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Influence of Olive Maturity Stage and Geographical Origin on Some Minor Components in Virgin Olive Oil of the Chemlali Variety

AIDA LAZZEZ,[†] ENZO PERRI,*[,‡] MARIA ANNA CARAVITA,[‡] MOHCEN KHLIF,[†] AND MONCEF COSSENTINI[§]

Institut de l'Olivier de Sfax, BP 1087, 3000 Sfax, Tunisie, CRA Experimental Institute for Olive Growing, C.da Li Rocchi, 87036 Rende (CS), Italy, and Institut Préparatoire aux Etudes d'Ingénieurs de Sfax, BP. 805,3000 Sfax, Tunisie

Minor compounds such as sterols, aliphatic alcohols, tocopherols, and chlorophylls in virgin olive oil from Chemlali cultivar were analyzed during the maturity process. The study concerns oils from the following three different olive growing areas of Tunisia: Sfax, Sidi Bouzid, and Enfidha. Analytical results showed that both the maturity process and the olive provenances influence the evolution of the content of these compounds.

KEYWORDS: Sterols; aliphatic alcohols; chlorophylls; tocopherols; provenance

INTRODUCTION

Olive oil occupies an important place in the Mediterranean diet due to its nutritional and dietetic qualities. Indeed, the acidic composition of olive oil gives a very good balance between saturated, mono, and poly unsaturated fatty acids that confer to it a much appreciated nutritional characteristic. The importance accorded to olive oil is not due to the presence of fatty acids only, but also to the richness of this product in minor components such as chlorophylls, polyphenols (*1–4*), and tocopherols, which are implied in the mechanisms of oil oxidation and which preserve the oil quality during storage (*5, 6*).

Other minor components such as sterols and aliphatic alcohols were detected in olive oil. Mariani et al. (*7*) and Vichi et al. (*8*) reported that sterols and aliphatic alcohols could be used to characterize virgin olive oil and especially to detect the adulteration of olive oil with hazelnut oil. Recently, it has been proposed that these profiles could also be used to classify virgin olive oil according to its variety (*9, 10*).

The impact of sterols on the human health is proved by several researches. Indeed, some authors showed that a sufficient quantity of β-sitosterol inhibits the intestinal absorption of LDL-cholesterol (*11*), this is why these compounds are being considered as ingredients of functional foods (*12*).

Because of the importance of these components, several workers have showed that sterols and aliphatic alcohols could be influenced by many factors such as the agronomic (*13, 14*), geographic (*15, 13*), harvesting time (*16–18*), and technological factors (*19, 20*). They have also proved that antioxidant compounds and especially the total polyphenols show a great variability depending on various factors such as cultivars (*21, 22*), geographical sites (*23*), climate, environmental factors, ripeness (*24–26*), processing (*27, 28*), and oil storage.

Because the minor compounds of the Tunisian olive oil have not been studied sufficiently, we are interested in identifying and quantifying some minor components in the olive oil extracted from the Chemlali variety and in studying the impact of the geographical site and the maturity stage on their evolution.

MATERIALS AND METHODS

Olive Samples. Olive samples were taken from the Chemlali variety. Olives at different maturity stages came from three different geographical sites in Tunisia (Sfax in the south, Sidi Bouzid in the central West, and Enfidha in the central East). This choice was based on the importance of these regions in olive oil production and on the variability of pedoclimatic conditions.

Oil samples were obtained by a cold extraction process using a laboratory mill equipped with a metal crusher, a mixer, and a basket centrifuge. The oil samples were immediately stored in the dark at 0 °C until the moment of analysis. The analysis of chlorophylls and tocopherols was carried out a few days after the oil extraction. As for the analysis of sterols and alcohols, no sample was stored longer than 4 months.

Determination of Sterols and Alcohols Content. The analysis of two sterolic and alcoholic fractions extracted from olive oil was determined, respectively, according to the method adopted by EC Regulations, (EC N° 2568/91, Annexes V and VI and EC N° L 248/10, Annex IV) and their integrations and modifications.

Sterols Analysis. Five grams of olive oil were added to α-cholestanol, used as an internal standard, and saponified with potassium hydroxide in the ethanol solution. After one hour of boiling, 100 mL of water was added, and the extraction of unsaponifiable was carried out by 200 mL of diethyl ether. Twenty milligrams of unsaponifiable were dissolved in 0.5 mL of chloroform, and then deposed on a basic silica gel plate. The elution was achieved by a mixture of hexane and diethyl

* Corresponding Author. E-mail: enzo.perri@entecra.it.
[†] Institut de l'Olivier de Sfax.
[‡] CRA Experimental Institute for Olive Growing.
[§] Institut Préparatoire aux Etudes d'Ingénieurs de Sfax.

