Pedologic Factors Affecting Virgin Olive Oil Quality of “Chemlali” Olive Trees (*Olea europaea* L.)

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Abstract: The aim of this study examined the characterization of extra virgin olive oil samples from the main cultivar Chemlali, grown in five olive orchards with different soil type (Sandy, Clay, Stony, Brown, Limestone and Gypsum). Volatile compounds were studied using headspace-solid phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) technics. Moreover, the sterol profile was established using gas chromatography-mass spectrometry. 35 different volatile compounds were identified: alcohols, esters, aldehydes, ketones and hydrocarbons. The chemical composition of the volatile fraction was characterized by the preeminence of 2-hexenal (32.75%) and 1-hexanol (31.88%). Three sterols were identified and characterized. For all olive oil samples, β -sitosterol (302.25 mg/kg) was the most abundant sterol. Interestingly, our results showed significant qualitative and quantitative differences in the levels of the volatile compounds and sterols from oils obtained from olive trees grown in different soil type.

Key words: Chemlali cultivar, extra virgin olive oil, soil, SPME, sterols, volatile compounds

1 Introduction

Extra virgin olive oil (EVOO) represents the principal source of noble fats in the Mediterranean basin as it shows positive effects on human health, in particular to prevent breast and colon cancers, and as regards diabetes accompanied by inflammatory and autoimmune diseases¹. The protective role of EVOO is the result of its specific composition including a high proportion of monounsaturated fatty acids (oleic acid), a balanced presence of polyunsaturated fatty acids and minor components, such as tocopherols, phenolic compounds, sterols and flavors².

EVOO has characteristic aromatic notes that distinguish it from other edible vegetable oils. Flavors of virgin olive oil are generated by a number of volatile constituents present at extremely low concentrations.

Unsaponifiable fraction of olive oil is mainly represented by phytosterols, exerting appreciable effects on health due to their anticarcinogenic and hypocholesterolemic properties³.

The Mediterranean area contributes to more than 95% of the olive oil worldwide production, with a percentage of 75% coming from European Union countries, primarily Italy, Spain and Greece⁴. Tunisia is the second country

after the European Union in the olive oil exportation. The variety ‘Chemlali’ contributes to 80% of the Tunisia national olive oil production and covers a wide geographical area where a wide range of edapho-climatic conditions are prevailing⁵. The quality and the particularity of the olive oil depend on several factors among those the cultivar is the main one. Thus, monovarietal olive oils are influenced by different factors clustered into four groups: environmental (climate), agronomic (irrigation and fertilization), cultivation (harvesting and ripeness) and technological factors (post-harvest storage and extraction system)⁶. Furthermore, olive oil classification according to its volatile composition has been considered to be an important tool for the monitoring of adulteration⁷. Several researches correlate the volatile composition of olive oil to varieties, geographical origin, and year of harvest^{8–10}. Other studies were performed to correlate the sterolic fraction to the olive ripening degree¹¹, agronomic and climatic conditions¹², geographical origin and cultivars^{13,14}.

Despite being the main Tunisian olive variety, there is a lack of information about the effects of the type of the soil of the growing area on the quality of olive oil produced and its volatile compounds and sterols composition. Therefore,

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the aim of this work was to attest the influence of soil type of growing area on volatile and sterolic composition of Chemlali extra virgin olive oil, as on other chemical parameters (free acidity, peroxide value, antioxidant capacity, total phenolic content) and consequently on its quality.

2 Materials and methods

2.1 Oil sample extraction

Olive oil samples were obtained from the fruits of the main Tunisian olive cultivar, Chemlali which were picked by hand at the same stage of maturity from three trees during two crop season 2014 and 2015 in 5 olive orchards with different soil type, (sandy, clay, stony, brown, limestone and gypsum), located in Sousse (Tunisia, 35°49'N, 10°30'E). Three different trees were selected in each orchard and were subjected to an identical fertilization regime and to all common olive cultivation practices. Oil was extracted using an Abencor laboratory oil mill equipped with a crusher, a mixer and a basket centrifuge. Only healthy fruits, without any kind of infection or physical damage, were processed. After harvesting, fresh olives (1.5–2.0 kg) were washed and de-leafed, crushed with a hammer crusher, and the paste mixed at 25°C for 30 min, centrifuged without the addition of warm water (oil produced from each extraction was 200–250 mL/kg) and then transferred into dark glass bottles, and stored at 4°C until analysis.

