International Scholars Journals

African Journal of Wood Science and Forestry ISSN 2375-0979 Vol. 8 (7), pp. 001-008, July, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of cultivar on minor components in Tunisia olive fruits cultivated in microclimate

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Accepted 15 October, 2019

This paper evaluates the usefulness of three chemical parameters (composition of volatiles compounds, total phenols and fatty acids) as a tool to discriminate the olive oils obtained from three varieties (Oueslati, Chemlali and Chetoui). These varieties are included among the cultivars permitted by the disciplinary for the production of the ''Kairouan olive oil'', a Tunisian protected designation of origin (PDO) product. The olives were collected during the year crop 2009/2010 from the same orchard (calcareous soil), in order to eliminate geographical and climatic influences. Analysis of the effect of cultivar on the different analytical values, revealed statistically significant differences in some parameters, mainly in free fatty acid and phenol contents and oxidative stability. Furthermore, most of the quality indices and fatty acid composition showed significant variations among olive varieties. Oueslati variety had the highest values of oleic acid, whereas Chetoui was noteworthy for its high content of phenolic compounds. The major volatile component was the C6 aldehyde fraction whose content varied greatly between the different varieties: the (E)-2-hexenal content ranged from 20.9% in the oil obtained from the Oueslati variety to 7.7% in the case of Chemlali one; the amount of hexanal ranged from 10.2% in Oueslati to 3.7% in Chetoui. These results suggest that the genetic factor (cultivar) influences the volatiles formation.

Key words: Cultivar, volatiles, quality indices, phenols.

INTRODUCTION

In Tunisia, the second VOO exporter and producer after the European Union (Haddada et al., 2007), the two most diffuse cultivars are Chemlali and Chetoui. Chetoui, the second main variety cultivated in Tunisia, is widespread in the North of the Country, occurring in plains as well as in mountain Regions, being highly adaptable to various pedo-climatic conditions. In addition, this oil has a very balanced fatty acid profile and an important amount of phenols, which make it quite astringent. However, this latter characteristic is not accepted by most consumers, especially by kids. Chemlali variety, which is mainly cultivated in the Central and Southern areas of the Country, contributes to 80% of the National olive oil production. It is a productive variety, well adapted to severe environmental conditions. In addition, other minor varieties such as Oueslati, are cultivated in Tunisia. The oil of Oueslati variety is characterised by a good content of total phenols, tocopherols and a good resistance to oxidation.

The extra virgin olive oil is the principal source of fat in the Mediterranean diet with important nutraceutic effects due to its abundance of oleic acid, a monounsaturated fatty acid controlling the cholesterol level and an adequate content of linoleic and linolenic acids, the major essential fatty acids that lower the risk of coronary heart diseases and cancers (Galli and Visioli, 1999). Virgin olive oil is the only vegetal fat that can be eaten raw (also called ''olive juice''), without refining operations. This allows the preserving of its natural composition, including the minor, non-saponificable compounds, making up to 1

– 2% of total content, e.g. hydrocarbons, phenols, alcohols, sterols, pigments, tocopherols and vitamins. These compounds are crucial both for the oil oxidative stability (improving the shelf life) and for its unique flavor.

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Aroma and phenols are the only parameters that consumers can appraise directly, while other quality features (e.g. chemical composition) are not always reported on the bottle.

Two recent works reviewed the several factors influencing aromatic quality of virgin olive oil, that is, biogenesis and composition of volatiles, relationships with sensory notes, possible influence of agronomic and processing factors and oil oxidation (Kalua et al., 2007; Angerosa et al., 2004). All these findings show that volatiles content, mainly C6- and C5-skeleton compounds from the lipoxygenate pathway, are strongly influenced by the genetic origin (cultivar) for the enzymatic expression and by horticultural and processing parameters for the enzyme activity. The unique flavor of virgin olive oil is mainly attributed to the volatiles that develop during and after oil extraction from the fruit. These compounds become less important during oil storage due to oxidetion. These changes on volatile composition, together with genetic, horticultural and processing influences, explain quality differences in olive oils (Harwood and Aparicio, 2000).

