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Fatty Acid, Triacylglycerol, and Phytosterol Composition in Six Tunisian Olive Varieties

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The physicochemical and stability properties as well as the fatty acid, triacylglycerol, sterol, and triterpenic dialcohol compositions of Tunisian olive oil varieties were analyzed. On the basis of our results, we classified all of the monovarietal oils into the extra virgin category. Oleic and linoleic acids were the most useful fatty acids to discriminate three cultivars, Neb Jmel, Chétoui, and Ain Jarboua, from the others. Of the six monovarietal virgin olive oils analyzed, the main triacylglycerols were OOO, POO, PLO plus SLL, and OLO, which was expected given the high oleic acid and low linoleic and linolenic acids content observed in total fatty acids. In total, these accounted for more than 80% of the total HPLC chromatogram peak area. The main sterols found were β-sitosterol, Δ5-avenasterol, and campesterol. The statistical analysis showed significant differences between oil samples, and the obtained results showed a great variability in the oil composition between cultivars, which is influenced exclusively by genetic factors.

KEYWORDS: Fatty acids; monovarietal olive oils; sterols; triacylglycerols; triterpenic dialcohols

INTRODUCTION

In contrast to other vegetable oils, extra virgin olive oil made from olive fruits (*Olea europaea* L.) is only obtained from mechanical processes (crushing, malaxation, and centrifugation) and then consumed without refinement, which preserves its original characteristics. The lack of refinement is important for its nutritional (*1*, *2*) and sensory (*3*) properties and its resistance to oxidative degradation (*4*).

In Tunisia, the commercial production of olive oil is an important economic activity. 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 second largest exporter of olive oil in the world.

This study focuses on the characterization of Tunisian virgin olive oils (*5*, *6*), because the chemical composition of olive oils varies widely depending upon fruit cultivar, degree of fruit ripeness, environmental conditions, growing region, processing techniques, and storage (*7*–*9*). Chemical characterization of monovarietal olive oil is imperative for the selection of quality cultivars that produce virgin olive oil, with good characteristics for potential use in the future. At present, the Tunisian olive cultivation is dominated by two main cultivars: Chemlali and Chétoui. To diversify our olive genetic resources and improve the quality of olive oil produced in Tunisia, research on additional cultivars needs to be conducted. This will allow for the identification of cultivars with good oil quality that are well-adapted to our environmental conditions. Today, introduction of different virgin olive oil types, with different sensory and chemical characteristics, into the market is of great interest.

In this study, we describe the composition of six Tunisian virgin olive oils from Chétoui, Jarboui, Ain Jarboua, Rekhami, Regregui, and Neb Jmel grown in the same pedoclimatic conditions. Many analytical parameters necessary for quality evaluation (*10*, *11*) have been considered in the characterization of these varieties, such as physicochemical and stability parameters, fatty acids, triacylglycerols, sterols, and dialcohols.

MATERIALS AND METHODS

Oil Samples. Six different monovarietal extra virgin olive oils (EVOOs) from Northern Tunisia were analyzed. The EVOO samples chosen for this study were from various olive cultivars: Regregui, Rekhami, Jarboui, Ain Jarboua, Neb Jmel, and Chétoui. These cultivars were grown in the mountainous locality of Nebeur (altitude of more than 500 m) from the region of El Kef, in a semiarid zone (rainfall average of 350 mm per year) under the same pedoclimatic conditions.

Only healthy fruits, without any kind of infection or physical damage, were processed. We tested three consecutive crop years (2003/2004, 2004/2005, and 2005/2006). Oil extraction was performed using an

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Abencor laboratory oil mill (MC2 Ingenierias y sitemas, Sevilla, Spain) and kneading the olive paste at 28 °C for 30 min, and oil samples were gravity-filtered in a glass funnel using filter paper (filters folded of 300 mm and type 132). All analyses were carried out within 7 days after production, and samples were stored in amber glass bottles at +4 °C.

Analytical Determination. Determination of the physicochemical quality parameters (free acidity, peroxide content, and UV absorption characteristics) was carried out, following the analytical methods described by Regulation EEC/2568/91 of the Commission of the European Union (EUC 1992) (*12*).

Free acidity, expressed as the percent of oleic acid, was determined by the titration of a solution of oil dissolved in ethanol/ether (1:1) with 0.1 M potassium hydroxide in ethanol.

Peroxide content, expressed in milliequivalents of active oxygen per kilogram of oil (mequiv of O_2 kg^{-1}), was determined by the reaction of a mixture of oil and chloroform/acetic acid with a solution of potassium iodide in darkness.

