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Chemical composition of virgin olive oils from the Chemlali cultivar with regard to the method of the olive tree propagation

By **M. Guerfel**[1,*], **M.B. Mansour**[1], **Y. Ouni**[2], **F. Guido**[3], **D. Boujnah**[4] and **M. Zarrouk**[2]

[1] Institut Supérieur de Biologie Appliquée de Medenine, Université de Gabes, B.P 522, 4100 Medenine, Tunisia
[2] Centre de Biotechnologie de Borj Cédria, B.P. 901, 2050 Hammam-Lif, Tunisia
[3] Dipartimento di Scienze Farmaceutiche, sede di Chimica Bioorganica e Biofarmacia, via Bonanno 33, 56126 Pisa, Italy
[4] Institut de l'Olivier, Station de Sousse, Rue Ibn Khaldoun, B.P. 14, 4061 Sousse,Tunisia
*Corresponding author: Guerfel_mk@yahoo.fr

RESUMEN

Composición química de aceites de oliva virgen de la variedad Chamlali en relación con el método de propagación del olivo.

En este trabajo se presenta por primera vez un estudio de discriminación basado en compuestos antioxidantes, estabilidad oxidativa y compuestos volátiles de muestras de aceites de oliva virgen obtenidos de frutos de la principal variedad de aceitunas tunecinas (Chemlali) a partir de dos métodos de propagación del olivo (chupones y estaquillas herbáceas). Se han encontrado diferencias significativas entre los aceites obtenidos por los dos métodos. Las muestras de aceites de oliva obtenidas de frutos de árboles de chupones tenían una mayor proporción de ácido oleico (63,8%), un mayor contenido de clorofila y de carotenoides (3,01 mg/kg y 1,9 mg/kg, respectivamente), un mayor contenido de (E)-2 hexenal (66,1%) y un mayor contenido en fenoles totales (890 mg/kg). Curiosamente, el aceite más estable se ha obtenido de las aceitunas de árboles de chupones, en comparación con las aceitunas de árboles de estaquillas herbáceas. Estos resultados pueden ser utilizados para discriminar y caracterizar los aceites de oliva Chamlali según el origen del olivo.

PALABRAS CLAVE: Ácidos grasos – Estaquilla herbácea – Estabilidad oxidativa – Chupones – Aceites de oliva virgen – Compuestos volátiles.

SUMMARY

Chemical composition of virgin olive oils from the Chemlali cultivar with regard to the method of the olive tree propagation.

This paper reports for the first time a discrimination study based on the antioxidant compounds, oxidative stability and volatile compounds of virgin olive oil samples obtained from fruits of the main Tunisian olive cultivar (Chemlali) using two methods of olive tree propagation (suckers and cuttings). There were significant differences between the oils from the two methods. Olive oil samples obtained from the fruits of trees from suckers had a higher content of oleic acid (63.8%), higher contents of chlorophyll and carotenoids (3.01 mg/ kg and 1.9 mg/kg respectively), a higher content of (*E*)-2 hexenal (66.1%) and a higher content in total phenols (890 mg/kg). Interestingly, more stable oil was obtained from the olives from suckers compared to the olives from cuttings. These results can be used to discriminate and to characterize the Chemlali olive oils from each origin of olive tree.

KEY-WORDS: Cuttings – Fatty acids – Oxidative stability – Suckers – Virgin olive oils – Volatile compounds.

1. INTRODUCTION

The chemical composition of virgin olive oil (VOO) depends on many factors: olive tree cultivation, harvesting, pedoclimatic conditions, olive cultivar, plant density and number of processing steps required; mainly crushing, malaxation and centrifugation (Salvador *et al.,* 2003; Cerretani *et al.,* 2005; Torres and Maestri, 2006; Guerfel *et al.,* 2010).

Tunisia is a very important country in the olive oil producing world, the largest African exporter and fourth worldwide after Spain, Italy and Greece. The olive tree (*Olea europaea* L.) is present in practically every region of the country, up to the border of the southern dessert. The conventional method of olive tree propagation in Tunisia is based on vegetative multiplication using cuttings, grafting, or suckers. These methods have been frequently used for the propagation of some highly valued cultivars, particularly the Chemlali cultivar. The Chemlali cultivar is ubiquitous in the Tunisian arable land and contributes to up to 80% of the national olive oil production. Chemlali olive oil is characterized by the relatively low levels of oleic acid (53-55%) and high levels of palmitic and linoleic acids (Manaï *et al.,* 2007). Recently, a major effort has been made to improve the quality of olive oil produced in Tunisia. Perhaps, production improvement can be made once the main drawbacks are known (cultivar type and/or the origin of olives used for oil extraction).