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Table 1. Sterol Composition (mg/kg) of Chemlali Olive Oil from the Region of Sfax during the Maturity Stage

	September[a]	October	November	December	January	February
cholesterol	2.2 ± 0.4 a	1.9 ± 0.5 a	2.3 ± 0.4 a	1.7 ± 0.6 a	2.5 ± 0.8 a	1.8 ± 0.3 a
24-methylene cholesterol	2.7 ± 0.5 a	2.4 ± 0.3 a	2.0 ± 0.5 a	1.7 ± 0.2 a	2.4 ± 0.5 a	2.0 ± 0.3 a
campesterol	69.8 ± 4.7 b	51.1 ± 2.4 a	47.3 ± 5.0 a	46.0 ± 4.4 a	45.7 ± 3.5 a	54.0 ± 3.8 a
campestanol	0.9 ± 0.2 a	1.7 ± 0.3 a	1.2 ± 0.2 a	2.1 ± 0.5 a	3.7 ± 1.0 b	3.8 ± 0.8 b
stigmasterol	12.7 ± 1.5 b	15.5 ± 1.9 b	9.9 ± 2.7 ab	10.4 ± 3.2 ab	4.2 ± 0.6 a	8.3 ± 1.6 ab
clerosterol	12.3 ± 1.4 a	13.1 ± 1.9 a	10.8 ± 2.0 a	12.2 ± 1.4 a	12.2 ± 1.5 a	11.5 ± 0.9 a
β-sitosterol	2361.0 ± 78.3 d	1753.7 ± 25 c	1522.4 ± 35.4 b	1398.2 ± 50.2 ab	1339.1 ± 18.8 a	1385.8 ± 35.7 ab
sitostanol	7.6 ± 0.8 b	4.9 ± 0.4 ab	4.5 ± 0.3 ab	4.4 ± 0.8 a	5.4 ± 1.2 ab	5.5 ± 1.6 ab
Δ5-avenasterol	77.1 ± 3.8 a	62.2 ± 7.5 a	118.7 ± 12.9 ab	180.4 ± 15.5 bc	207.0 ± 22.9 c	200.5 ± 18.3 c
Δ5,24-stigmastadienol	14.3 ± 1.6 c	9.3 ± 1.3 b	5.4 ± 1.3a	4.2 ± 0.7 a	5.0 ± 0.9 a	4.1 ± 1.2 a
Δ7-stigmastenol	5.4 ± 0.9 a	4.0 ± 0.5 a	5.0 ± 1.1 a	5.4 ± 0.6 a	4.2 ± 0.7 a	4.8 ± 0.9 a
Δ7-avenasterol	16.2 ± 0.9 c	8.4 ± 2.0 a	6.8 ± 1.4 a	8.8 ± 2.2 a	11.1 ± 2.4 ab	5.9 ± 3.0 a
total Sterol	2580 ± 68.3 c	1924.8 ± 33.5 b	1738.9 ± 38.5 ab	1676.9 ± 65.6 a	1643.9 ± 42.3 a	1663.4 ± 61.0 a

[a] Columns followed by the same letter were not significantly different according to the multiple range Duncan test at $P = 0.05$.

ether (65/35, v/v), then the plate was pulverized by a solution of 2,7-dichlorofluoresceine (0.2% in the ethanol), and the band corresponding to sterols was scraped. Sterols recovered from the plate were dissolved in chloroform and filtered through a paper filter. The solvent was evaporated under N2, the sterols were transformed into trimethylsilyl ethers, and the mixture was analyzed by gas chromatography using a chromatograph Shimadzu set 17A equipped with a capillary column (30 m length × 0.32 mm i.d.) coated with a stationary phase formed by 5% of biphenyl and 95% of dimethyl polysiloxane (0.25 μm thickness).

The analytical conditions were as follows: type of detector: FID (flame ionization detector); vector gas: nitrogen; flow rate: 1 mL/mn; column temperature: 260 °C; injector temperature: 280 °C; detector temperature: 290 °C; and quantity injected: 5 μl.