K_{270} and K_{232} extinction coefficients were calculated from absorption at 270 and 232 nm, respectively, with a UV spectrophotometer (Model SECOMAM ANTHELIE Advanced, France), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

The maturation index (MI) was determined according to Hermoso et al. (*13*) and varied between 0 and 7.

Oxidative Stability. Oxidative stability was evaluated by the Rancimat method (*12*). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 743 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g that was heated to 101.6 °C and an air flow of 10 L h^{-1}.

Determination of Total Phenol and *o*-Diphenol Compounds. Total phenol compounds were isolated by extraction of a solution of oil in hexane 3 times and with a water/methanol (60:40, v/v) mixture 3 times. Total phenols were determined by adding the Folin−Ciocalteau reagent to a suitable aliquot of the combined extracts and measuring the absorbance at 725 nm 2 h later (*15*). Total phenols are expressed as milligrams of cafeic acid per kilogram of oil. A total of 5 mL of the combined extract was mixed with 1 mL of a 5% solution of sodium molybdate dehydrate in ethanol/water (1:1, v/v). The mixture was shaken vigorously for 15 min, and the absorbance at 370 nm was measured to determine the *o*-diphenol content (*16*). Total *o*-diphenol is expressed as milligrams of cafeic acid per kilogram of oil.

Chlorophyll and Carotenoid Compounds. The amounts of chlorophyll and carotenoid compounds were determined at 470 and 670 nm in cyclohexane, using the specific extinction coefficients according to the method of Minguez-Mosquera et al. (*17*). The extinction coefficients applied were $E_0 = 613$ for pheophytin as the major component in the chlorophyll fraction and $E_0 = 2000$ for lutein as the major component in the carotenoid fraction. Thus, pigment contents were calculated as follows:

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

$$[\text{carotenoid}]\ (\text{mg/kg}) = (A_{470} \times 10^6)/(2000 \times 100 \times d)$$

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

Chlorophyll and carotenoid contents were expressed as milligrams of pheophytin "*a*" or lutein per kilogram of oil, respectively.

Fatty Acid Composition. The analytical methods for the determination of fatty acid composition are described in regulation EEC 2568/91 (corresponding to AOCS method Ch 2-91). Fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g of oil and 3 mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide. The converted fatty acid methyl esters were analyzed with a Hewlett-Packard (HP 4890D) chromatograph, equipped with a capillary column (Supelcowax; 30 m × 0.53 mm; 0.25 μm), a split-splitless injector, and a flame ionization detector (FID). The carrier gas was nitrogen and used at a flow rate of 1 mL/min. The temperatures of the injector, detector, and oven were held at 220, 250, and 210 °C, respectively.

Iodine values (IV) were calculated from fatty acid percentages using the following formula (*18*):

$$\text{IV} = (\%\ \text{palmitoleic} \times 1.001) + (\%\ \text{oleic} \times 0.899) + (\%\ \text{linoleic} \times 1.814) + (\%\ \text{linolenic} \times 2.737)$$

Total Sterol Content. The content of sterols (%) was determined according to procedures described by Sánchez-Casas et al. (*19*). We used a Hewlett-Packard, model HP 6890 gas chromatograph, equipped with a FID, a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm), and a 6890 Agilent automatic injector. The working temperatures of the chromatograph were 300 °C at the injector, 260 °C at the isothermal column, and 325 °C at the detector. Quantification was made by the addition of an internal standard (α-cholestanol), and apparent β-sitosterol was calculated as the sum of β-sitosterol, Δ5,23-stigmastadienol, clerosterol, sitostanol, and Δ5,24-stigmastadienol.

Triacylglycerol Composition. The analysis of triacylglycerols was performed according to the official chromatographic method of the European Economic Community Regulations number 2568/91 (*12*). A Hewlett-Packard high-performance liquid chromatography (HPLC, HP 1050, Agilent Technology) quaternary pump instrument, equipped with a refractometer detector, was employed using a Lichrosorb RP18 column (250 × 4.6 mm, 5 μL particle size; Teknocroma, Barcelona, Spain), and the following settings were used: column oven, 45 °C; elution solvent, acetone/acetonitrile (60:40, v/v); flow rate, 1.2 mL/min. The standards used were trilinolein (LLL), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolenin (LnLnLn), and tripalmitolein (PoPoPo) of purity greater than 98% and purchased from Sigma (St. Louis, MO). The abbreviations used for the fatty acids were Po for palmitoleic, L for linoleic, Ln for linolenic, O for oleic, P for palmitic, S for stearic, and A for arachidic.