This is the first evaluation of the chemical composition of Chemlali virgin olive oils in relation to the method used for the olive tree propagation (cuttings, suckers). Because of the importance of this cultivar for Tunisian oil production, the aim of this work was to characterize virgin olive oils of

Chemlali trees from cuttings and suckers based on the study of major (fatty acids) and minor compounds (phenols, chlorophylls, carotenoids and volatiles) as well as on the oxidative stability.

2. MATERIALS AND METHODS

2.1. Oil Sample Extraction

Olive oil samples were obtained from fruits of the main Tunisian olive cultivar, Chemlali, which were picked by hand at the same stage of maturity from three trees during the crop season 2009/2010 (October) in a 4 ha olive orchard located in the Souassi center of Tunisia (35°, 49' N, 10°, 30' E). The olive trees were planted in 1988 and were subjected to an identical fertilization regime and to all common olive cultivation practices. The same laboratory mill was used to prepare the olive oil samples. Only healthy fruits, without any kind of infection or physical damage, were processed in triplicate from trees from cuttings and from suckers. After harvesting, fresh olives (1.5-2.0 kg) from each fruit sample were washed and the leaves were removed. They were then crushed with a hammer crusher, and the paste was mixed at 25 °C for 30 min, centrifuged without the addition of warm water (oil produced from each extraction was 200-250 mL/kg), transferred to dark glass bottles, and stored for one week in the dark at 4 °C until analysis.

2.2. Determination of Oil Quality Parameters

Free acidity, expressed as percent of oleic acid (%18:1); peroxide value, given as milliequivalents of active oxygen per kilogram of oil (meqO2/kg); and UV absorption characteristics (K_{232} and K_{270}) were determined according to the analytical methods described in the European Union Commission Regulations EEC/2568/91 and EEC/1429/92.

2.3. Fatty Acid Composition

The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g oil and 3 mL of hexane with 0.4 mL of 2-N methanolic potassium hydroxide, and analyzed using a Hewlett-Packard (HP 4890D; Hewlett-Packard Company, Wilmington, DE) chromatograph equipped with a capillary column (Supelcowax: 30 m × 0.53 mm; 0.25 mm), a split/splitless injector and a flame ionization (FID) detector. The carrier gas was nitrogen at a flow rate of 1 mL/min. The temperatures of the injector, the detector and the oven were held at 220, 250 and 210 °C, respectively. The injection volume was 1 μL.

2.4. Pigment Content

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

$$\text{Chlorophyll (mg/ kg)} = \frac{(A_{670} \times 10^6)}{(613 \times 100 \times d)}$$

$$\text{Carotenoid (mg/ kg)} = \frac{(A_{470} \times 10^6)}{(2{,}000 \times 100 \times d)}$$

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

2.5. Total phenolic content

Total phenol contents were quantified colorimetrically (Ranalli *et al.,* 1999). Phenolic compounds were isolated by the triple extraction of a solution of oil (10 g) in hexane (20 mL) with 30 mL of a methanol and 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).

2.6. Volatile compound analyses

Solid phase micro extraction was used as a technique for headspace sampling of virgin olive oils. 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 glass septum 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 (25 °C). Once sampling was finished, the fiber was withdrawn into a needle and transferred to the injection port of the GC-FID and GC–MS system. GC-EIMS separations 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^{-1}; carrier gas, helium at 1 mL min^{-1}; 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 homemade library mass spectra built from pure substances and components of known oils and MS literature data (Jennings and Shibanoto1980; Davies, 1990; Adam, 1995). Moreover, the molecular weights of all the identification

substances were confirmed by GC-CIMS, using MeOH as the CI ionizing gas.

2.7. Oil Stability

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

2.8. Statistical Analysis

Significant differences between means were determined by an analysis of variancewhich applied a Duncan's test. Differences were considered statistically significant when the probability was greater than 99% ($P < 0.01$). The statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., 2004).

3. RESULTS

3.1. Fatty Acid Composition

Compared to the olive oil samples obtained from cuttings, the olive oil samples obtained from suckers had a higher content of oleic acid (63.8%) (Table 1). The palmitic acid content varied between 14.3% and 17.5%. The olive oil samples also contained low amounts of linolenic acid (C18:3), arachidic acid (C20:0) and palmitoleic acid (C16:1). The olive oil samples obtained from trees cuttings were found to be rich in total saturated fatty acids (21.1%), essentially due to their high content of palmitic acid. The olive oil samples obtained from suckers were found to show a higher content in total monounsaturated fatty acids (64%), due to their high percentage of oleic acid. The olive oil samples obtained from trees from cuttings were found to have a higher percentage of polyunsaturated fatty acids (20%) due to their high content in linoleic acid.