Alcohols Analysis. Five grams of olive oil were added to 1-eicosanol as internal standard (0.1% in the ethanol), saponified with potassium hydroxide in ethanol solution, and then the unsaponifiable was extracted with ethyl ether. The alcoholic fraction was separated from the unsaponifiable by thin layer chromatography, then the plate was sprayed with the 2,7-dichlorofluoresceine, and the pink bands of the alcohols could be observed under UV light. This band was scraped off and the silica gel containing the alcohols was dissolved in chloroform and then filtered through a paper filter. The solvent was evaporated under N_2, and the alcohols were treated with a dramatizing reagent to obtain the trimethylsilyl derivatives, which were then analyzed by gas chromatography in the following conditions: The column was held at 180 °C for 8 min and then programmed to increase at 5 °C/min until 260 °C and stabilized at this temperature for 15 min.; type of detector: FID (flame ionization detector); evaporator temperature: 280 °C; detector temperature: 290 °C; gas vector: nitrogen; flow rate: 1 mL/min; quantity injected: 5 μl.

The qualitative analysis of the sterolic and alcoholic fraction was performed after the determination of the retention time of their pure compounds, which had been analyzed in the same conditions.

Determination of Chlorophylls. The chlorophylls contents in olive oil were determined according to the method of A.O.C.S. described by Wolff (*29*). Briefly, the method consists in measuring the absorbance at a different wavelengths (630, 670, and 710 nm) against a vat containing carbon tetrachloride as a control. The content of chlorophylls is given by eq 1,

$$\text{Total chlorphylls (ppm)} = (A_{670} - (A_{630} + A_{710})/2)/(0.1086L) \quad (1)$$

where L is the thickness of the vat (1 cm), and 0.1086 is the variable coefficient according to the spectrophotometer.

Tocopherols Analysis. Samples were analyzed using an Agilent 1100 (Waldbronn, Germany) HPLC equipped with FLD detector; the column was a Purospher STAR NH2 (5 mm) (VWR International, Milano, Italy). The peak integration and the quantitative calculations were performed with the relative software; the calibration curve was obtained by injecting standard solutions of tocopherol at different concentrations. The HPLC analyses were performed using a mobile phase composed of n-hexane and ethyl acetate (8:2). The flow rate was 1 mL/min; the injection volume was 20 μl of a solution obtained by diluting 600 mg of olive oil in 10 mL of n-hexane. The fluorescence detector was set as follows (lex = 295 nm, lem = 323 nm). The time of analysis was 20 min. The detection limit of HPLC analysis of tocopherols was 0.045, 0.050, 0.035, and 0.100 for α-, β-, γ- and δ-tocopherol, respectively.

Statistical Analysis. Statistical analysis was performed using the statistical package SPSS Base 11. All analyses were carried out in triplicate, and the results are presented as means ± standard deviation. Duncan test was used to compare the means and to reveal significant differences for each parameter taking maturity and the olive provenance into account.

RESULTS AND DISCUSSION

Impact of Olive Maturity Stage and Geographical Sites on Sterolic Compounds Evolution. Sterol compositions of olive oil from the three regions, Sfax, Sidi Bouzid, and Enfidha are reported, respectively, in **Tables 1**, **2** and **3**. Twelve sterolic compounds were identified in the Chemlali olive oil. These compounds were cholesterol, 24-methylene-cholesterol, campesterol, campestanol, stigmasterol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol, Δ5,24-stigmastadienol, Δ7-stigmastenol, and Δ7-avenasterol.

As **Tables 1**, **2**, and **3** show, the total sterol content of the Chemlali olive oil samples exceeded 1000 mg/Kg, which represents the threshold for extra virgin olive oil, established by International Olive Oil Council (*30*), the content ranging between 2580 and 1644 mg/Kg. The total sterols content in the oil from Enfidha did not show significant variation through the maturity stage. In fact, the tenors are practically constant and, in general, are superior to 2000 mg/Kg, except for the sample ripening in February where the content was 1850 mg/Kg. With respect to the oil from Sidi Bouzid, the content was highest between October and December and decreased at the end of maturity. In the oil of Sfax olives, the recorded data revealed that the total sterol content decreased significantly during the maturity process ($P < 0.05$). Indeed, it declined from 2580 to 1644 mg/Kg between September and January. These results were consistent with those of earlier findings (*31, 32*), suggesting that the synthesis of sterols occurred in the first stages of the fruit development, and with ripening these components became diluted as more oil was being produced.

With respect to the amount of β-sitosterol, which represents the most abundant compound in the sterolic fraction, the maximum content (about 2361 mg/Kg) was observed during maturation, whereas the lowest content was about 1340 mg/Kg.