α-Tocopherol. Tocopherols were evaluated following the AOCS method Ce 8-89 (*20*). A solution of oil in hexane was analyzed on an Agilent Technologies HPLC (1100 series) with a silica gel Lichrosorb Si-60 column (particle size of 5 μm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain). Compounds of interest were eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 mL/min. A fluorescence detector (Thermo-Finnigan FL3000) was used with excitation and emission wavelengths set at 290 and 330 nm, respectively.

Statistical Analysis. The results are reported as the means ± standard deviation (SD) of independent measurements. In addition, Duncan's multiple range tests were used to determine significant differences among the data. All collected data were submitted to hierarchical clustering analysis. The associations obtained were based on the similarity in Euclidian distances. Statistical analysis was performed using the SPSS program 13.0 for Windows (SPSS, Inc.).

RESULTS AND DISCUSSION

For all olive oil samples analyzed, the values of the physicochemical parameters evaluated (acidity ≤ 0.8%, peroxide index ≤ 20 mequiv of O_2 kg^{-1}, $K_{270} \leq 0.22$, and $K_{232} \leq 2.5$) (**Table 1**) fell within the ranges established for the highest quality category of "extra virgin" olive oil, as stated by Regulation EC/1989/2003 (*21*). This is not surprising because the raw material was carefully selected, picked, and processed. These results show that cultivar had no significant influence on these analytical parameters, which are indicative of damage inflicted upon the fruits (e.g., olive fly attacks or improper systems of harvesting, transport, and storage of olives) (*9*).

The fatty acid composition has previously been used as a parameter for oil classification (*22*, *23*) because of its importance in the description and determination of adulteration. There is a relatively wide range of fatty acid compositions during the ripening of the olives at harvest because of genetic and environmental factors that exist during the development of the fruit.

The fatty acids and their levels in the analyzed oils are shown in **Table 2**. Palmitic acid (C16:0) is the major saturated fatty acid in olive oil and its content ranged between 13.22 and

Table 1. Means and Standard Deviations for the Analytical Parameters Evaluated in the Olive Oil Samples Studied[a]

components	olive varieties						EVOO (EEC)
	Chétoui	Jarboui	Ain Jarboua	Neb Jmel	Rekhami	Regregui	
free acidity (% C18:1)	0.22 ± 0.07 a	0.22 ± 0.09 a	0.28 ± 0.08 a	0.25 ± 0.10 a	0.23 ± 0.05 a	0.25 ± 0.09 a	≤0.8
peroxide value (mequiv of O_2 kg^{-1})	3.00 ± 1.04 a	6.08 ± 2.25 cd	5.20 ± 0.82 bcd	6.68 ± 2.78 d	4.34 ± 1.32 abc	3.66 ± 0.52 ab	≤20
K_{232}	1.84 ± 0.04 b	2.11 ± 0.06 c	1.74 ± 0.05 b	1.64 ± 0.05 b	1.40 a	1.82 ± 0.19 b	≤2.5
K_{270}	0.20 c	0.11 ± 0.01 ab	0.13 ± 0.01 b	0.11 ± 0.01 a	0.11 ± 0.01 a	0.13 ± 0.01 b	≤0.22
maturity index	1.65 ± 0.46 a	2.18 ± 0.50 ab	2.23 ± 0.41 b	2.36 ± 0.36 bc	2.90 ± 0.58 c	2.84 ± 0.37 c	
carotenoids (mg kg^{-1})	1.89 ± 0.42 ab	1.82 ± 0.46 ab	2.03 ± 0.43 c	1.37 ± 0.54 a	1.64 ± 0.34 ab	1.42 ± 0.50 a	
chlorophylls (mg kg^{-1})	3.74 ± 0.70 c	3.66 ± 0.56 c	3.53 ± 0.64 c	2.12 ± 0.68 a	2.98 ± 0.29 bc	2.51 ± 0.59 ab	
phenols totaux (mg kg^{-1})	321.68 ± 16.26 d	189.57 ± 18.17 b	253.7 ± 3.2 c	260.7 ± 4.9 c	210.4 ± 5.0 b	149.9 ± 11.5 a	
o-diphenols (mg kg^{-1})	33.17 ± 0.24 d	14.62 ± 0.63 a	27.05 ± 0.31 c	25.63 ± 2.66 c	19.36 ± 0.24 b	11.33 ± 3.13 a	
α-tocopherols (mg kg^{-1})	362.93 ± 6.19 e	267.75 ± 4.7 c	269.74 ± 0.3 c	231.85 ± 1.72 a	304.72 ± 0.78 d	250.31 ± 0.72 b	
oxidative stability (h)	68.49 ± 4.92 c	45.94 ± 19.95 ab	43.06 ± 9.50 ab	48.40 ± 6.08 b	30.45 ± 3.70 a	32.46 ± 6.54 a	

[a] Data are means of 18 independent samples ± SD. Different letters for the same quality parameter indicate significant differences among varieties ($p < 0.05$). EVOO = extra virgin olive oil. All analyses were carried out within 7 days after production. Samples were stored in amber glass bottles at +4 °C.

