 4 and Table 4). F2 was dominated by the following variables; (+)-1-acetoxypinoresinol; hexyl acetate; (*Z*)-3-hexenyl-acetate; 1-penten-3-one; (*E*)-2-heptenal; (*E*,*E*)-2,4-hexadienal; and phenol. The results obtained were confirmed by performing an HCA on these principal components. In fact, the dendrogram in Fig. 5 shows three distinct blocks, with a high similarity between *Oueslati* Kairouan and *Chemlali* Sahel, between *Arbosana* and *Arbequina* and between *Koroneiki* and *Chétoui* Béja, whereas *Oueslati* Tunis and *Chétoui* Zaghouan presented an extreme difference in their composition in comparison to the other cultivars. In this case, the formation of clusters and its linkage could be compared and related to the groups formed in PCA. Comparison between score plot and loading plot indicated that the variables 3,4-DHPEA-EDA, 3,4-DHPEA-EA, total phenols, 3,4-DHPEA, 6-methyl-5-hepten-2-one, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, *p*-HPEA, *p*-HPEA-EDA, (+)-1-pinoresinol, 1-hexanol and 1-pentanol were mainly responsible for discrimination of *Chétoui* Béja and *Koroneiki*. Varieties composing the second cluster are mainly discriminated by (*E*)-2-hexenal, phenol, (*E*)-2-pentenal and 1-penten-3-ol while the last group, which is composed of *Chétoui* Zaghouan and *Oueslati* Tunis, was mainly differentiated by the variables 1-penten-3-one, (*E*)-2-heptenal, hexanal, 1-butanol, (*E*,*E*)-2,4-hexadienal and (+)-1-acetoxypinoresinol.

CONCLUSION

The results of the present study may be of particular interest in the official control of VOO and are expected to attract the attention of the appropriate officials. The antioxidant profile was primarily influenced by cultivar and secondarily by environment (area of origin). European cultivars grown in Tunisia produced oils with some differences from those obtained in their traditional growing areas. In comparing the studied varieties, *Arbosana, Arbequina* and *Oueslati* (Kairouan) cvs had a good aromatic profile and an important volatile compound content. However, a noticeable difference in the phenolic fraction was observed between cultivars: *Chétoui* cv. exhibits a good phenolic profile whereas *Arbequina* and *Chemlali* cvs show a similar profile for phenolic fraction and volatile compounds. These differences may be explained by genetic factors and geographic area, particularly altitude. Since geographic area has a great effect, additional work will be addressed to assess how these European introduced cultivars can be grown in the south of Tunisia.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, UR03ES08, Nutrition Humaine et Désordres Métaboliques and DGRST-USCR–Spectrométrie de masse. Part of this work was carried out at the Dipartimento di Scienze degli Alimenti, Sezione di Tecnologie e Biotecnologie degli Alimenti, Università degli Studi di Perugia, Italia. The authors are grateful to the staff of experimental farms of the National Office of Oil (Tunis) for their help and advice. We are also grateful to the anonymous reviewers for their valuable comments and constructive criticisms of the manuscript.