Table 2. Sterol Composition (mg/kg) of Chemlali Olive Oil from the Region of Sidi Bouzid during the Maturity Stage

	September[a]	October	November	December	January	February
cholesterol	1.2 ± 0.8 a	2.3 ± 0.5 b	1.9 ± 0.5 ab	1.8 ± 0.4 ab	2.6 ± 0.5 b	1.4 ± 0.7 a
24-methylene cholesterol	2.0 ± 0.3 a	1.9 ± 0.4 a	1.8 ± 0.7 a	3.0 ± 1.0 a	1.6 ± 0.7 a	1.5 ± 0.4 a
campesterol	65.6 ± 4.5 b	62.4 ± 6.2 b	54.2 ± 3.2 ab	57.2 ± 3.2 ab	53.4 ± 3.6 ab	48.3 ± 3.8 a
campestanol	1.9 ± 0.5 ab	1.3 ± 0.3 a	1.3 ± 0.3 a	3.1 ± 0.4 b	2.4 ± 1.2 ab	2.3 ± 0.4 ab
stigmasterol	13.2 ± 0.9 c	9.1 ± 0.5 b	8.6 ± 1.0 b	8.0 ± 0.7 b	5.9 ± 1.1 a	6.6 ± 1.2 ab
clerosterol	6.6 ± 1.2 a	4.9 ± 0.9 a	7.6 ± 1.3 a	6.8 ± 1.9 a	5.4 ± 0.8 a	5.5 ± 1.1 a
β-sitosterol	1631.1 ± 52.3 ab	1898.5 ± 82.5 b	1763.5 ± 90.8 ab	1810.2 ± 36.0 ab	1624.0 ± 33.1 ab	1514.2 ± 72.7 a
sitostanol	5.7 ± 0.9 b	4.8 ± 0.6 ab	5.3 ± 0.5 ab	4.6 ± 0.8 ab	4.7 ± 1.0 ab	3.6 ± 0.7 a
Δ5-avenasterol	60.4 ± 9.6 a	76.3 ± 3.8 ab	111.7 ± 6.9 bc	170.0 ± 13.0 d	139.3 ± 13.8 cd	115.2 ± 12.1 bc
Δ5,24-stigmastadienol	11.6 ± 1.5 c	8.3 ± 0.8 b	5.4 ± 0.7 a	4.5 ± 0.7 a	3.1 ± 0.4 a	3.7 ± 0.4 a
Δ7-stigmastenol	5.1 ± 0.5 a	5.8 ± 1.4 a	5.5 ± 0.5 a	8.3 ± 1.8 b	6.2 ± 1.6 b	4.3 ± 0.5 a
Δ7-avenasterol	8.3 ± 0.8 a	12.0 ± 1.1 a	6.0 ± 0.7 a	13.8 ± 1.1 a	11.7 ± 1.2 a	9.4 ± 0.8 a
total sterol	1812.9 ± 52.3 ab	2088.4 ± 71.1 b	1972.8 ± 65.5 ab	2090.9 ± 47.7 b	1861.1 ± 51.6 ab	1718.5 ± 61.7 a

[a] Columns followed by the same letter were not significantly different according to the multiple range Duncan test at $P = 0.05$.

Table 3. Sterol Composition (mg/kg) of Chemlali Olive Oil from the Region of Enfidha during the Maturity Stage