In a previous study (Tura et al., 2007), the antioxidant profile was found to be firstly influenced by the cultivar and then by the site of cultivation. The oxidative stability was correlated to phenols, tocopherols and saturated on unsaturated fatty acids. In another study several olive accessions from a local cultivars collection in the Garda lake area (Northern Italy) were successfully classified by oil sensory profile (Tura et al., 2002). Moreover, these sensorial attributes results correlated to some volatile and phenolic compounds. A similar cultivar assessment was scored by a principal components analysis and eight aromatic compounds were found to be the more significant in varietal characterization, e.g. (Z)-3-hexen-1-ol, hexan-1-ol, pentan-3-one and (E)-2-hexen-1-ol (Pedo et al., 2002).

A study on the volatile profiles of Australian virgin olive oils has shown that the cultivar is the single-most important factor in determining aroma oil quality (Tura et al., 2004). Other works confirmed the cultivar strong effect on the aroma quality. Dhifi et al. (2005) found that the different volatile composition in four Tunisian oils was affected by the cultivar, also showing a close relation with the enzymatic profile that is genetically determined. Luna et al. (2006) characterized many virgin olive oils from several countries by their volatile compounds and sensory attributes. Berlioz et al. (2006) analyzed the volatile and flavor compositions of several French oils from Protected Designation of Origin (PDO) districts and standard commercial olive oils, developing a chemometric method able to discriminate the oils. Baccouri et al. (2007) demonstrated that the volatile profiles of oleaster oils (olive cultivars selected from wild olives, Olea europea var. oleaster) were different from standard European and Tunisian virgin oils from Olea europea var. sativa. Other peculiar differences in the composition of volatiles in Tunisian and French PDO oils were found by Haddada et al. (2007) who demonstrated that the building up of metabolites in oils from different cultivars was related to genetic origin. Vichi et al. (2003) found signifycant differences in volatile composition in oils of different cultivars and geographical origin in Northern Italy.

Some authors compared different analytical techniques to assess the volatile compounds and/or sensory attributes in olive oils. Procida et al. (2005) arranged a chemometric approach to correlate volatile molecules with oil sensory defects. Also, Morales et al. (2005) studied the correlations between volatile profiles and defects by olfactometry techniques. Cavalli et al. (2003) and Kanavouras et al. (2005) compared several methods for volatiles extraction from oils, that is, static and dynamic headspace, solid phase micro-extraction (SPME), sorptive extraction and thermal desorption. Garcia-Gonzalez et al. (2004) tested the electronic nose coupled with SPME to distinguish different olive oils. Contini and Esti (2006) checked the effectiveness of HS-SPME for the volatiles analysis of virgin olive oils at different dilutions and Jimenez et al. (2006) applied this method to carry out a quality control of virgin olive oils from fruit picked either from the tree or from the ground.

In Tunisia, the presence of typical varieties, the peculiar microclimate conditions and precise olive orchard management, lead to the production of very valuable olive oils with a distinctive taste. The aim of this study is the global characterization of some olive varieties, cultivated in microclimate calcareous soil in the centre of Tunisia, by analysis of their antioxidants content, oxidetive stability, antiradical activity, volatile compounds and chemometric analysis of their virgin olive oils. To avoid the influence of other factors in the characterization, olive trees were cultivated under the same pedoclimatic conditions, olive fruits were picked at the same maturity stage, and their oils were extracted with the same processing system.

MATERIALS AND METHODS

Samples

The olives were hand-picked, at the same stage of maturity, from 3 varieties cultivated in the same condition in the Centre of Tunisia and immediately processed with the same laboratory mill to obtain the olive oil samples. Only drupes not damaged, fresh and healthy were selected. The olives were washed and deleafed, crushed with a hammer crusher, and the paste mixed at 2°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 until analysis.

Quality parameters

Determination of the regulated physicochemical quality parameters (free acidity, peroxide value and UV absorption characteristics, K270 and K232), was carried out according to the analytical methods described by Regulation EEC/ 2568/91 and EEC/ 1429/92 of European Union Commission (EUC, 1991, 1992).

Free acidity, given as percentage of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) with

0.1 M potassium hydroxide in ethanol.

Peroxide value, expressed as milli-equivalents 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 the darkness; the free iodine was then titrated with a sodium thiosulfate solution.

K270 and K232 extinction coefficients were calculated from absorption at 270 and 232 nm, respectively, with a UV spectrophotometer (Hewlett–Packard, HP 8452 A), using pure cyclohexane as a blank.