Table 2. Fatty Acid and Triacylglycerol Compositions (%) of Olive Oil Samples Studied[a]

fatty acids and triacylglycerols (%)	olive varieties					
	Regregui	Rekhami	Jarboui	Neb Jmel	Chetoui	Ain Jarboua
C16:0[b]	15.36 ± 1.05 a	14.86 ± 2.18 a	14.34 ± 2.44 a	15.42 ± 2.16 a	13.22 ± 2.49 a	14.43 ± 2.09 a
C16:1	0.80 ± 0.23 b	0.64 ± 0.32 b	0.73 ± 0.17 b	1.13 ± 0.31 c	0.26 ± 0.17 a	0.93 ± 0.33 bc
C18:0	1.94 ± 0.12 a	2.38 ± 0.59 bc	2.22 ± 0.41 ab	2.58 ± 0.27 bc	2.73 ± 0.12 c	2.53 ± 0.27 bc
C18:1	62.37 ± 1.29 a	64.57 ± 5.58 a	62.93 ± 6.79 a	69.98 ± 2.06 b	70.23 ± 4.26 b	70.35 ± 1.75 b
C18:2	18.34 ± 0.55 c	16.09 ± 5.13 bc	18.40 ± 4.96 c	9.85 ± 1.10 a	12.28 ± 3.35 ab	10.42 ± 1.08 a
C18:3	0.68 ± 0.18 a	0.76 ± 0.11 a	0.82 ± 0.12 a	0.65 ± 0.11 a	0.76 ± 0.18 a	0.81 ± 0.12 a
C20:0	0.45 ± 0.04 a	0.40 ± 0.14 a	0.38 ± 0.09 a	0.50 ± 0.05 a	0.46 ± 0.06 a	0.48 ± 0.04 a
C18:1/C18:2	3.40 ± 1.35 a	4.54 ± 0.81 ab	3.72 ± 0.16 a	7.18 ± 0.70 c	6.22 ± 2.00 bc	6.81 ± 2.40 c
IV	87.49 ± 4.84 ab	96.1 ± 2.48 c	83.46 ± 1.98 a	82.28 ± 2.46 a	86.82 ± 3.55 ab	92.17 ± 1.03b bc
LLnLn[c]	0.36 ± 0.003 a	0.35 ± 0.03 a	0.50 ± 0.02 bc	0.44 ± 0.07 ab	0.59 ± 0.003 c	0.88 ± 0.004 d
LLLn	0 a	0.06 ± 0.01 b	0 a	0 a	0 a	0 a
LLL	0.62 ± 0.006 b	1.38 ± 0.05 c	0.27 ± 0.02 a	0.18 ± 0.04 a	0.20 ± 0.01 a	0.21 ± 0.01 a
OLLn	0.36 b	0.44 ± 0.03 c	0.32 ± 0.02 ab	0.31 ± 0.001 ab	0.29 a	0.29 ± 0.02 a
PLLn	0.14 ± 0.01 ab	0.17 ± 0.03 c	0.10 ± 0.04 ab	0.10 ± 0.01 ab	0.08 b	0.07 ± 0.01 a
LLO	6.13 ± 0.01 e	8.78 ± 0.14 f	4.19 ± 0.04 d	2.81 ± 0.05 a	3.59 ± 0.07 c	3.23 ± 0.005 b
OLnO	3.13 ± 0.07 c	5.31 ± 0.04 d	2.17 ± 0.01 b	1.96 ± 0.05 a	1.92 ± 0.07 a	2.06 ± 0.01 ab
PLL	0.44 ± 0.02 a	0.49 ± 0.03 ab	0.52 ± 0.03 b	0.53 ± 0.01 b	0.42 ± 0.03 a	0.55 ± 0.01 b
OLO	18.54 ± 0.11 e	18.16 ± 0.03 d	18.51 ± 0.01 e	16.30 ± 0.08 a	17.92 ± 0.05 c	16.85 ± 0.01 b
PLO plus SLL	12.05 ± 0.04 d	14.07 ± 0.15 e	8.71 ± 0.02 c	7.63 ± 0.06 b	7.22 ± 0.01 a	7.76 ± 0.06 b
PPL	1.71 ± 0.03 b	2.59 ± 0.11 c	0.91 ± 0.04 a	0.35 ± 0.41 a	0.56 ± 0.02 a	0.70 ± 0.10 a
OOO	25.54 ± 0.002 b	21.70 ± 0.05 a	34.07 ± 0.05 c	39.37 ± 0.04 f	39.03 ± 0.05 e	37.23 ± 0.07 d
POO	22.01 ± 0.01 f	18.84 ± 0.07 a	20.99 ± 0.02 d	20.65 ± 0.05 c	19.48 ± 0.01 b	21.54 ± 0.01 e
PPO	4.43 ± 0.04 e	3.96 ± 0.06 d	3.11 ± 0.08 c	2.87 ± 0.04 b	2.35 ± 0.01 a	2.88 ± 0.02 b
PPP	0.53 ± 0.05 a	0.33 ± 0.06 a	0.46 ± 0.06 a	0.45 ± 0.07 a	0.61 ± 0.11 a	0.54 ± 0.12 a
SOO	3.03 ± 0.01 b	2.56 ± 0.08 a	4.10 ± 0.03 c	4.76 ± 0.05 d	4.75 ± 0.12 d	4.22 ± 0.13 c
SLS plus POS	0.99 ± 0.07 b	0.78 ± 0.07 a	1.06 ± 0.04 b	0.98 b	0.96 ± 0.05 ab	0.95 ± 0.04 ab