	September[a]	October	November	December	January	February
cholesterol	1.3 ± 0.6 a	1.1 ± 0.6 a	1.2 ± 0.2 a	1.7 ± 0.8 a	2.1 ± 0.5 ab	2.9 ± 0.9 b
24-methylene cholesterol	2.1 ± 0.4 a	1.3 ± 0.3 a	1.8 ± 0.4 a	1.2 ± 0.6 a	1.5 ± 0.5 a	1.5 ± 0.4 a
campesterol	68.2 ± 4.5 b	62.7 ± 4.9 ab	62.0 ± 4.6 ab	64.3 ± 5.2 ab	62.6 ± 6.3 ab	55.6 ± 3.9 a
campestanol	1.2 ± 0.5 a	1.2 ± 0.4 a	1.2 ± 0.2 a	1.6 ± 0.3 a	3.1 ± 0.6 b	2.1 ± 0.4 a
stigmasterol	12.4 ± 3.1 ab	16.0 ± 3.3 b	9.5 ± 0.6 ab	11.6 ± 1.2 ab	8.7 ± 2.2 a	6.1 ± 1.0 a
clerosterol	4.6 ± 0.6 a	4.6 ± 1.2 a	4.2 ± 0.9 a	4.7 ± 1.5 a	6.3 ± 0.8 a	5.7 ± 1.0 a
β-sitosterol	1987.2 ± 25.0 b	1902.2 ± 77.7 b	1803.3 ± 62.8 b	1901.4 ± 57.7 b	1906.5 ± 45.3 b	1589.1 ± 89.6 a
sitostanol	3.0 ± 0.5 a	4.7 ± 0.6 b	5.1 ± 0.9 b	5.3 ± 1.3 b	4.8 ± 1.0 b	2.9 ± 0.8 a
Δ5-avenasterol	54.7 ± 4.7 a	59.5 ± 5.8 a	100.1 ± 8.8 b	136.3 ± 7.8 c	172.9 ± 9.2 d	158.9 ± 10.7 d
Δ5–24-stigmastadienol	9.7 ± 1.7 d	9.0 ± 1.0 cd	5.4 ± 0.6 abc	4.3 ± 0.5 ab	2.8 ± 0.6 a	7.5 ± 2.0 bcd
Δ7-stigmastenol	5.7 ± 0.9 bc	4.0 ± 0.6 a	5.0 ± 0.5 ab	6.2 ± 0.6 bc	5.4 ± 0.5 abc	7.1 ± 0.5 c
Δ7-avenasterol	9.0 ± 1.7 a	12.0 ± 2.5 a	10.9 ± 1.4 a	10.0 ± 0.9 a	8.2 ± 2.1 a	9.4 ± 2.2 a
total sterol	2159.8 ± 60.9 b	2080.1 ± 48.9 b	2011.9 ± 88.4 ab	2150.5 ± 74.2 b	2186.0 ± 87.0 b	1851.0 ± 56.3 a

[a] Columns followed by the same letter were not significantly different according to the multiple range Duncan test at $P = 0.05$.

The evolution of β-sitosterol during the maturity process in olive oil from the three regions was similar to that of total sterol content (**Tables 1**, **2**, and **3**). The statistical analysis showed a significant difference ($P < 0.05$) in the amount of β-sitosterol in relation to the maturity stage, especially in the oil from Sfax. In fact, a regular decrease from 2361 mg/Kg in September to 1339 mg/Kg in January was observed (**Table 1**). However, β-sitosterol showed no significant difference in relation to the maturity stage in the oils coming from Enfidha. The content was constant and decreased only in February. As for the oil of Sidi Bouzid, the highest content was observed in October and was followed by a decrease until the end of maturity (**Tables 2** and **3**).

Koutsaftakis et al. (*18*) proved in their study on the influence of the variety, implantation zones, and maturity stage on sterolic composition of olive oil that the Italian oils from Khania and Messara were characterized by a strong content in β-sitosterol, whereas oils of Sitia in Spain contained a low quantity of these sterolic components.

The Δ5-avenasterol is considered the second most abundant sterolic compound in olive oil after the β-sitosterol. It may reach 207 mg/Kg during the maturity process (**Tables 1**, **2**, and **3**). The main significant differences in the amount of Δ5-avenasterol ($P < 0.05$) was observed during the maturity stage. For the oils of Enfidha and Sidi Bouzid, we detected a similarity in Δ5-avenasterol evolution during the maturity process. In fact, from September to January the content in Δ5-avenasterol increased from 50 to 170 mg/Kg and then decreased at the end of maturity. This result confirms that obtained by Fiorino et al. (*17*), who proved that Δ5-avenasterol, which has a weak quantity in September, showed a more important increase afterward and reached its maximum between the end of October and the beginning of November, then decreased later on. In the oils from Sfax, the amount of Δ5-avenasterol increased during the maturity processes, between September and February, the content varying from 62 to 207 mg/Kg.

The third sterolic compound of olive oil, which represents a high content, is the campesterol. It can reach 70 mg/Kg during the process maturation. The content of this compound is always less than the 4% limit value established by the virgin olive oil norms (*30*). This result was similar to that found by Sanchez Casas et al. (*33*). In fact, they showed that campesterol was always inferior to limits in the Spanish varieties, except for the Corniche variety whose campesterol content was close to these limits and slightly superior in some cases.

During the maturity process, the content of campesterol passed from 70 to 46 mg/Kg, from 66 to 48 mg/Kg, and from 68 to 56 mg/Kg, in the oils from Sfax, Sidi Bouzid, and Enfidha, respectively. In previous studies (*13, 34*), authors have already confirmed a decrease in campesterol content during the maturity process.