Fatty acids composition

The determination of fatty acids was performed as described in the EEC regulation (European Union Commission Regulation 2568/91). Methyl-esters were prepared from olive oil after room temperature 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 hydroxide and analysed by gas chromatography (GC) with a Hewlett–Packard (HP 4890 D, Hewlett-Packard Company, Wilmington, DE) chromatograph equipped with a capillary column (Supelcowax 10: 30 × 0.53 × 0.25 µm), a split-splitless injector and a FID detector.

Pigment content

Chlorophyll and carotenoids were determined colorimetrically operating as described by Minguez-Mosquera et al. (1991). The maximum absorption at 670 nm is related to the chlorophyll fraction and that at 470 nm is related to carotenoid fraction. The values of the coefficients of specific extinction applied were E0 = 613 for pheophytin as a major component in the chlorophyll fraction and E0 = 2,000 for lutein as a major component in the carotenoids fraction. Thus the pigment contents were calculated as follows:

Chlorophyll (mg/kg) = (A670 × 106) / (613 × 100 × d)

Carotenoid (mg/kg) = (A470 × 106) / (2,000 × 100 × d)

Where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

Total phenols

Total phenols and o-diphenols were quantified colorimetrically (Ranalli et al., 1999). Phenolic compounds were isolated by triple extraction of a solution of oil (10 g) in hexane (25 ml) with a methanol-water mixture (60:40, v/v). The Folin-Ciocalteau reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts and the absorption of the solution at 725 nm was measured. Values are given as milligrams of caffeic acid per kilogram of oil (Gutfinger, 1981). Ortho-diphenols were also measured colorimetrically at 370 nm after adding 5% (w/v) sodium molybdate in 50% ethanol to the extract (Gutfinger, 1981). Results are given as milligrams of caffeic acid per kilogram of oil.

Volatile compound analyses

Sampling

Solid phase micro extraction 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 2 ml of virgin olive oil inserted into a 5 ml glass vial and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC-MS system (Campeol et al., 2001).

Identification

GC analyses were accomplished with an HP-5890 series II instrument equipped with a DB-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, helium (2 ml/min); detector FID; splitless injection. 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 as follows: injector and transfer line temperature at 250 and 240°C respectively; oven temperature was programmed from 60 to 240°C at 3°C/min; carrier gas, helium at 1 ml/min; splitless injection. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices 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 MS literature data (Massada, 1976; Jennings and Shibamoto, 1980; Davies, 1990; Adams, 1995). Moreover, the molecular weights of all the identification substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas (Flamini et al., 2003).

Oil stability

Oxidative stability was evaluated by the Rancimat method (Gutierrez, 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 743 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g warmed to 100°C and air flow of 10 l/h.

RESULTS

Determination of oil quality parameters

The values of the quality parameters were comprised within the ranges established for “extra virgin olive oil” (EVOO) category for all the oils. At the sensory analysis, acidity is considered an important quality index, which has been exclusively used as a traditional criterion for classifying olive oil. The percentage of free acidity (Table 1), did not exceed the upper limit of 0.8% established by the IOOC norm and corresponding to the EVOO class. The lowest value was found for Chetoui with 0.25% while for the oil obtained from Oueslati it was higher (0.83%). These low values of acidity were due the use of fresh and healthy olives, harvested at the optimal ripening stage,

Table 1. Quality parameters of the monovarietal olive oils from plants cultivated in microclimate.

Parameters	Minor varieties	Major varieties		EVOO
	Oueslati	Chetoui	Chemlali	
FA (%C18:1)	0.83 ± 0.03	0.25 ± 0.10	0.3 ± 0.02	0.8
Pv (meq O2 kg^{-1})	6.2 ± 0.73	2.92 ± 0.36	5.83 ± 1.03	20
K_{270}	0.2 ± 0.01	0.18 ± 0 .03	0.09 ± 0.01	0.22
K_{232}	2.09 ± 0.06	1.69 ± 0.16	1.77 ± 0.04	2.5
Chlorophyll (mg/kg)	5.15 ± 0.29	4.51 ± 0.39	1.83 ± 0.29	-
Carotenoids (mg/kg)	2.5 ± 0.06	2.15 ± 0.30	0.72 ± 0.06	-
Phenols (mg/kg)	510.42 ± 0.40	828.27 ± 13.2	190.09 ± 0.4	-
o-diphenols (mg/kg)	185.62 ± 0.20	282.82 ± 40.95	91.13 ± 0.20	-
Stability (h)	103.62 ± 0.02	73.25 ± 5.81	41.12 ± 0.52	-

Table 2. Fatty acids composition (%) of the monovarietal olive oils from plants cultivated in moicroclimate.