3.2. Oil Quality Parameters and Pigment Contents

The olives from both methods of propagation yielded extra virgin olive oils, but the profile of the analytical parameters (e.g., free fatty acid content, peroxide value and extinction coefficients at 232 and 270 nm) showed some slight differences (Table 2). The oils from suckers had higher contents of chlorophyll and carotenoids.

3.3. Changes in Oxidative Stability

The olive oil samples obtained from the fruits of the trees from suckers were found to have higher contents in total phenols (890 mg/kg) (Table 2). Therefore, significant differences between the two propagation methods were observed with regard to the total phenol contents, with more stable oil being obtained from the trees from suckers (50.2 h).

3.4. Volatile Compounds Analyses

The aromatic composition of the different samples is reported in Table 3. Fifteen compounds have been characterized by GC-FID and GC–MS analysis. (*E*)-2-Hexenal was the major constituent, accounting for about 55% of all volatiles. Other compounds present in relatively high concentrations were hexenal (2.7-4.8%), (*E,Z*)-2,4-heptadienal (9.2-11.9%), (*E*)-β-ocimene (0.7-1), nonanal (0.7%), and (*E,E*)-α-farnesene (0.6-2.1%). The chemical composition of the volatile fraction of Chemlali olive oils was quite variable,

Table 1
Fatty acid composition of virgin olive oil samples from the two ways of olive tree propagation

	Olives from cuttings	Olives from suckers
Palmitic acid C16 :0	14.32 ± 0.11a	17.51 ± 0.12b
Palmitoleic acid C16 :1	0.33 ± 0.08a	0.51 ± 0.01a
Stearic acid C18 :0	2.96 ± 0.16a	3.20 ± 0.15a
Oleic acid C18 :1	60.16 ± 1.28a	63.86 ± 0.41b
Linoleic acid C18 :2	19.84 ± 0.69b	16.0 ± 0.24a
Linolenic acid C18 :3	0.76 ± 0.09a	0.52 ± 0.04a
Arachidic acid C20 :0	0.44 ± 0.02a	0.38 ± 0.04a
Saturated fatty acids (SFAs)	17.72 ± 0.29a	21.09 ± 0.31b
Monounsaturated fatty acids (MUFAs)	60.49 ± 1.36a	64.37 ± 0.42b
Polyunsaturated fatty acids (PUFAs)	20.60 ± 0.78b	16.52 ± 0.28a

[a, b] Mean ± S.D. (n = 6). Significant differences within the same row are shown by different letters ($P < 0.01$).

Table 2
Quality parameters of Chemlali olive oil Samples from the two ways of olive tree propagation

	Olives from cuttings	Olives from suckers
Acidity (%C18 :1)	0.8 ± 0.1b	0.64 ± 0.03a
PV (Meq O_2 /Kg)	17 ± 1b	15 ± 0.58a
$^{K}270$	0.08 ± 0.01a	0.07 ± 0.08a
$^{K}232$	0.55 ± 0.18a	2.13 ± 0.09b
Oxidative stability (h)	46.7 ± 2.30a	50.2 ± 1.80b
Chlorophylls (mg/kg)	2.18 ± 0.25a	3.01 ± 0.15b
Carotenoids (mg/kg)	1.13 ± 0.22a	1.9 ± 0.19b
Phenols (mg/kg)	730 ± 13.5a	890 ± 9.5b

[a, b] Mean ± S.D.(n = 6). Significant differences within the same row are shown by different letters ($P < 0.001$). PV, peroxide value; K_{232} and K_{270}, values of specific extinction given as absorbance at 232 and 270 nm, respectively.

Table 3
Composition of the volatile fraction obtained from Chemlali virgin olive oils extracted by HS-SPME

	LRI	Olives from cuttings	Olives from suckers
Hexanal	800	4.8b	2.7a
(*E*)-2-hexenal	851	59.8a	66.1b
1-hexanol	871	–	5.5a
(*E,Z*)-2,4-heptadienal	998	9.2a	11.9b
(*E*)-β-ocimene	1051	1a	0.7a
nonanal	1104	0.7a	0.7a
Decanal	1206	7.5b	4.9a
Tridecane	1300	3.9a	–
α-copaene	1377	0.2a	–
Isocaryophyllene	1407	1.1a	0.5a
β-caryophyllene	1418	2.5b	1.2a
dihydro-b-ionone	1435	0.1a	–
α-humulene	1456	1.3b	0.7a
(*E,E*)-α-farnesene	1505	2.1b	0.6a
Cedrol	1597	0.7a	0.2a
Total identified		94.9	95.7