No change was noticed in clerosterol content during the maturity stages ($P > 0.05$) in the oils of the three regions. Differences in the content of this compound came from the difference of provenances, thus the oil of Sfax contained the highest quantity (about 12 mg/Kg), whereas for the other regions the contents were less than 7.6 mg/Kg.

As for the stigmasterol, Garcia (*19*) and Gutierrez et al. (*31*) demonstrated a relationship between the stigmasterol and various parameters of the virgin olive oil quality. They showed that high levels were correlated with high acidity and low organoleptic quality. Koutsaftakis et al. (*20*) also showed that samples presenting low levels of these sterols came from healthy fruits

and were not obtained from forcing systems. Our samples presented low levels of this sterol and were found to conform to the norm established by the olive oil council (tenor of stigmasterol is inferior to campesterol) (*30*).

The campestanol is considered as a minor sterolic compound; all samples contained a low quantity, but we observed an increase in the tenors of this compound during maturity.

The obtained results reveal the absence of the brassicasterol in all samples; it was difficult to quantify this compound because it had a very low peak area in the chromatograms. This result had been found by other researchers, (*18, 31*), who also confirmed that the brassicasterol had a negligible presence in these virgin olive oils.

The other sterolic compounds, such as 24-methylene cholesterol, sitostanol, Δ5,24-stigmastadienol, Δ7-stigmastenol, and Δ7-avenasterol, were weak in content and did not change significantly during the maturity process and in relation to the source of olives ($P > 0.05$).

Impact of Maturity Stage and Geographical Sites on Aliphatic Alcohol Evolution. The main aliphatic alcohol components found in the Chemlali olive oil were the docosanol (C22), the tetracosanol (C24), the hexacosanol (C26), and the octacosanol (C28). Other aliphatic alcohols like the tricosanol (C23), pentacosanol (C25) and heptacosanol (C27), were detected in low or negligible content.

The quantitative analysis of aliphatic alcohols in the olive oil Chemlali revealed the abundance of the alcohol C26 in the oils of the three regions, followed by C28 and C24, whereas the C22 represented the weakest contents.

As reported in **Figure 1**, the origin of the oil affects the total aliphatic alcohol content significantly. Indeed, the oils from Enfidha had an elevated total aliphatic alcohol content, whereas those from Sfax and Sidi Bouzid had a weaker content. Also, several studies showed the impact of the geographical site on aliphatic alcohols content evolution. A similar survey that was carried out on the evolution of aliphatic alcohols in the oil of the Picholine showed that the oils of the South had the most elevated contents, which ranged between 73 and 96 mg/Kg, whereas the oil of the North had the lowest proportions with 46−55 mg/kg of oil (*10*).

When the chemlali variety was compared with the Moroccan Picholine oils, a similarity in the content of aliphatic alcohols was detected in the two varieties; total aliphatic alcohols contents varied from 46 to 96 mg/Kg between December and January (*13*). Salvador et al. (*35*) showed that the oil extracted from the Spanish variety Cornicabra contained 130 mg/kg of total aliphatic alcohol.

In the present study, all the samples represent high levels in the first stages of maturity (September). They reach 267 mg/kg in the oil of Sfax and 308 and 317 mg/kg in Sidi Bouzid and Enfidha oils, respectively. During the olive maturation, a decrease in the total aliphatic alcohol content was observed; in fact, it decreased progressively to 70 mg/Kg in January. At the end of the maturity process, the content in total aliphatic alcohols tended to increase and exceeded 100 mg/Kg in February in both Sidi Bouzid and Enfidha oils (**Figure 1**).

Figure 2 shows the evolution of the alcohol C28 during the maturity process. For the three regions, a clear relationship between the content in C28 and the harvest period was observed. The content of C28 decreased during the maturity process; indeed, the high value of 100 mg/Kg was detected in September and decreased to 20 mg/Kg in February.

The obtained results also showed a progressive reduction in the alcohol C26 during the maturity stage; in the oil from

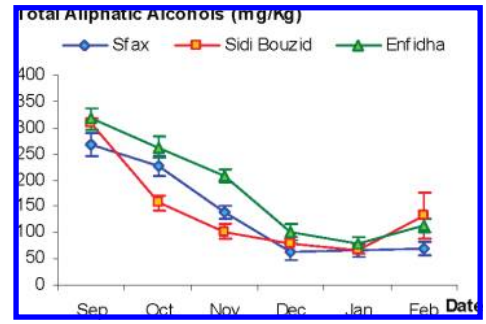

Figure 1. Evolution of total aliphatic alcohols during maturity.