Composition	Minor varieties	Major varieties		EVOO
	Oueslati	Chetoui	Chemlali	
C16:0	12.08 ± 0.20	13.55 ± 0.16	18.59 ± 0.26	7.5 – 20.0
C16:1	0.62 ± 0.35	0.31 ± 0.00	2.20 ± 0.24	0.3 -3.50
C18:0	3.55 ± 0.03	2.28 ± 0.17	2.71 ± 0.87	0.5 – 5.0
C18:1	70.51 ± 0.90	68.85 ± 0.43	55.87 ± 0.56	55.0 – 83
C18:2	12.58 ± 0.23	14.01 ± 0.34	18.05 ± 0.52	3.5 - 21.0
C18:3	0.63 ± 0.03	0.55 ± 0.02	0.97 ± 0.09	≤1.0
C20:0	0.32 ± 0.11	0.40 ± 0.05	0.51 ± 0.05	≤ 0.6
C18:1/C18:2	5.60 ± 0.170	4.91 ± 0.14	3.09 ± 0.170	-
SFA	15.95 ± 0.32	16.23 ± 0.12	21.81	-
MUFA	71.13 ± 0.62	69.16 ± 0.21	58.07 ± 0.62	-
PUFA	13.21 ± 0.79	15.56 ± 0.18	19.02 ± 0.79	-
MUFA/ PUFA	5.51	4.38	3.05	-

C16:0: palmitic; C16:1 palmitoleic; C18:0: stearic, C18:1: oleic; C18:2: linoleic, C18:3; linolenic, C20:0: arachidic; SFA: Saturated fatty acid, MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; EVOO: Extra virgin olive oil.

followed by immediate extraction without olive storage. Some authors reported that at the later stage of olive ripening, the oil obtained presents a higher percentage of free acidity because of the increase of enzymatic activity, especially by lipolytic enzymes, favored by olive tissue damages (Salvador et al., 2001). The peroxide value and UV characteristics are the two main parameters that represent the assessment of the progress state of the different stages that lead to oil rancidity. As indicated in Table 3, all the oil samples presented lower peroxide values, ranging from 2.92 to 6.2 meqO2/kg, belonging to the EVOO category. UV values were generally markedly lower than the limit established for the EVOO (Table 1).

Fatty acid composition

The fatty acid composition of the oil may differ depending on the variety. As shown in Table 2, numerous fatty acids were detected in the EVOOs of the examined varieties. Palmitic, oleic and linoleic acids were the major ones, while palmitoleic, stearic, linolenic and arachidic acids were present in smaller amounts. The levels of palmitic acid ranged from 12.08 ± 0.20% for Oueslati olive oil to 18.59 ± 0.26% for the Chemlali one. These values are potentially high, generally with an average of 13.55% for Chetoui olive oil. Oleic acid, the major MUFA, was high especially in Oueslati and Chetoui oils (70.51 ± 0.9 and 68.85 ± 0.43%, respectively), while it was lower for Chemlali (55.87 ± 0.44%). Concerning linoleic acid, which is much more susceptible to oxidation than MUFAs, its highest percentage was observed in Chemlali oil (18.05 ± 0.52%), whereas its lowest one is in Oueslati (12.58 ± 0.23%). For the other fatty acids, palmitoleic (C16:1), stearic (C18:0), linolenic (C18:3) and arachidic (C20:0), although their percentages changed from one olive oil to the other, they were quite small. All these levels are close to that of the IOOC norm (2001). These findings are in

Table 3. Composition of volatile obtained from plants cultivated in moicroclimate.

