



Effect of the growing area conditions on differentiation between Chemlali and Chétoui olive oils

Manel Issaoui^a, Guido Flamini^{b,*}, Faten Brahmi^a, Samia Dabbou^a, Kaouther Ben Hassine^a, Amani Taamali^d, Hechmi Chehab^c, Myriem Ellouz^a, Mokhtar Zarrouk^d, Mohamed Hammami^{a,*}

^aLaboratory of Biochemistry, UR: "Human Nutrition and Metabolic Disorder" Faculty of Medicine, Monastir 5019, Tunisia

^bDipartimento di Chimica Bioorganica e Biofarmacia, via Bonanno 33, 56126 Pisa, Italy

^cOlive Tree Institute of Sousse, Tunisia

^dLaboratoire Caractérisation et Qualité de l'huile d'olive, Centre de Biotechnologie de Borj Cedria, Hammam-lif, Tunisia

ARTICLE INFO

Article history:

Received 21 February 2009

Received in revised form 6 May 2009

Accepted 9 June 2009

Keywords:

Tunisian olive oil varieties

Fatty acids

Oxidative stability

Volatile compounds

Sensory characteristics

Growing areas

ABSTRACT

This paper reports a discrimination study based on the antioxidant compounds, oxidative stability, aroma and sensory profiles of virgin olive oils from the main Tunisian cultivars, Chemlali and Chétoui, grown in two different locations, north and south Tunisia, with important differences in altitude, latitude and climatic conditions. There were significant differences between the oils from both cultivars when grown in the different environments. At higher altitude, the oils showed a greater amount of oleic acid, phenols and a higher stability, whilst in the open the oils had higher saturated and linoleic acid content. Aroma profiles were also influenced by the pedoclimatic conditions; hence, oils from the South had the highest level of (*E*)-2-hexenal and 1-hexanol, whereas varieties from the North were higher in (*E*)-3-hexenyl acetate and hexyl acetate. In general, and independently of the growing area conditions, oils from Chétoui olives had higher levels of antioxidants, greater oxidative stability, higher antiradical activities and more marked intensity of bitterness. These results can be used to discriminate and to characterise the Chemlali and Chétoui olive oils from each region.

© 2009 Published by Elsevier Ltd.

1. Introduction

Olive oil plays an important role in the Tunisian agronomy and economy. Olive trees cover an area of 1,611,200 ha and account for more than 4% of the olive oil produced in the world. Indeed, Tunisia is the fourth largest exporter of olive oil in the world (Dabbou et al., 2009). Due to the diversity of Tunisian olive oil cultivars, the olive-growing areas spread from north to south, where a wide range of pedoclimatic conditions prevail, from mild semi-arid to arid conditions (Issaoui et al., 2009). More than 50 different cultivars are found throughout Tunisia; however, two important varieties dominate most of the arable land: Chétoui and Chemlali (Dabbou et al., 2009). The Chemlali cultivar, which contributes 80% of national olive oil production, is characterised by relatively low levels of oleic acid (53–60%), high levels of palmitic and a low oleic/linoleic acid ratio. In contrast, the Chétoui variety can be found mostly in the north of the country covering an area of 176,000 ha (Ben Temime et al.,

2006). Its fruit is medium to large in size, with a characteristically elongated and asymmetric shape. The fat yield is 20–30% of fresh weight and the oil is valued for its high amounts of total phenols and tocopherols (Ben Temime et al., 2006). It has long been known that the chemical composition of virgin olive oil (VOO) is influenced by genetic (cultivar) and environmental factors (edaphic characteristics and climatological conditions), so that the olive production area is greatly responsible for the specific characteristics of olive oil. In the last few years, there has been increasing interest in the geographical identification of virgin olive oil, as a reliable criterion for its authentication and quality.

Many studies have been carried out to categorise virgin olive oils from different geographical origins according to their different chemical properties. However, there is no study on the effect of altitude and geographic location on antioxidant levels and sensory profiles, particularly for the Tunisian cultivars. The present research aimed (i) to study antioxidant compounds, aroma profiles and sensory properties, and (ii) to attest the oxidative stability of virgin olive oils from the two main Tunisian cultivars (Chemlali and Chétoui) planted in north and south Tunisia, in order to evaluate their adaptation to different geographic growing area conditions.

* Corresponding authors. Tel.: +216 73 462 200; fax: +216 73 460 737 (M. Hammami), tel.: +39 05044074; fax: +39 05043321 (G. Flamini).

E-mail addresses: flamini@farm.unipi.it (G. Flamini), mohamed.hammami@fmm.rnu.tn (M. Hammami).