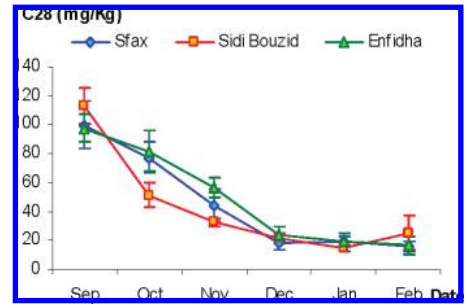

Figure 2. Evolution of alcohol C28 during maturity.

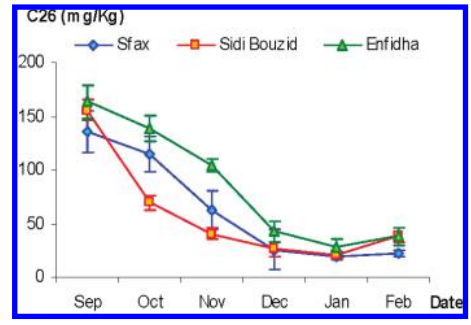

Figure 3. Evolution of alcohol C26 during maturity.

Sfax, the tenor of C26 was about 135 mg/Kg at the beginning of maturity, and it decreased to 22 mg/Kg in February. The same variation was recorded in the oils of the two other regions. Indeed, the tenor in C26 passed from 155 to 21 mg/Kg and from 164 to 28 mg/Kg in the oils from Sidi Bouzid and Enfidha, respectively, from September to January. Then, the tenor increased to 39 mg/Kg in the last period (**Figure 3**).

It was quite observable that the decrease in total aliphatic alcohols and in the contents of C28 and C26 was more pronounced in the oils from Sidi Bouzid than in those of the two other regions.

As for the alcohol C24, the content of this compound was less important than those of C26 and C28. In most samples, C24 content was higher than 20 mg/Kg, according to the results found in the aliphatic alcohol of the three regions. We detected a sensible variation in the content of the C24 during the maturity stages that decreased and then increased at the end of maturity (**Figure 4**).

With respect to the content of C22, all samples showed a tenor inferior to 20 mg/Kg, and the oil from Sfax contained the lowest quantity that varied from 5 mg/Kg in September to 9 mg/Kg in February. The oils from Sidi Bouzid and Enfidha contained the highest tenors. At the end of the maturity process the tenor of this alcohol tended to increase (**Figure 5**).

Impact of the Maturity Stage and of the Geographical Sites on Chlorophylls Evolution. Olive oil contains chloro-

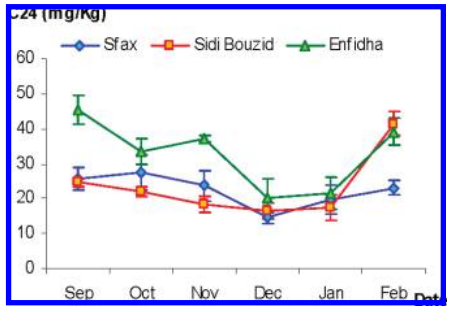

Figure 4. Evolution of alcohol C24 during maturity.

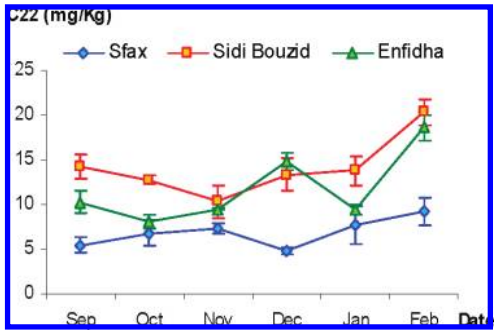

Figure 5. Evolution of alcohol C22 during maturity.

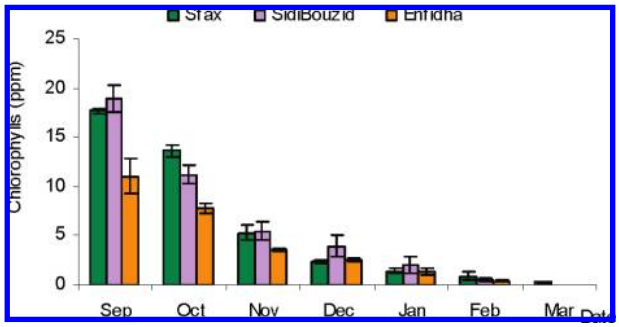

Figure 6. Evolution of total chlorophylls during maturity.

phylls a and b and pheophytins a and b. These pigments are responsible for the olive oil color, which is the basic attribute for evaluating olive oil sensorial quality. Furthermore, these compounds are involved in the auto-oxidation and photo-oxidation of the oil during storage (*36, 37*).