Constituent	I.r.i.	Minor varieties	Major varieties	
		Oueslati	Chemlali	Chetoui
hexanal	804	10.2	4.8	3.7
(E)-2-hexenal	855	20.9	7.7	–
(E)-3-hexenol	857	–	–	26.1
(E)-2-hexenol	862	–	–	–
1-hexanol	873	1.2	63.7	19.9
1-heptanol	967	1.2	–	0.5
1-octen-3-ol	980	–	–	–
3-octanone	982	–	–	–
6-methyl-5-hepten-2-one	987	0.7	1.4	0.7
decane	1000	–	–	–
octanal	1003	–	–	–
(E,Z)-2,4-heptadienal	1006	6.3	4.7	–
(Z)-3-hexenyl acetate	1009	5.9	–	13.0
1-hexyl acetate	1011	3.8	–	3.7
(E,E)-2,4-heptadienal	1015	–	–	–
limonene	1032	0.3	0.2	0.9
(Z)-β-ocimene	1041	tr	0.4	–
(E)-β-ocimene	1052	1.2	2.6	2.4
α-terpinene	1062	0.8	0.5	0.3
1-octanol	1070	–	–	–
(Z)-6-nonenal	1097	0.8	2.1	–
undecane	1100	0.5	tr	0.3
nonanal	1103	1.0	0.9	0.6
(E)-2-nonenal	1162	0.6	0.8	–
dodecane	1200	0.4	0.2	–
decanal	1206	1.9	3.0	14.2
(E)-2-decenal	1266	1.8	0.6	–
(E,Z)-2,4-decadienal	1295	0.1	–	–
tridecane	1300	–	0.1	–
(E,E)-2,4-decadienal	1318	–	–	–
cyclosativene	1370	1.9	–	–
longicyclene	1373	1.3	–	–
α-copaene	1376	17.1	0.2	tr
1-tetradecene	1393	0.3	–	0.1
tetradecane	1400	2.7	1.5	1.8
dodecanal	1409	0.3	–	–
(Z)-α-farnesene	1444	–	–	0.4
(E)-geranylacetone	1457	–	–	–
pentadecane	1500	–	–	–
trans-g-guaiene	1502	3.7	–	–
(E,E)- α -farnesene	1508	6.8	1.9	7.4
hexadecane	1600	1.9	0.7	0.8
Total identified		95.8	98.0	96.8

LRI: linear retention indices; tr: < 0.1%. Data values expressed in mg/kg.

good agreement with those of other authors working on other Tunisian olive oil varieties, like Sayali, Gerboui and Zalmati (Abaza et al., 2002). In comparison to the EVOO of the main Tunisian varieties (Chemlali and Chetoui), the Oueslati variety produced oils with a higher level of oleic acid (around 70%) and lower linoleic and palmitic acids,

(both about 12%).

Furthermore, some differences in the fatty acid percentages were present with respect to Cornicabra olive oil, the main cultivar grown in Castilla La-Mancha (Spain). Its oil contains a high percentage of oleic acid, with an average value of 80.4% and a low percentage of palmitic and linoleic acids, with mean values of 9.22 and 4.46%, respectively (Aranda et al., 2004). The percentages of saturated fatty acids (SFA), MUFA and PUFA of the studied olive oils were also calculated and it was observed that Chemlali was rich in total SFA (21.81 ± 0.23%) essentially because of its higher content in palmitic acid, which represents the main acid of the SFA fraction. Regarding the total MUFA, Oueslati olive oil contained the highest percentage (71.13 ± 0.62%) because of its high oleic acid content. These values are in good agreement with to those reported by some authors for other olive oil varieties (Ranalli et al., 2002; Aranda et al., 2004).

Pigment contents

The olive oil color is directly related to their chlorophyll and carotenoid content and it has been proposed as a characterizing factor and as a quality index related to the oil extraction method and to the olive variety (Minguez-Mosquera et al., 1991). In the studied oils, chlorophyll pigments were found at concentrations comprised between 1.83 and 5.15 mg/kg (Table 1). Oueslati oils contained the highest amount, while Chemlali samples the lowest one. Carotenoid pigments contents varied between 0.72 and 2.50 mg/kg according to cultivars (Table 1). The highest amounts were observed in the oils obtained from Oueslati and Chetoui varieties, while the lowest ones were noted in Chemlali oils. Therefore, the different cultivars were characterized by significant differences in their pigments contents. The chlorophyll content of EVOO from Oueslati was 5.15 mg/kg, while EVOO from Cornicabra variety presented higher amounts than the Tunisian varieties; in fact chlorophyll and carotenoids have been found to range from 2 to 27 mg/kg and from 2 to 14 mg/kg, respectively (Salvador et al., 2001).