REFERENCES

1 Vinha AF, Ferrerei F, Silva BM, Valentào P, Gonçalves A, Pereira JA, *et al*, Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): influences of cultivar and geographical origin. *Food Chem* **89**:561–568 (2005).
2 Kalua CM, Allen MS, Bedgood Jr DR, Bishop AG, Prenzler PD and Robards K, Olive oil volatile compounds, flavour development and quality: a critical review. *Food Chem* **100**:273–286 (2007).
3 Aparicio R, Morales MT and Alonso MV, Authentication of European virgin olive oils by their chemical compounds, sensory attributes, and consumers' attitudes. *J Agric Food Chem* **46**:1076–1083 (1997).
4 Kiritsakis AK, Flavour components of olive oil. *J Am Oil Chem Soc* **75**:673–681 (1998).
5 Kiritsakis AK and Christie WW, Analysis of edible oils, in *Handbook of Olive Oil Analysis and Properties*, ed. by Harwood J and Aparicio R. Aspen, Gaithersburg, MA, pp. 129–158 (2000).
6 Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S and Montedoro GF, Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *J Chromatogr A* **1054**:17–31 (2004).
7 Angerosa F, Basti C and Vito R, Virgin olive oil compounds from lipoxygenase pathway and characterization of some Italian cultivars. *J Agric Food Chem* **47**:836–839 (1999).
8 Campeol E, Flamini G, Chericoni S, Catalano S and Cremonini R, Volatile compounds from three cultivars of *Olea europea* from Italy. *J Agric Food Chem* **49**:5409–5411 (2001).
9 Aparicio R and Morales MT, Characterization of olive ripeness by green aroma compounds of virgin olive oil. *J Agric Food Chem* **46**:1116–1122 (1998).
10 Ranalli A, Tombesi A, Ferrante ML and De Mattia G, Respiratory rate of olive drupes, during their ripening cycle and quality of oil extracted. *J Sci Food Agric* **77**:359–367 (1998).
11 Solinas M, Marsilio V and Angerosa F, Behaviour of some components on virgin olive oil flavour in connection with the ripening of olives. *Riv Ital Sost Grasse* **64**:475–480 (1987).
12 Angerosa F, Mostallino R, Basti C and Vito R, Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem* **72**:19–28 (2001).
13 Ranalli A, Costantini N, De Mattia G and Ferrante ML, Evaluating two kinds of centrifuged virgin oils arising from continuous olive processing. *J Sci Food Agric* **80**:673–683 (2000).
14 Ranalli A and Angerosa F, Integral centrifuges for olive oil extraction: the qualitative characteristics of product. *J Am Oil Chem Soc* **73**:417–422 (1996).
15 Salas JJ and Sanchez J, The decrease of virgin olive oil flavour produced by high malaxation temperature is due to inactivation of hydroperoxide lyase. *J Agric Food Chem* **47**:809–812 (1999).
16 Ranalli A, Tombesi A, De Mattia G, Ferrante ML and Giansante L, Incidence of olive cultivation area on the analytical characteristics of the oil. *Riv Ital Sost Grasse* **74**:501–508 (1997).
17 Matos LC, Pereira JA, Andrade PB, Seabra RM and Oliveira BPP, Evaluation of a numerical method to predict the polyphenols content in monovarietal olive oils. *Food Chem* **102**:976–983 (2007).
18 Gallina-Toschi T, Cerretani L, Bendini A, Bonoli-Carbognin M and Lercker G, Oxidative stability and phenolic content of virgin olive oil: an analytical approach by traditional and high resolution techniques. *J Sep Sci* **28**:859–870 (2005).
19 Beltran G, Aguilera MP, Del Rio C, Sanchez S and Martinez L, Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chem* **89**:207–215 (2005).
20 Dhifi W, Hamrouni I, Ayachi S, Chahed T, Saidani M and Marzouk B, Biochemical characterization of some Tunisian olive oils. *J Food Lipids* **11**:287–296 (2004).
21 Issaoui M, Dabbou S, Echbili A, Rjiba I, Gazzah N, Trigui A, *et al*, Biochemical characterisation of some Tunisian virgin olive oils obtained from different cultivars growing in Sfax National Collection. *J Food Agric Environ* **5**:17–21 (2007).
22 Montedoro GF, Servili M, Baldioli M, Selvaggini R, Maniati E and Macchioni A, Simple and hydrolyzable compounds in virgin olive oil. 3: spectroscopic characterization of the secoiridoids derivatives. *J Agric Food Chem* **41**:2228–2234 (1993).
23 European Union Commission Regulation 2568/91, Characteristics of olive and olive pomace oils and their analytical methods. *Off J Eur Commun* **L248**:1–82 (1991).