[a] Data are means of 18 independent samples ± SD. Different letters for the same quality parameter indicate significant differences among varieties ($p < 0.01$). [b] C16:0, palmitic; C16:1, palmitoleic; C18:0, stearic; C18:1, oleic; C18:2, linoleic; C18:3, linolenic; C20:0, arachidic; IV, iodine value. [c] LLL, 1,2,3-trilinoleylglycerol; LLO, 1,2-dilinoleyl-3-oleylglycerol; OOLn, 1,2-dioleyl-3-linolenoylglycerol; PLL, 2,3-dilinoleyl-1-stearoylglycerol; OOL, 1,2-dioleyl-3-linoleyglycerol; POL, palmitoyl-2-oleyl-3-linoleylglycerol; SLL, 2,3-dilinoleyl-1palmitoylglycerol; OOO, 1,2,3-trioleylglycerol; POO, 2,3-dioleyl-1-palmitoylglycerol; PPO, 1,2- dipalmitoyl-3-oleylglycerol; PPP, 1,2,3-tripalmitoylglycerol; SLS, 1,3-distearoyl-2-linoleylglycerol; SOO, 2,3-dioleyl-1-stearoylglycerol; SPO, 1-stearoyl-2-palmitoyl-3-oleylglycerol.

15.42% according to cultivars. The highest percentage was observed in Neb Jmel oils (15.42%), whereas the lowest was in Chétoui oils (13.22%).

The varieties Regregui and Jarboui showed the highest linoleic (C18:2) values (18.34 and 18.40%, respectively). The oleic (C18:1) and linoleic acid contents were the most useful and significant parameters for differentiating the predominant cultivars, Chétoui, Neb Jmel, and Ain Jarboua, from the others. These three cultivars showed high oleic and low linoleic values, unlike the others.

The ratio of oleic/linoleic acids (C18:1/C18:2) has the most marked relationship with stability and was used in the characterization of olive oil cultivars (*24*). The linoleic acid content and the ratio of oleic/linoleic acids were used to detect mixtures of virgin olive oil with 5–10% refined seed oils.

The contents of the other fatty acids, including palmitoleic (C16:1), stearic (C18:0), linolenic (C18:3), and arachidic (C20:0), changed from one olive oil to another, but the changes were fairly small.

Results show that the distribution of fatty acid composition (**Table 2**) covered the normal ranges expected for olive oil (EEC), and significant differences are also observed between analyzed cultivars ($p < 0.01$). These findings are in good agreement with those of other authors working on Tunisian olive oil varieties, such as Sayali, Gerboui, and Zalmati (*25*).

Triacylglycerol composition has also been established as a measurement of the quality and purity of vegetable oils. Because of the specificity of the composition in different kinds of fats and oils, it is increasingly used in the food industry to confirm authenticity, despite the laborious nature of the analysis.