2. Material and methods

2.1. Samples

The study was carried out on monovarietal virgin olive oils from the two main Tunisian cultivars, namely Chétoui and Chemlali. Olive samples were hand picked, in triplicate, from good quality fresh and healthy fruits. In order to eliminate the influence of maturation state on olive oil quality, the ripening degree was the same for all studied olive cultivars (maturation indices were 5). Both varieties were planted in the north and south (Sfax: 13 m; 34°44'N, 10°36'E) of Tunisia. In the north, Chemlali and Chétoui were cultivated at an altitude of about 222 m in Beja (36° 44'N, 09°11'E). The mean precipitation registered in the north was about 650 mm/year, with a mean temperature of approximately 18 °C. In the south the mean precipitation and temperature registered were 190 mm/year and 31 °C.

2.2. Oil isolation

Oil isolation was carried out using an Abencor analyser (Abencor, Cordoba, Spain). The fresh olives (1.5–2.0 kg) were crushed with a hammer mill and were then slowly mixed for 30 min at 25 °C. The resulting paste was centrifuged at 3500 rpm for 3 min. The oil was separated by decanting. All samples were subsequently filtered over anhydrous Na₂SO₄, placed in amber glass bottles and stored in the dark at 4 °C.

2.3. Analytical indices

Free acidity, peroxide value and UV absorbances at 232 and 270 nm (K232 and K270) were determined according to the European Official Methods of Analysis (EEC 2568/91).

2.4. Fatty acid composition

Fatty acid methyl esters (FAMES) from the oil samples were prepared as described by Issaoui et al. (2008). Individual FAMES were separated and quantified by gas chromatography using a Model 5890 Series II instrument (Hewlett–Packard, Palo Alto, CA) equipped with a flame ionisation detector, and a fused silica capillary column (HP-Innowax; 30 m × 0.25 mm × 0.25 μm).

2.5. Carotenoids and chlorophylls

Carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane, using specific extinction values, according to the method of Minguez-Mosquera, Rejano, Gandul, Sanchez, and Garrido (1991).

2.6. Phenols and *o*-diphenols

The total phenols were determined colorimetrically at 765 nm using the Folin–Ciocalteu reagent and were expressed as 3,4-dihydroxyphenylethanol (3,4-DHPEA) equivalents in a methanolic extract of virgin olive oil obtained as follows. Ten millilitres of a solution of methanol/water (80:20 v/v) plus Tween 20 (2% v/w) were added to 10 g of olive oil and mixed with an Ultra-Turrax T25 (IKA Werke GmbH & Co., Staufen, Germany) at 15,000g for 1 min and centrifuged at 5000g for 10 min; the extraction was repeated twice. To eliminate the oil droplets, the methanolic extract was kept for 24 h at –20 °C (Montedoro, Servili, Baldioli, & Miniati, 1992). The *o*-diphenols were determined colorimetrically at 500 nm, using 1 ml of a solution of HCl (0.5 N), 1 ml of a solution of a mixture of NaNO₂ (10 g) and NaM₆O 4.2H₂O (10 g) in 100 ml

H₂O and finally 1 ml of a solution of NaOH (1 N) with 100 μl of the methanolic extract. The mixture was allowed to react for 30 min, after which the extract was filtered (on 0.45 μm cellulose acetate). The *o*-diphenols were also expressed as 3,4-DHPEA equivalents (Dabbou et al., 2009; Montedoro et al., 1992).

2.7. Volatile compound analyses

Supelco (Bellefonte, PA) SPME devices coated with polydimethylsiloxane (PDMS, 100 μm) were used to sample the headspace of 2 ml of olive oil inserted into a 5-ml 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–MS system. GC–EIMS analyses were performed with a Varian (Palo Alto, CA) CP 3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures were 250 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; carrier gas was helium at 1 ml/min; splitless injector. The identification of the constituents was based on a comparison of the retention times with those of authentic samples, comparing their linear retention indices (LRI) relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and Adams) and homemade library mass spectra, built from pure substances, components of known oils, and MS literature data (Adams, 1995; Davies, 1990; Jennings & Shibanoto, 1980; Massada, 1976; Stenhagen, Abrahamsson, & Mc Lafferty, 1974; Swigar & Silvestein, 1981). Moreover, the molecular weights of all the substances identified were confirmed by GC–CIMS, using methanol as the ionising gas.

2.8. Sensory evaluation

The sensory analyses were performed by a fully trained analytical taste panel, members of staff of “Office National Huile (ONH)”, composed of twelve assessors trained to perform VOO sensory analysis, recognised by the International Olive Oil Council (IOOC of Madrid, Spain). Sensory profiles were determined using a standard profile sheet, according to the IOOC method T20/Doc. N.15/Rev.1. The sensory profiles of VOO were reported as spider plots to compare the samples' differences, examining the effect of the growing area conditions on Chemlali and Chétoui olive oils.