Total phenols and *o*-diphenols

The phenolic compounds contained in EVOOs are one of the bases of the nutritional importance of this oil (Beltran et al., 2005). In Table 1 the changes in total phenols and *o*-diphenols are reported to the variety. In these oils, the phenol content varied between 190.09 to 828.27 mg/kg. Chemlali varieties contained the lowest amount of total phenols in comparison with Chetoui and Oueslati ones. Chetoui olive oil contained the highest value, followed by the Oueslati variety (510.42 mg/kg). Thus, these samples were relatively rich in total phenols (> 500 mg/kg). The bitterness of olive oils is related to their phenols content; however, this characteristic is not appreciated by the majority of the consumers, despite their beneficial effect. In the case of *o*-diphenols, the highest content of was observed in Chetoui oil (282.82 mg/kg), followed by Oueslati (185.62 mg/kg), while Chemlali had the lowest amount (91.13 mg/kg). These observations suggest the existence of a genotype effect, as the extraction system, the olive ripeness and pedoclimatic and agronomic conditions were all the same for all the varieties.

Oxidative stability

Rancid is a common sensory characteristic of oils and fats that have undergone a process of autoxidation caused by a prolonged contact with the air. The defects are due to inadequate fruit preservation before olive oil processing while the last one is produced during olive oil storage (Morales et al., 2005). The oxidative stability index (OSI) measured by the Rancimat apparatus is a very useful parameter for the prediction of the shelf life of the sample. Under the accelerated conditions, Oueslatii oil showed a quite high resistance to oxidation (103.62 h), followed by Chetoui oil (73.25 h). The oil obtained from Chemlali varieties showed intermediate values, (41.12 h) (Table 1).

Aroma profiles

Volatile compounds are low molecular weight compounds (less than 300 Da), which readily vaporise at room temperature. Some volatile compounds reach the olfactory epithelium, dissolve into the mucus and may bond with olfactory receptors to give an odour sensation (Angerosa, 2002). The aroma of olive oil is attributed to aldehydes, alcohols, esters, hydrocarbons, ketones, furans and probably, other as yet unidentified volatile compounds. The major volatile compounds reported in virgin olive oils are the C6 and the C5 volatile compounds. Hexanal, *(E)*-2-hexenal, hexan-1-ol and 3-methylbutan-1-ol are found in most virgin olive oils in Tunisia (Issaoui et al., 2009).

For the Tunisian monovarietal olive oils involved in this study, *(E)*-2-hexenal was the major volatile in Oueslati olive oils (20.9%). Our results are in agreement with those of Haddada et al. (2007). Chemlali contained a considerably lower amount (7.7%) of this compound that was not found at all in Chetoui olive oil (Table 3). Other C6 compounds were identified in the headspace of the, samples, such as hexanal. However, Oueslati contained higher amounts of this chemical (10.2%) with respect to the major varieties cultivated in Tunisia, such as Chetoui and Chemlali (3.7 and 4.8% respectively). This compound is usually associated to oxidation in vegetable oils. However, it was also detected in the initial virgin olive oil flavor and produced from linoleic acid through the LOX

pathway (Morales et al., 1997). In the studies of Aparicio et al. (1996) and Morales et al. (1995, 1996), authors demonstrated that hexanal is an important flavor compound of virgin olive oil and contributes to a sweet perception. Furthermore, it has been postulated that hexanal content in virgin olive oil is positively correlated with the overall acceptability of potential and habitual consumers of virgin olive oil (McEwan, 1994). The third C6 compound detected in the oils was hexanol (1.2 - 63.7%). The highest percentage was observed in Chemlali oil (63.7%), followed by Chetoui (19.9%), while Oueslati contained the lowest amount (1.2%), (Table 3). The C6 aldehydes hexanal and *(E)*-2-hexenal, as well as hexanol, contribute to the typical green sensory perception of Tunisian virgin olive oils. Produced via the LOX pathway from polyunsaturated fatty acids (linolenic acids and linoleic acids), hexanal and *(E)*-2-hexenal accumulate in virgin olive oils during physical extraction procedures (Olias et al., 1993). The latter is derived from *(Z)*-3-hexenal, which undergoes isomerization to a more stable compound that can then be further reduced to *(E)*-2-hexen-1-ol (Iraqi et al., 2005). Hexyl acetate and *(E)*-3-hexenyl acetate are present in the aroma of volatiles of all Tunisian olive oil samples, but at different levels according to the respective varieties. These esters are synthesized by alcohol acyltransferase within the LOX pathway. Moreover, the low level of esters in the Chemlali and Oueslati cultivars also indicates a lower content of alcohol acyltransferase in the olive oils compared with Chetoui and its fruits. Other minor volatile compounds were observed in some of the virgin olive oils samples.