Figure 6 shows that the total amount of chlorophylls did not exceed 20 ppm during the maturity process. It ranged between 19 and 0.2 ppm between September and March in the Chemlali single cultivar olive oils from the three concerned geographical sites.

During the maturity process, a progressive decrease was detected in the total chlorophylls content. This result confirms what has already been found by other authors (*38*). **Figure 6** proves the influence of geographical sites on the total chlorophylls content. Indeed, the Chemlali olive oil from Enfidha contains the lowest tenors in chlorophylls, whereas the highest tenors were recorded in the oil from the two other regions, Sfax and Sidi Bouzid. This result was consistent with that of Temime et al. (*23*), who showed a great variability in the total chlorophylls extracted from the olive oil Chetoui from different geographical sites.

Impact of the Maturity Stage and of the Geographical Sites on Tocopherols Evolution. The tocopherols, or Vitamin E, are important components in the olive oil because they contribute to its oxidative stability during storage and to its

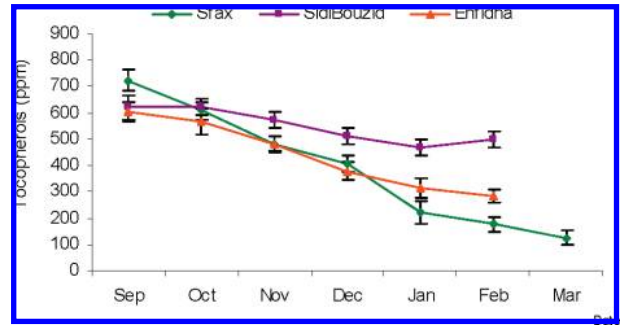

Figure 7. Evolution of total tocopherols during maturity.

nutritional quality. The major tocopherol compound present in the olive oil is the α-tocopherol, which represents 98% of total tocopherols.

Rahmani et al. (*39*) showed that the protective effect of α-tocopherol is more important when the concentration of this compound is very high. This protective effect appears only in the first hours of the photo-oxidation, because it is quickly decomposed.

Results showed that total tocopherols content was influenced by the maturity stage (**Figure 7**) in the olive oil Chemlali, which ranged from 722 to 125 ppm, from 620 to 499 ppm, and from 606 to 283 ppm in Sfax, Sidi Bouzid, and Enfidha oils, respectively. This finding indicates that the Chemlali olive oil represents a considerable amount of tocopherols as compared with other Tunisian varieties. Indeed, Temime et al. (*40*) found that tocopherols contents in Chetoui oil from different geographical sites varied from 341 to 405 ppm.

Figure 7 shows a regular decrease in total tocopherols content during the maturity process in the Chemlali olive oil variety from the three concerned regions. But this decrease differed according to the region; the decline of the total tocopherols content in the oil of Sidi Bouzid was less evident than in the oils of the two other regions.

According to Rahmani et al. (*39*), the content of tocopherols in olive oil is variable (5–300 ppm). Generally, it is greater than 100 ppm in the oils of good quality and depends mainly on the variety (*41*). Other groups of researchers (*42, 43*) revealed that the mean content of tocopherols in olive oil ranged between 100 and 300 ppm, depending on the variety, the maturity stage of the olive, and its conservation conditions.

In conclusion, analytical data demonstrated that single cultivar virgin olive oil of the Chemlali variety had excellent nutritional characteristics in terms of sterol, aliphatic alcohol, and tocopherol content. The reported results also demonstrated that the influences of the olive maturity stage and cultivation place are very important for the chemical composition of the olive oils. As was expected, during the maturity process a significant decline was recorded in the above minor components, especially in the oil from Sfax. However, although a similar trend in the content of total sterol and aliphatic alcohols of virgin oils from Enfidha and Sidi Bouzid regions was observed, the content of these compounds in the oil from Sfax showed a different evolution. In particular, the sterol and aliphatic alcohol content of the olive oil from Enfidha and Sidi Bouzid regions was higher. The observed variability of the minor components may foster the assessment of the characteristics of typicalness of the corresponding olive oils. These results will be useful to characterize the Chemlali cultivar olive oils from each region. For this reason, the chemometric analysis of analytical data will be explored.

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