2.9. Antiradical activities

Trolox-equivalent antioxidant capacity assay (TEAC) is based on the ability of antioxidant molecules (phenolic compounds) to quench ABTS^{•+}, compared with that of Trolox, a water-soluble vitamin E analogue (Re et al., 1999). The radical was generated using potassium persulfate. The solution was diluted with ethanol until absorbance reached 0.70 at 734 nm.

The radical-scavenging activity (RSA) was measured following the methodology described by Brand-Williams, Cuvelier, and Brest (1995). The bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), was monitored in the presence of the sample. A volume of 3.9 ml of 6 × 10^{–5} M DPPH[•] methanol solution was used. The reaction was started by the addition of 100 μl of phenolic extract. For each phenolic extract, a dilution series with methanol was prepared, to determine the antiradical activity. Absorption at 515 nm was monitored at 25 °C for 120 min.

2.10. Rancimat assay

Oxidation stability was evaluated by the Rancimat apparatus (Mod. 743, Metrohm, Schweiz AG, Zofingen, Switzerland) using an oil sample of 3 g warmed to 120 °C and an air flow of 20 l/h. Stability was expressed as induction time (hours).

2.11. Statistical analysis

All parameters were determined in triplicate for each sample. Variance and linear regression analyses were processed by SPSS statistical package (Version 12.00 for Windows, SPSS Inc., Chicago, IL, 2003). The significance of differences at a 5% level amongst means was determined by one-way analysis of variance (ANOVA), using Tukey's test. ANOVA was applied in order to evaluate the influence of cultivar and pedoclimatic conditions. Correlation analysis was performed with Pearson's test.

3. Results and discussion

3.1. Merceological parameters

The extra-virgin olive oils (EVOO) greatly differed in their degradation levels, determined according to EEC 2568/91 (Table 1). The oils from cultivars Chemlali (1% acidity) and Chétoui (0.9% acidity) from the south of Tunisia exceeded the acidity limit of 0.8%, which represented an advanced level of degradation. On the contrary, oil produced from the varieties grown in the North exhibited a low percentage of acidity. The peroxide values were below the limit of 20 meq O₂/kg of oil for all oils, which is accepted as the limit for 'extra-virgin' olive oil quality. Except for the South Chétoui olive oil, the absorbance measurements at 232 and 270 nm did not (K232 and K270) exceed the respective values of 2.5 and 0.22, the limits established for extra-virgin olive oil (Table 1).

3.2. Fatty acid composition

The mean fatty acid methyl esters (FAMES) composition of the oils of both cultivars, from each location, is shown in Table 1. The palmitic acid mean value for the Chemlali cultivar was 18.4% in the

Table 1
Mean values of analytical parameters, and fatty acids composition (%) of virgin olive oils of Chemlali and Chétoui cultivars from two locations in Tunisia.

	Chemlali cv.		Chétoui cv.	
	Southern	Northern	Southern	Northern
Acidity (oleic acid, g/100 g)	1 ^a	0.1 ^c	0.9 ^a	0.3 ^b
PV (meq O ₂ /kg)	8.3 ^a	3.3 ^b	3.2 ^b	8.2 ^a
K232 nm	1.9 ^b	1.4 ^c	2.2 ^a	2.1 ^a
K270 nm	0.2 ^b	0.2 ^b	0.3 ^a	0.2 ^b
Palmitic acid	18.4 ^a	10.6 ^d	13.7 ^b	11.9 ^c
Palmitoleic acid	2.8 ^a	0.3 ^b	0.3 ^b	0.2 ^c
Stearic acid	1.9 ^c	3.1 ^a	2.7 ^b	3.1 ^a
Oleic acid	54.6 ^d	66.8 ^a	61.8 ^c	65.5 ^b
Linoleic acid	20.1 ^a	15.8 ^c	19.0 ^b	16.0 ^c
Linolenic acid	0.6 ^a	0.7 ^a	0.7 ^a	0.7 ^a
Arachidic acid	0.3 ^b	0.8 ^a	0.7 ^a	0.7 ^a
Gadoleic acid	0.3 ^c	0.4 ^c	0.7 ^b	1.1 ^a
SFA	21.0 ^a	15.8 ^d	17.4 ^c	16.4 ^b
MUFA	57.7 ^d	67.6 ^a	62.8 ^c	66.9 ^b
PUFA	20.7 ^a	16.5 ^c	19.7 ^b	16.7 ^c
MUFA/PUFA	2.8 ^c	4.1 ^a	3.2 ^b	4.0 ^a

Values followed by identical letters are not significantly different (Tukey's test, $p < 0.05$).

PV: peroxide value.

SFA: saturated fatty acids.

MUFA: monounsaturated fatty acids.