Table 3. Sterol and Triterpenic Dialcohol Compositions of Studied Olive Oil Samples (Results Expressed as a Percentage of Total Sterols)[a]

components	olive varieties						EVOO (EEC)
	Chétoui	Jarboui	Ain Jarboua	Neb Jmel	Rekhami	Regregui	
cholesterol	0.13 ± 0.02 bc	0.08 ± 0.01 a	0.10 ± 0.003 ab	0.07 ± 0.004 a	0.14 ± 0.01 c	0.10 ± 0.01 ab	≤0.5
24-M-cholesterol	0.30 ± 0.01 d	0.14 ± 0.01 b	0.20 ± 0.001 c	0.13 ± 0.01 b	0.28 ± 0.01 d	0.05 ± 0.01 a	
campesterol	2.59 ± 0.01 a	2.89 ± 0.01 d	2.76 ± 0.003 c	2.88 ± 0.002 d	2.62 ± 0.02 a	2.65 ± 0.01 b	≤4
β-sitosterol	77.975 ± 0.011 a	85.24 ± 0.08 d	82.03 ± 0.10 b	85.35 ± 0.01 d	83.00 ± 0.43 c	90.24 ± 0.25 e	
sitostanol	0.27 ± 0.02 ab	0.22 ± 0.01 a	0.33 ± 0.01 bc	0.22 ± 0.001 a	0.48 ± 0.02 d	0.38 ± 0.032 c	
avenasterol	15.42 ± 0.01 e	8.15 ± 0.02 b	11.45 ± 0.04 d	8.14 ± 0.01 b	10.550 ± 0.413 c	3.79 ± 0.14 a	
campestanol	0.08 ± 0.01 bc	0.04 ± 0.004 a	0.09 ± 0.003 bc	0.05 ± 0.001 a	0.10 ± 0.01 d	0.06 ± 0.01 ab	
estigmasterol	1.12 ± 0.01 d	0.56 ± 0.01 a	0.97 ± 0.004 c	0.55 ± 0.002 a	0.64 ± 0.04 b	0.71 ± 0.03 b	campesterol
clerosterol	0.99 ± 0.01 a	1.01 ± 0.09 a	0.96 ± 0.02 a	0.95 ± 0.01 a	1.06 ± 0.10 a	0.96 ± 0.02 a	
Δ5,24-estigmastadienol	0.69 ± 0.01 c	0.69 ± 0.003 c	0.64 ± 0.02 c	0.68 ± 0.003 c	0.48 ± 0.01 b	0.23 ± 0.02 a	
Δ7-estigmastenol	0.14 ± 0.02 a	0.24 ± 0.01 b	0.14 ± 0.02 a	0.24 ± 0.01 b	0.34 ± 0.02 c	0.28 ± 0.03 bc	≤0.5
Δ7-avenastenol	0.31 ± 0.01 a	0.75 ± 0.01 c	0.33 ± 0.001 a	0.74 ± 0.004 c	0.32 ± 0.01 a	0.53 ± 0.06 b	
sitosterol apparent[b]	95.33 ± 0.03 a	95.31 ± 0.01 a	95.41 ± 0.02 ab	95.33 ± 0.02 a	95.57 ± 0.09 bc	95.62 ± 0.09 c	≥93
total sterol mg/kg	1287.90 ± 3.28 a	1964.28 ± 21.75 c	1600.24 ± 62.20 b	2047.98 ± 10.45 c	1661.98 ± 81.60 b	2291.95 ± 94.20 d	≥1000
erythrodiol	1.25 ± 0.03 a	1.15 ± 0.01 a	2.16 ± 0.05 d	1.17 ± 0.03 a	1.70 ± 0.01 c	1.50 ± 0.10 b	
uvaol	0.52 ± 0.03 c	0.24 ± 0.03 a	0.44 ± 0.002 bc	0.25 ± 0.01 a	0.81 ± 0.07 d	0.32 ± 0.003 ab	
erythrodiol plus uvaol	1.76 ± 0.003 b	1.38 ± 0.04 a	2.60 ± 0.05 c	1.42 ± 0.02 a	2.51 ± 0.09 c	1.81 ± 0.10 b	≤4.5

[a] Different letters for the same quality parameter indicate significant differences among varieties ($n = 18$; $p < 0.01$). EVOO = extra virgin olive oil. [b] Apparent β-sitosterol = β-sitosterol + Δ5-avenasterol + clerosterol + sitostanol + Δ5,24-stigmastadienol.

The mean values of triacylglycerols (TAGs) for the analyzed oils are also shown in **Table 2**. Nine triacylglycerols (in order of most representative to least), OOO, POO, OLO, PLO plus SLL, OLL, PPO, SOO, and OLnO, accounted for ~95% of the total triacylglycerols. A total of 10 other triacylglycerols, LLL, LLnLn, LLLn, PLL, PPP, PPL, PLLn, OLLn, and SLS plus POS, were also observed in all samples, except for LLLn, which was observed in only the Rekhami oil sample.