24 Montedoro GF, Servili M, Baldioli M and Miniati E, Simple and hydrolysable compounds in virgin olive oil. 1. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. *J Agric Food Chem* **40**:1571–1576 (1992).
25 Selvaggini R, Servili M, Urbani S, Esposto S, Taticchi A and Montedoro GF, Evaluation of phenolic compounds in virgin olive oil by direct injection in HPLC with fluorometric detection. *J Agric Food Chem* **54**:2832–2838 (2006).
26 Psomiadou E and Tsimidou M, Simultaneous HPLC determination of tocopherols, carotenoids and chlorophylls for monitoring their effect on virgin olive oil oxidation. *J Agric Food Chem* **46**:5132–5138 (1998).
27 Servili M, Selvaggini R, Taticchi A and Montedoro GF, Headspace composition of virgin olive oil evaluated by solid phase microextraction: relationship with the oil sensory characteristics, In *Food flavours and chemistry: advances of the new millenium*, ed. by Spainer AM, Shaidi F, Parliment TH, Mussinan C, Ho CT and Tratratas Contis E. The Royal Society of Chemistry Publisher, UK, pp. 236–247 (2001).
28 European Union Commission Regulation 1989/2003, Characteristics of olive and olive pomace oils and their analytical methods. *Off J Eur Commun* **L295**:57–77 (2003).
29 Ben Temine S, Campeol E, Cioni PL, Daoud D and Zarrouk M, Volatile compounds from Chétoui olive oil and variations induced by growing area. *Food Chem* **99**:315–325 (2006).
30 Boskou D, Blekas G and Tsimidou M, Olive oil composition, in *Olive Oil, Chemistry and Technology*, ed. by Boskou D. AOCS Press, Champaign IL, pp. 41–72 (2006).
31 Psomiadou E, Tsimidou M and Boskou D, α-Tocopherol content of Greek virgin olive oils. *J Agric Food Chem* **48**:1770–1775 (2000).
32 Paz Romero M, Jesús Tovar M, Ramo T and José Motilva M, Effect of crop season on the composition of virgin olive oil with protected designation of origin 'Les Garrigues'. *J Am Oil Chem Soc* **80**:423–430 (2003).
33 Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro GF and Morozzi G, Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *J Chromatogr A* **1054**:113–127 (2004).
34 Brenes M, Garcia A, Garcia P and Garrido A, Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J Agric Food Chem* **49**:5609–5614 (2001).
35 Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Gallina-Toschi T and Fernandez-Gutiérrez A, Analytical determination of polyphenols in olive oils. *J Sep Sci* **28**:837–858 (2005).
36 Contini M and Esti M, Effect of the matrix volatile composition in the HS-SPME analysis of extra virgin olive oil: analytical nutritional and clinical methods. *Food Chem* **94**:143–150 (2006).
37 Sanchez J and Salas JJ, Biogenesis of olive oil aroma, in *Handbook of Olive Oil: Analysis and Properties*, ed. by Harwood J and Aparicio R. Aspen, Gaithersburg, MD, pp. 79–100 (2000).
38 Angerosa F and Di Giacinto L, Caratteristiche di qualità dell'olio di oliva vergine in relazione ai metodi di frangitura. Nota II. *Riv Ital Sost Grasse* **72**:443–450 (1995).
39 Sanchez J and Harwood JL, Biosynthesis of triacylglycerols and volatiles in olives. *Eur J Lipid Sci Technol* **104**:564–573 (2002).
40 Morales MT, Rios JJ and Aparicio R, Changes in the volatile composition of virgin olive oil during oxidation: flavors and off-flavors. *J Agric Food Chem* **45**:2666–2673 (1997).

41 Aparicio R and Luna G, Characterisation of monovarietal virgin olive oils. *Eur J Lipid Sci Technol* **104**:614–627 (2002).
42 Aparicio R, Morales MT and Alonso MV, Relationship between volatile compounds and sensory attributes of olive oils by sensory wheel. *J Am Oil Chem Soc* **73**:1253–1264 (1996).
43 Salas JJ, Characterization of alcohol acyltransferase from olive fruit. *J Agric Food Chem* **52**:3155–3158 (2004).
44 Dhifi W, Angerosa F, Serraiocco A, Oumar I, Hamrouni I and Marzouk B, Virgin olive oil aroma: characterization of some Tunisian cultivars. *Food Chem* **93**:697–701 (2005).
45 Cavalli JF, Fernandez X, Lizzani-Cuvelier L and Loiseau M, Characterization of volatile compounds of French and Spanish virgin olive oils by HS–SPME of quality freshness markers. *Food Chem* **88**:151–157 (2004).