PUFA: polyunsaturated fatty acids.

south and 10.6% in the north. With respect to the effect of the environment, as described by Stefanoudaki, Kotsifaki, and Koutsafakis (1999), a significant ($p < 0.001$) higher content was observed in the oils from low altitude (south Tunisia: Sfax) for Chemlali cultivars. However, in Chétoui olive oil there were slight decreases of palmitic acid from south (13.7%) to north (11.9%). Stearic acid was within the range 1.9–3.1% for Chemlali and 2.7–3.1% for Chétoui oils. Chemlali olive oil exhibited significant differences between the two locations ($p < 0.001$). The olive cultivars showed different mean values for oleic acid, with Chétoui North having the highest value (65.5%) and Chemlali South the lowest (54.6%). When the oils from the different locations were compared, a trend showing greater oleic content for northern oils, and significant differences between cultivars ($p < 0.001$) was evident (Table 1).

The linoleic acid content was higher in Chemlali South oils, as previously described (Manai, Haddada, Trigui, Daoud, & Zarrouk, 2007); the same was true for Chétoui oils. This result does not agree with the so-called Ivanov rule, i.e., "the amount of linoleic acid rises when the temperature decreases, contrary to oleic acid" (Ivanov, 1927, 1929). The ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA), for both cultivars, was higher in oils from the north. The differences observed between locations for the fatty acid composition may be explained by the different altitudes of the locations. This agrees with the results described by other authors for oils from olives growing at different altitudes (Aguilera et al., 2005; Mousa, Gerasopoulos, Metzidakis, & Kristakis, 1996).

3.3. Chlorophylls and carotenoids

Table 2 shows that the total amount of chlorophylls did not exceed 20 mg/kg for both Chétoui and Chemlali cultivars. Independently of the effect of growing area, data showed that Chétoui had the higher level of chlorophylls (17.3 mg/kg) and β -carotene (8.4 mg/kg), whilst lower levels of pigments were detected in Chemlali oils (8.8 and 3.7 mg/kg for chlorophylls and β -carotene, respectively). The growing area conditions also had a significant effect on the amount of chlorophylls, olive oils from the north had the highest level (9.1 and 17.3 mg/kg for Chemlali North and Chétoui North olive oils, respectively). The same phenomenon was observed with β -carotene. Data proved the influence of geographical location on β -carotene content: both Chemlali and Chétoui olive oils from the south contained the lowest levels (3.7 and 5.2 mg/kg, respectively).

3.4. Total phenols and o-diphenols

The effect of the geographic growing area on phenols was clearly observed because of the different behaviour exhibited by each cultivar. It was noticeable that cultivars from the north pro-

Table 2
Mean values of oxidative stability, antioxidant content (phenols, o-diphenols and pigments) and antiradical activities of virgin olive oils of Chemlali and Chétoui cultivars from two locations in Tunisia.

	Chemlali cv.		Chétoui cv.	
	Southern	Northern	Southern	Northern
OSI (h)	2.5 ^d	6.4 ^c	7.2 ^b	12.7 ^a
Chlorophylls (mg/kg)	8.8 ^d	9.1 ^c	12.5 ^b	17.3 ^a
β -Carotene (mg/kg)	3.7 ^c	5.6 ^b	5.2 ^b	8.4 ^a
Phenols (mg/kg)	172.5 ^c	572.5 ^a	274.0 ^b	551.1 ^a
o-Diphenols (mg/kg)	133.6 ^d	283.4 ^b	151.4 ^c	264.9 ^a
EC ₅₀ (mg/kg)	793.4 ^a	177.4 ^c	297.4 ^b	6.9 ^d
ABTS (mM)	0.12 ^d	0.50 ^b	0.37 ^c	0.74 ^a

Values followed by identical letters are not significantly different (Tukey's test, $p < 0.05$).

OSI: oxidative stability index.

EC₅₀: effective concentration at 50% response.

duced oils with higher phenols content, in comparison to the oils from the south of Tunisia (Table 2). For Chemlali, the phenol levels in the olive oil from the north (573 mg/kg) were 3 times greater than those in the same oil from the south (173 mg/kg). However, for Chétoui, the olive oil from the north had twice the phenols value (551 mg/kg), compared to the same oil from the south (274 mg/kg). Therefore, different responses to the geographic growing area conditions were observed for each variety. Differences were also found in *o*-diphenols contents (Table 2). Their variation was parallel to that of total phenols ($r = 0.97$, $p < 0.001$). It is important to note that northern cultivars had the highest concentration of *o*-diphenols; hence varieties planted at higher altitude produced phenols in greater amounts than those planted at lower altitude.