Among them, nonanal is obtained via autoxi-dation reactions (Morales et al., 1997) that inevitably start after virgin olive oil has been extracted. For the varieties involved in this study, mono- and sesquiterpene hydrocarbons were detected as the main aroma chemical class of the oils. The role of these components in the definition of the flavor is not clear (Baccouri et al., 2007). In fact, in the literature, only very few papers (Bentivenga et al., 2001; Flamini et al., 2003; Vichi et al., 2003; Baccouri et al., 2007) report the presence of these compounds, which could play a very important role in the fragrance of this valuable food (Baccouri et al., 2007). The presence of 6-methyl-5-hepten-2-one is attributed to the activity of some microorganisms (*Pseudomonas* sp.) present on all the fruits before extraction. Also, the levels of the alkanes from C9 to C17 found in the samples did not allow their differentiation, in spite of their absence in some of the samples.

The volatile fraction of the Oueslati oil was the richest in aroma compounds in comparison with the other major varieties of Tunisia (29 compounds in its aroma profiles) in which *(E)*-2-hexanal was the dominant one (20.9%) followed by α copaene (17.1%), hexanal (10.2%), (*E,E)*-α-farnesene (6.8%), *(E,Z)*–2-4-heptadienal (6.3%) and *(Z)*-3-hexenyl acetate (5.9%). The second aroma compounds-richest oil was that of the Chemlali variety, in which 1-hexanol (63.7%) was the main volatile, followed by *(E)*-2-hexenal (7.7%) and hexanal (4.8%). In the Chetoui olive oil headspace, 18 different compounds were identified, accounting for 96.8% of total volatiles. It contained *(E)*-3-hexanol, 1-hexanol, decanal, (*E)*-2-hexenal, *(Z)*-3-hexenyl acetate and (*E,E)*-α-farnesene among its major components (26.1, 19.9, 14.2, 13.0, 7.4 and 19.0%, respectively) (Table 3). It is also important to note the increasing level of 1-hexanol (detected at considerable lower levels in Oueslati), which emphasizes the perception of green pleasant attributes. As reported by Angerosa and Basti (2003), these results should be considered in a very positive way because the activity of alcohol acyltransferase seems to be little compromised, since esters contribute to the smooth green notes that are generated from the LOX pathway (Grosch, 1993). In fact, Angerosa and Basti (2003) studying the strict dependence on the enzymatic store for the production of the volatile compounds suggested that, when more cultivars are milled, some interactions and/or synergisms could occur among the enzymes involved in the LOX cascade. It is likely to attribute these interactions and synergisms to their changed activity in the olive paste, probably ascribable to the new relative concentration of each enzyme involved in the LOX pathway fixed by the genetic stores of the two cultivars.

Conclusion

The chemical characterization of EVOO from some major varieties and one minor varieties cultivated in an arid Region in the center of Tunisia, showed that, with the exception of the Chemlali variety, which presented a high level of linoleic acid, all the other varieties produced EVOO with good chemical characteristics with regard to their fatty acid composition, which complies with commercial standards, as well as for their appreciable amounts of phenolic and volatiles compounds. Furthermore, the Oueslati and Chetoui varieties presented an improved oil fatty acid composition as compared to Chemlali, the main olive variety in Tunisia. These two cultivars could be recommended to Tunisian olive growers for a large-scale plantation in the future.

ACKNOWLEDGEMENT

This work had been done as apart of a National Research Project. We thank the Ministry of Higher Education, Scientific Research and Technology for financially supporting this program. This work was supported by our Ministry of High Education, Scientific Research and Technology. Part of this work was carried out at the Dipartimento di Chimica Bioorganicae Biofarmacia, Universita‘ di Pisa, Italy.

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