Triacylglycerol composition varies greatly as previously described for fatty acids. For example, OOO varies between 21.70 and 39.37%, POO varies between 18.84 and 22.01%, OOL varies between 16.30 and 18.54%, POL plus SLL varies between 7.22 and 14.07%, SOO varies between 5.74 and 6.42%, and LLO varies between 2.81 and 8.78% (**Table 2**). The presence of a high 1,2,3-trioleylglycerol (OOO) level in olive oil constitutes a favorable authenticity indicator.

Again, statistical differences were found between cultivars in terms of TAG content, and these results agree with those found for fatty acid composition. The obtained results showed that triacylglycerol composition was the most useful parameter for discriminating the cultivars ($p < 0.01$).

The amount of phenolic compounds is an important factor when evaluating the quality of virgin olive oil because of their involvement in resistance to oxidation and the sharp bitter taste of the oil. The research conducted on olive oil chemical composition highlights that the polyphenols are remarkably variable according to the variety, the agronomic conditions, the state of ripeness, and the technology of conservation (*17, 26*). As shown in **Table 1**, the amounts of total phenols and *o*-diphenols in the analyzed oils show significant differences ($p < 0.05$) according to the cultivars. Chétoui oil showed the highest values for phenols and *o*-diphenols (321.68 and 33.17 mg kg^{-1}, respectively) (**Table 1**).

As suggested by several authors, the tocopherol fraction in virgin olive oils consists mainly of α-tocopherol; these substances exert both vitamin potency and antioxidant properties. These natural antioxidant compounds, which provide stability for the oils, vary from one edible vegetable oil to another.

The Tunisian olive oil samples have various α-tocopherol amounts, ranging from 231.85 mg kg^{-1} (Neb Jmel variety) to 362.93 mg kg^{-1} (Chétoui variety) (**Table 1**). Duncan's test showed significant ($p < 0.05$) differences in the α-tocopherol content between the studied cultivars. Similar results have been found by other authors (*5, 7, 27*), suggesting that tocopherol content is highly cultivar-dependent (*5, 28*).

The presence of natural pigments (chlorophylls and carotenoids) is relevant to the product with regards to technological characteristics and product stability. The obtained results show that both chlorophylls and carotenoids vary in a similar way between cultivars. The maximum pigment concentrations were observed in Chétoui oil, while Neb Jmel and Regregui oils showed the lowest mean values of carotenoids and chlorophylls, respectively (**Table 1**). Significant differences between cultivars ($p < 0.05$) were also observed in pigment contents.

It is known, in fact, that these substances also have biological and health properties (*8*) and occur in the oil at concentrations that usually correlate with those of phenols and volatiles (*29*). The concentrations of these pigments are a function of several variables, such as olive cultivar, fruit ripeness, the soil and climate conditions, and agronomic and technological procedures (*17, 30, 31*). Moreover, olive oils that are green in color are often appreciated by consumers.

The oxidative stabilities of the oils, measured as the induction time determined using the Rancimat method, are shown in **Table 1**. The oxidative stability time values were highly variable among the samples analyzed (30.45–68.49 h). The oils from Regregui and Rekhami had lower stabilities compared to the other oil cultivars. The highest stability value was detected in Chétoui oil, which can be explained by its richness in phenols, *o*-diphenols, and α-tocopherol (**Table 1**). Virgin olive oil provides a rich source of natural antioxidants. These include carotenoids and phenolic compounds that confer an effective defense system against free-radical attack that may act by different mechanisms.

Sterols, which comprise a major portion of the unsaponifiable matter, are found in almost all fats and oils and are also characteristic of the purity of vegetable oils. The composition of the steroidal fraction of the olive oil is a very useful parameter for detecting adulterations or verifying authenticity, because it can be considered as a distinct fingerprint (*32*).

Table 3 reports the value limits of sterol and triterpenic dialcohol compositions suggested by the European Union (EU) and the International Olive Oil Council (IOOC) and the corresponding values found in the extra virgin olive oils analyzed.

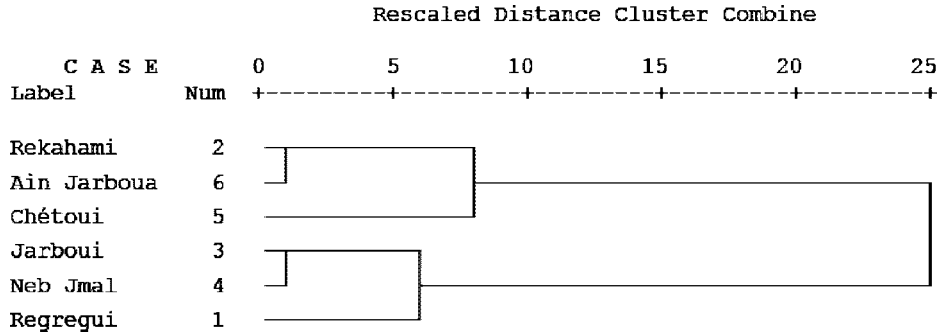

Figure 1. Clustering of the studied olive oil samples using all of the collected data.