3.5. Antiradical activity and antioxidant capacity

All samples were tested with the Trolox-equivalent antioxidant capacity assay. All the samples tested were able to scavenge the ABTS^{•+} radical cation. Table 2 shows that results were statistically different between olive oil varieties ($p < 0.001$). Antiradical activities of the Chemlali and Chétoui olive oils analysed varied according to their growing areas. Southern Chemlali olive oils had the lowest value (0.12 mM), whilst oils from both Northern Chétoui and Chemlali had higher values (0.74 and 0.50 mM, respectively). The same results were observed with another assay, with all extracts able to react directly with and quench the DPPH[•] radical (Table 2). The efficient concentration required to scavenge 50% of the free radicals in the reaction mixture (called EC_{50} value) was determined. A higher DPPH radical-scavenging activity is associ-

ated with a lower EC_{50} value. The lowest EC_{50} value (6.9 mg/kg) was detected in oils from northern Chétoui olives. Overall, the northern oils had lower EC_{50} values than the corresponding ones from the south. The largest difference was found for the antiradical activity of the northern Chemlali olive oil (177 mg/kg), which was nearly 5-fold more efficient than the southern Chemlali olive oil (793 mg/kg). Therefore, oils from northern cultivars are more effective as DPPH radical-scavenging agents. In addition, DPPH radical-scavenging activities were found to be both time and dose-dependent. Phenols undoubtedly play an important role in EVOO stability and could be considered the most effective antioxidants.

The influence of the growing area conditions on the oxidative stability of the oils was greater for Chemlali, with significant differences between the locations (from 2.5 h in the south to 6.4 h in the north), with the most stable oils obtained in the north (Table 2). On the other hand, Chétoui oils had a good oxidative stability in both the south and the north (7.2 and 12.7 h, respectively). The differences observed in the oxidative stability of studied oils from different locations can be explained by their antioxidant profiles. Oils produced from the north had an important amount of phenols and a high level of oleic acid. As described by many authors (Aguilera et al., 2005; Aparacio, Roda, Albi, & Gutiérrez, 1999) the last two parameters are the main factors responsible for the oxidative stability of olive oils.

3.6. Aroma profiles

The aromatic composition of the different samples is reported in Table 3. Twenty-seven compounds were identified, accounting

Table 3

Composition^a of volatiles obtained from Chétoui and Chemlali olive oils according to their different geographical origin (north and south).

Compounds	LRI ^b	Chemlali cv.		Chétoui cv.	
		Southern	Northern	Southern	Northern
Hexanal	801	tr ^c	tr	tr	–
(<i>E</i>)-2-Hexenal	851	37.6	–	28.5	–
(<i>E</i>)-3-Hexen-1-ol	852	–	–	–	12.0
(<i>E</i>)-2-Hexen-1-ol	862	–	–	–	8.4
1-Hexanol	871	12.8	–	20.2	–
1-Nonene	892	–	–	–	0.9
Unknown	895	0.2	–	–	–
α -Pinene	940	–	4.4	–	2.4
3-Octanone	984	–	–	tr	–
6-Methyl-5-hepten-2-one	996	–	3.9	–	–
Mesitylene	998	–	–	–	tr
(<i>E</i>)-3-Hexenyl acetate	1004	–	21.9	6.6	16.1
Hexyl acetate	1010	–	10.2	3.2	9.2
(<i>E</i>)-2-Hexenyl acetate	1017	–	–	–	tr
(<i>E</i>)- β -Ocimene	1050	tr	22.6	–	7.6
Linalool	1101	–	–	–	tr
Nonanal	1104	tr	4.7	1.5	5.4
Perillene	1112	–	3.0	–	–
(<i>E, E</i>)-2,4-Octadienal	1115	–	–	–	3.0
Phenylethyl alcohol	1116	–	–	–	tr
1-Dodecene	1193	15.8	3.0	16.3	4.4
Decanal	1206	–	–	–	3.0
Cyclosativene	1370	–	2.6	–	–
α -Copaene	1376	–	5.4	tr	2.7
Unknown	1466	–	3.4	–	–
Valencene	1490	4.0	tr	8.9	11.9
(<i>Z, E</i>)- α -Farnesene	1491	tr	–	–	–
(<i>E, E</i>)- α -Farnesene	1508	28.9	14.9	8.8	9.8
Unknown	1587	0.4	–	–	–
Unknown	2049	–	1.4	–	–
Total identified %		9(99.1)	13(96.6)	11(94.0)	18(96.8)
Non identified %		2(0.6)	2(3.4)	0(6.0)	0(3.0)
Total compounds		11	15	11	18

^a Percentages obtained by FID peak area normalisation (HP-5 column).

^b Linear retention indices (DB-5 column).