LRI: linear retention indices; Data values expressed in mg/kg. [a, b] Mean ± S.D.(n = 3). Significant differences within the same row are shown by different letters ($P < 0.005$). – compound not detected.

depending on the method used for olive tree propagation.The volatile fraction of the oil from trees from cuttings was characterized by the pre-eminence of two compounds: (*E,Z*)-2,4-heptadienal (9.2%) and (*E*)-2-hexenal (59.8%). The other main compounds detected were decanal (7.5%), hexanal (4.8%), tridecane (3.9%), β-caryophyllene (2.5%), (*E,E*)-α-farnesene (2.1%), isocaryophyllene (1.1%), α-humulene (1.3%) and cedrol (0.7%). The volatile fraction of the oil from suckers was characterized by the pre-eminence of two compounds: (*E*)-2-hexenal (66.1%) and (*E,Z*)-2,4-heptadienal (11.9%) which may be used as markers to differentiate the VOO obtained from the two methods of olive tree propagation. The other main compounds detected were 1-hexanol (5.5%), decanal (4.9%), hexanal (2.7%), β-caryophyllene (0.6%), (*Z*)-3-hexenyl acetate (4.5%), cyclosativene (3.0%), (*E*)-2-decenal (1.2%), nonanal (0.7%) and α-humulene (0.7%). Differences in the levels of esters in olive oil samples were not clearly observed between the methods adopted for olive tree propagation. Moreover, in the oils from the two methods of olive tree propagation differences were found in the content of (*E, E*)-α-farnesene.

4. DISCUSSION

The fatty acid composition of Chemlali from trees from suckers and cuttings was characterized by relatively high levels of oleic and linoleic acids. These results are similar to those reported by several authors for other olive oil varieties cultivated in Tunisia (Ben Temime *et al.*, 2006; Krichene *et al.*, 2007). The differences in the method of olive tree propagation led to differences in the fatty acid composition of virgin olive oil. Morello *et al.* (2004) reported that several agronomic parameters could modify the fatty acid composition of olive oil. The most studied aspects include cultivar and origin, fruit ripening, harvest period, climatic conditions and soil characteristics. However, our results show that the origin of the tree had little influence on the analytical parameters (free fatty acid content, peroxide value and extinction coefficients at 232 and 270 nm) (Table 2), which are affected by factors causing damage to the fruits (e.g. olive fly attacks or improper methods of harvesting, transport and storage of olives) (Kiritsakis *et al.,* 1998). In addition, olive oil samples obtained from the fruits of trees from both propagation methods were found to have varied chlorophyll and carotenoids contents. The total pigment content in olive oil is an important parameter for evaluating olive oil quality. Furthermore, pigments are involved in auto-oxidation and photo-oxidation mechanisms (Gutiérrez, 1989).

Stability to oxidation is an important property of olive oil, which is improved by synergistic interactions between the various antioxidants present in the oil itself, and also depends on the lipid composition. Oxidative stability of the Chemlali olive oils varied according to the method adopted for olive tree propagation (Table 2). Moreover, the two studied olive oils presented a good correlation between total phenols ($r = 0.80$), and oxidative stability measured by Rancimat. A good direct correlation between oxidative stability and total phenolic content has been previously reported by other authors (Gutiérrez *et al.,* 2001). Significant differences among the two propagation methods were observed with regard to the total phenol contents. Olive oil is the only vegetable oil which contains appreciable amounts of phenolic compounds (which were represented basically by o-diphenols) acting as antioxidant substances and conferring to it a greater stability against oxidation during storage (Bendini *et al.,* 2007).

(*E*)-2-hexenal was the principal volatile identified in the oils from the two propagation methods. (*E*)-2-hexenal was also the main volatile reported among the constituents of olive oil aroma (Ben Temime *et al.,* 2006; Vichi *et al.,* 2003). From the levels of the esters in the olive oils samples it can be hypothesized that levels of alcohol acetyl transferase (AAT) are not dependent on the origin of the olive tree. However, some differences in the levels of some terpenes hydrocarbons were observed in our olive oils samples. 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 (Vichi *et al.*, 2003; Aparicio and Luna, 2002; Bortolomeazzi *et al.*, 2001; Guinda *et al.*, 1996).

5. CONCLUSION

In conclusion, the olive oils samples from both methods of olive tree propagation were within the limits established in the European Regulation, allowing them to be classified as extra virgin olive oils. However, our results showed that olive oil quality was different when the olive tree is from suckers or from cuttings. The oils obtained from the fruits of trees from suckers and compared to oils from trees from cuttings showed high levels of antioxidants along with an increased oxidative stability and high level of oleic acid.

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