Results show that the main sterols found in all of the olive oils studied were β-sitosterol, Δ5-avenasterol, and campesterol. β-Sitosterol is the most abundant sterol in olive oil and has a recognized effect on lowering cholesterol levels by opposing the absorption of cholesterol in the intestinal tract (*33*). Thus, the Regregui cultivar showed a high level of β-sitosterol (90.24%) and a low level of Δ5-avenasterol (3.78%). The Δ5-Avenasterol content for the Chétoui cultivar was significantly higher than the values for the other cultivars. The health aspects of β-sitosterol, the major sterol of olive oil, have also been reported in several studies (*34*).

Moreover, the campesterol content varied from one cultivar to another, but its level did not exceed the upper limit established by the EU regulation (4%). The percentages of stigmasterol in all tested samples were lower than those of campesterol, which shows that all of the oil samples came from healthy fruits that were not obtained by forcing systems (*35*). Thus, the stigmasterol content for Chétoui was significantly higher than the values of other cultivars.

The Δ5,24-stigmastadienol content for the Regregui cultivar (0.23%) was significantly lower than the values for the other varieties. Cholesterol and Δ7-stigmastenol were quantified and compared to other authenticity indices established by the current legislation (*21*). Hence, in all cultivars, the individual percentages were below the established limit of 0.5%.

On the other hand, the apparent β-sitosterol, expressed by the sum of the contents of β-sitosterol and the other four sterols formed by the degradation of β-sitosterol (sitostanol, Δ5,24-stigmastadienol, clerosterol, and Δ5-avenasterol), was higher than the minimum established (93%) in all oil samples. The highest sitostanol content was found in the Rekhami sample.

Concerning total sterols, all of the monovarietal oils studied contained more than 1000 mg kg^{-1}, the minimum value established by the EU regulation for the "extra virgin" olive oil category (**Table 3**). Regregui oil showed the highest value (2291.95 mg kg^{-1}), while Chétoui had the lowest value (1287.90 mg kg^{-1}). Significant differences between cultivars were also observed in total sterol contents. Δ5-Avenasterol, stigmasterol, and total sterol contents were useful only to discriminate Chétoui from the remaining cultivars. β-Sitosterol, apparent sitosterol, and total sterol contents were useful only to discriminate Regregui from the remaining cultivars, because it had the highest amounts of these sterols. It is well-known that sterol composition can be used to identify adulteration of olive oil, and it has recently been suggested that it may be used to classify virgin olive oils according to their fruit variety (*24*, *36*).

The triterpenic dialcohols, which are also part of the unsaponifiable fraction of the olive oil, are usually analyzed together with the sterol fraction. Their content in extra virgin olive oil must not exceed 4.5% of total sterols (*12*), because higher values would indicate blending with olive−pomace oil. For all samples analyzed, the sum of erythrodiol and uvaol was within the established limit for the "extra virgin" olive oil category and there was a clear difference between analyzed oils ($p < 0.01$).

The health aspects of phytosterols and triterpenoids have also been reported by Sánchez-Perona et al. (*1*), who suggested that these components have anti-inflammatory and vasorelaxant effects, respectively. This study showed that the olive cultivar significantly affected the sterol and triterpenic dialcohol contents of all six samples analyzed but not the qualitative compositions of these unsaponifiable fractions.

All of the collected data was submitted to hierarchical clustering analysis, which was capable of distinguishing between the six olive cultivars. This technique produced a hierarchy of partitions such that any cluster of a partition is fully included in one of the clusters of the later partitions. Such partitions are best represented by a dendrogram (binary tree).

The dendrogram produced by the hierarchical clustering analysis is shown in **Figure 1** and indicates that, at a rescaled distance of 5, the samples are distributed into four major clusters. The first group consists of two oil samples, Rekhami and Ain Jarboua; the second group is composed of the Chétoui oil sample; the third group consists of the Jarboui and Neb Jmel oil samples; and the fourth group includes Regregui oil. At a rescaled distance of 10, the monovarietal olive oils analyzed are distributed into two major clusters: one group consists of the Rekhami, Ain Jarboua, and Chétoui oil samples, while the second group includes the remaining samples, Jarboui, Neb Jmel, and Regregui. This study solely addressed the influence of cultivar specific factors, because the harvesting period and extraction conditions were similar for all of the samples studied.

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