^c tr < 0.1%.

for 94.0–99.1% of the aroma extract. The number of aromatic compounds differed according to the environmental conditions: olive oils from the northern varieties produced a higher numbers of aromatic compounds (15 and 18 different aromatic compounds for Chemlali North and Chétoui North, respectively), in comparison to the southern oils (Table 3). These results indicate that olive oil aroma compounds accumulate differently according to the geographic area and the cultivar kind (Aparicio & Morales, 1998; Baccouri et al., 2008). The major constituents of the volatile fraction of Chemlali South oils were (*E*)-2-hexenal (37.6%), 1-dodecene (15.8%), 1-hexanol (12.8%) and valencene (4%) (Table 3). The volatile fraction of Chemlali North oil was characterised by four main compounds: (*E*)-3-hexenyl acetate (21.9%), (*E*)- β -ocimene (22.6%), hexyl acetate (10.2%) and (*E*, *E*)- α -farnesene (14.9%). It is important to note, that the major sesquiterpene (*E*, *E*)- α -farnesene, present in all the oils studied was significantly more abundant in Chemlali olive oil (28.9% and 14.9% for southern and northern oils, respectively) than in Chétoui olive oils (8.8% and 9.8% for southern and northern oils, respectively). The main constituents characterising the volatile fraction of Chétoui South oil were (*E*)-2-hexenal (28.5%), 1-hexanol (20.2%), 1-dodecene (16.3%), valencene (8.9%) and (*E*, *E*)- α -farnesene (8.8%). Dominant volatiles in Chétoui North oil were (*E*)-3-hexenyl acetate (16.1%), (*E*)-3-hexen-1-ol (12%) and valencene (11.9%). Northern Chétoui olive oil had a high percentage of alcohol compounds, especially (*E*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol, which were absent in Chemlali (North and South) and Chétoui from the south. Valencene was detected in all the main aroma fractions of the oils tested. The role of this compound in the flavour of olive oil is not clear. In fact, in the literature, only very few papers (Flamini, Cioni, & Morelli, 2003) report the presence of this compound, which could play a very important role in the fragrance of this valuable food.

Taking into consideration the effect of the growing area conditions, it is important to note that varieties grown at low altitude and high temperature (south) had the highest level of aldehydes; (*E*)-2-hexenal was not detected in the varieties grown at higher

altitude and lower temperature (north). Hence, we can claim that the (*E*)-2-hexenal (37.6% and 28.5% for Chemlali South and Chétoui South, respectively) and the alcoholic compounds, 1-hexanol, (12.8% and 20.2% for Chemlali South and Chétoui South, respectively) may be used as markers to differentiate VOO of different geographical origins. Our results are in agreement with the study of Vichi, Pizzale, Conte, Buxaderas, and López-Tamames (2003), which found that levels of hexanal, 1-hexanol, (*E*)-2-hexenal, (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol showed a strong dependence on geographical origin. 1-Dodecene was present at a low percentage in oils from the north (3.0% and 4.4% for Chemlali and Chétoui, respectively) in comparison to oils produced in the south (15.8% and 16.3% for Chemlali and Chétoui, respectively). (*E*)-3-Hexenyl acetate (21.9% and 16.1% for Chemlali North and Chétoui North, respectively) and hexyl acetate (10.2% and 9.2% for Chemlali North and Chétoui North, respectively) dominated oils extracted from northern olives. Thus, these two esters seem to characterise the oils from high altitude and low temperature. It can be hypothesised that levels of alcohol acetyl transferase (AAT) are also dependent on pedoclimatic conditions.

The hydrocarbons of olive oil have been studied by different authors as possible markers to distinguish virgin olive oil from different olive varieties or geographical origins (Aparicio & Luna, 2002; Bortolomeazzi, Berno, Pizzale, & Conte, 2001; Guinda, Lanzón, & Albi, 1996; Vichi et al., 2003). In oils from the two varieties great differences were found mainly in the content of (*E*, *E*)- α -farnesene (Table 3). Moreover, pedoclimatic conditions seem to influence the contents of α -copaene (Vichi et al., 2003). In oils from the north, the levels of α -copaene, α -pinene and (*E*)- β -ocimene were significantly higher than in oils from the South (Table 3).

3.7. Sensory

Both Chétoui and Chemlali grown in the two different locations gave virgin olive oils (EC Regulation, 1991). Fig. 1 shows the three positive descriptors: fruity, bitter and pungent. Our results showed

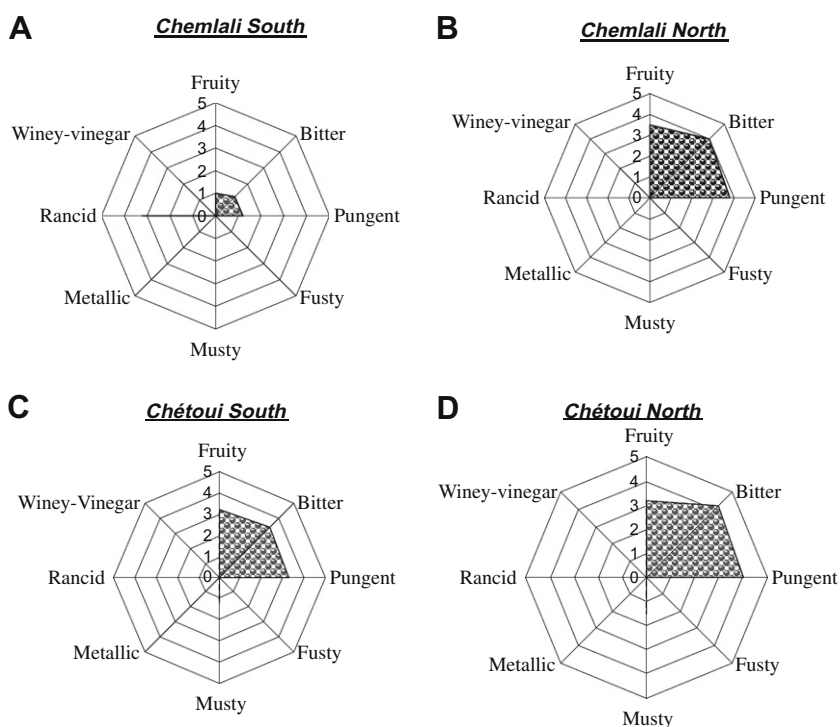


Fig. 1. Sensory wheels of virgin olive oils for the Tunisian olive cultivars Chemlali (A: south, B: north) and Chétoui (C: south D: north) for two locations in Tunisia (Sfax, south and Béja, north).

that the intensities of these three attributes vary according to the geographic area of origin and the cultivar. In fact, the panel test of Chemlali olive oil showed significant differences between the two locations. Northern Chemlali olive oil (Fig. 1B) was fruitier, but also with very bitter and pungent attributes, whereas Southern Chemlali had less fruity attributes (Fig. 1A). In contrast, sensory profiles of Chétoui olive oils were similar for both locations (Fig. 1C and D): 'fruity', with attributes, such as 'pungent' and 'bitter', varying only slightly in intensities. In fact, it became clear that sensory quality was the result of the synergistic effect of the oils' various components, whose composition was influenced by the geographic growing area conditions and cultivar kind.

4. Conclusion

In conclusion, the geographic area of origin appears to play a more significant role for the qualitative characteristics and the sensory attributes of Chemlali olive oil than for the Chétoui olive oil. In fact, all the results obtained showed that Chétoui olive trees adapt well to different pedoclimatic conditions to produce an oil of constant quality. In contrast, Chemlali olive oil showed a low tolerance against variation in climate and plantation zone. Northern Chemlali olive oil demonstrated excellent nutritional characteristics, in terms of antioxidant compounds. It possessed a very bitter taste, whereas the southern Chemlali had 'sweet' amongst its attributes and a lower oxidative stability.

Acknowledgements

This research was supported by the Tunisian Ministry of Enseignement Supérieur, Scientific Research and Technology (UR03/ES-08). Part of this work was carried out at the Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Italy. We wish to thank the personnel of the laboratory of "Human Nutrition and Metabolic Disorder" Faculty of Medicine of Monastir, Institute of Olivier of Sousse". We thank the reviewers for their constructive criticisms of the manuscript. The authors are thankful to Dr. R. Décor for her constructive criticisms of the manuscript.

References

- Adams, R. P. (1995). *Identification of essential oil components by gas chromatography-mass spectroscopy*. Carol Stream, IL: Allured Publishing Company.
- Aguilera, M. P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A., & Uceda, M. (2005). Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. *Food Chemistry*, 89, 387–391.
- Aparicio, R., & Morales, M. T. (1998). Characterization of olive ripeness by green aroma compounds of virgin olive oil. *Journal of Agricultural and Food Chemistry*, 46, 1116–1122.
- Aparicio, R., Roda, L., Albi, M. A., & Gutiérrez, F. (1999). Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural and Food Chemistry*, 47, 4150–4155.
- Aparicio, R., & Luna, G. (2002). Characterisation of monovarietal virgin olive oils. *European Journal of Lipid Science and Technology*, 104, 614–627.
- Baccouri, O., Bendini, A., Cerretani, L., Guerfel, M., Baccouri, B., Lercker, G., Zarrouk, M., & Daoud Ben Miled, D. (2008). Comparative study on volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils. *Food Chemistry*, 111, 277–296.
- Ben Temime, S., Taamalli, W., Baccouri, B., Abaza, L., Daoud, D., & Zarrouk, M. (2006). Changes in olive oil quality of Chétoui variety according to origin of plantation. *Journal of Food Lipids*, 13, 88–99.
- Bortolomeazzi, R., Berno, P., Pizzale, L., & Conte, L. S. (2001). Sesquiterpene, alkene, and alkane hydrocarbons in virgin Olive oils of different varieties and geographical origins. *Journal of Agricultural and Food Chemistry*, 49, 3278–3283.
- Brand-Williams, W., Cuvelier, M. E., & Brest, C. (1995). Use of free radicals method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft Und Technologie*, 28, 25–30.
- Dabbou, S., Issaoui, M., Servili, M., Taticchi, A., Sifi, S., Montedoro, G. F., et al. (2009). Characterisation of virgin olive oils from European olive cultivars introduced in Tunisia. *European Journal of Lipid Science and Technology*, 111, 292–401.
- Davies, N. W. (1990). Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20M phases. *Journal of Chromatography*, 503, 1–24.
- EEC (1991). Characteristics of olive oil and olive pomace and their analytical methods. Regulation EEC/2568/91 and latter modifications. *Official Journal of European Communities*, L248, 1–82.
- Flamini, G., Cioni, P. L., & Morelli, I. (2003). Volatiles from leaves, fruits, and virgin oil from *Olea europaea* Cv. Olivastra Seggianese from Italy. *Journal of Agricultural and Food Chemistry*, 51, 1382–1386.
- Guinda, A., Lanzón, A., & Albi, T. (1996). Differences in hydrocarbons of virgin olive oils from several olive varieties. *Journal of Agricultural and Food Chemistry*, 44, 1723–1726.
- International Olive Oil Council (IOOC) T20/Doc. 2000. N. 15 Rev.n.1.
- Issaoui, M., Mechri, B., Echbili, A., Dabbou, S., Yangui, A.M., Belguith, H., Trigui, A., & Hammami, M. (2008). Chemometric characterization of five Tunisian varieties of *olea europaea* l. Olive fruit according to different maturation indices. *Journal of Food Lipids*, 15, 322–328.
- Issaoui, M., Ben Hassine, K., Flamini, G., Brahmi, F., Chehab, H., Aouni, Y., et al. (2009). Discrimination of some monovarietal olive oils according to their oxidative stability, volatiles compounds and sensory analysis. *Journal of Food Lipids*, 16, 164–186.
- Ivanov, S. (1927). Dependence of the chemical composition of oil containing plants on the climate. *Oil and Fat Industries*, 5, 29.
- Ivanov, S. (1929). The factors in the process of oil formation in plants. *Osterreichische Chemiker Zeitung*, 32, 89.
- Jennings, W., & Shibanoto, T. (1980). *Qualitative analysis of flavor and fragrance volatiles by glass capillary chromatography*. New York: Academic Press.
- Manai, H., Haddada, F. M., Trigui, A., Daoud, D., & Zarrouk, M. (2007). Compositional quality of virgin olive oil from two new Tunisian cultivars obtained through controlled crossing. *Journal of the Science of Food and Agriculture*, 87, 600–606.
- Massada, Y. (1976). *Analysis of essential oils by gas chromatography and mass spectrometry*. New York: Journal Wiley and Sons.
- Minguez-Mosquera, M. I., Rejano, L., Gandul, B., Sanchez, A. H., & Garrido, J. (1991). Color-pigment correlation in virgin olive oil. *Journal of the American Oil Chemists' Society*, 68, 332–336.
- Montedoro, G. F., Servili, M., Baldioli, M., & Miniati, E. (1992). Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry*, 40, 1571–1576.
- Mousa, Y. M., Gerasopoulos, D., Metzidakis, I., & Kristakis, A. (1996). Effect of altitude on fruit and oil quality characteristics of mastoids olives. *Journal of the Science of Food and Agriculture*, 71, 345–349.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an important ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231–1237.
- Stefanoudaki, E., Kotsifaki, F., & Koutsafakis, A. (1999). Classification of virgin olive oils of the two major Cretan cultivars based on their fatty acid composition. *Journal of the American Oil Chemists' Society*, 76, 623–626.
- Stenhagen, E., Abrahamsson, S., & Mc Lafferty, F. W. (1974). *Registry of mass spectral data*. New York: John Wiley and Sons.
- Swigar, A. A., & Silvestein, R. M. (1981). *Monoterpenes*. Milwaukee: Aldrich Chemical Company.
- Vichi, S., Pizzale, L., Conte, L. S., Buxaderas, S., & López-Tamames, E. (2003). Solid-phase microextraction in the analysis of virgin olive oil volatiles fraction: Characterisation of virgin olive oils from two distinct geographical areas of northern Italy. *Journal of Agricultural and Food Chemistry*, 57, 6572–6577.