2.2 Determination of oil quality parameters

Free acidity expressed as percentage of oleic acid (% 18:1), peroxide value given as milli-equivalents of active oxygen per kilogram of oil (meqO₂/kg) and UV absorbance at 232 and 270 nm (K232 and K270) were determined according to the analytical methods described in the European Union Commission Regulations EEC/2568/91¹⁵⁾.

2.3 Determination of total phenolic content

Total phenolic content was determined colorimetrically at 765 nm using the Folin–Ciocalteu method¹⁶⁾. 10 mL of methanol/water (70:30) solution was added to 10 g of olive oil and the mixture was vortexed vigorously, shaken at 200 strokes/minute for 30 minutes and centrifuged for 10 min at 4000 rpm at room temperature. The extraction was repeated twice. After this step, 10 mL of Folin–Ciocalteu reagent (1:10) and 9 mL of Na₂CO₃ (7.5%) were added to 1 mL of the phenolic fraction. The mixture was incubated for 2 h in the dark at room temperature and then the absorbance of the mixture was measured at 765 nm. Results are expressed as mg gallic acid equivalent per kg of olive oil (mgGAE/kg).

2.4 Characterization of volatile profile by HS-SPME/GC-MS techniques

The volatile fraction of Chemlali olive oil was analyzed by headspace sampling, using the solid phase microextraction technique. For each SPME analysis, 3 g of olive oil were placed in a 30 mL glass vial in a warm water bath (40°C) and stirred for 45 min. The fiber, Divinylbenzene–Carboxen–Polymethylsiloxane (DVB/ Carboxen/PDMS) (Supelco, Bellefonte, PA, USA), was inserted and maintained in the sample head space for 45 min; then, it was removed and immediately inserted into the GC–MS injector (230°C for 2 min) for the desorption of compounds¹⁷⁾.

All the analyses were performed on a Thermo Scientific Trace 1300 gas-chromatograph coupled with a Thermo Scientific ISQ mass spectrometer equipped with electron impact ionization (EI) source, using a SUPELCOWAX 10 capillary column (Supelco, 30 m × 0.25 mm, f.t. 0.25 µm) and Helium as mobile phase (total flow of 18 mL min⁻¹). The temperatures of the injector and the auxiliary were set at 230°C. Splitless mode was chosen as injection modality maintaining the valve closed for 2 minutes. A temperature gradient was applied for the separation of volatile compound: oven temperature started from 50°C and then, after an initial holding of 3 minutes, temperature was increased of 5°C per minute until 200°C; this final value was kept for 18 minutes with a total run time of 45 minutes.

The MS source temperature was 230°C and the MS acquisition mode was full scan (from 40 *m/z* to 500 *m/z*). Moreover, a mixture of *n*-alkanes (C8–C20) dissolved in *n*-hexane was analyzed with the same gas-chromatographic method and employed for determination of linear retention indices. The retention indices were calculated for components eluting under experimental conditions between *n*-octane and *n*-eicosane. The main volatile compounds of the oil aromatic profiles were identified on the basis of their mass spectra compared with the reference mass spectra libraries (NIST) and of their calculated retention indices. The integrated peak areas were expressed as relative percentages, taking the sum of total areas as 100%¹⁷⁾.

2.5 Determination of sterol profile

0.2 g of oil sample was dissolved in *n*-hexane and treated with methanolic KOH (5%). The sterolic fraction was separated by chromatography on a silica gel cartridge, using ethyl acetate to recover sterols. The purified sterolic fraction was then silylated adding 0.3 mL of trimethylchlorosilane and 0.6 mL of hexamethyldisilazane and analyzed by GC-MS technique on the same instrument used for the determination of volatile compounds. For the analysis of sterols a BP5MS (SGE Analytical Science, 30 m × 0.25 mm, f.t. 0.25 µm) capillary column was used in the following chromatographic conditions: oven temperature was set at 240°C and, after an initial holding of 3 minutes, it was increased of 20°C per minute; the final temperature was set

at 280°C and maintained for 20 minutes. Injector and auxiliary temperatures were set at 290°C. Full scan modality was applied as detection mode. Quantification was performed by the addition of an internal standard (Cholestanol, 128 ppm) and expressed as mg/kg.

2.6 Statistical analysis

A One-way analysis of variance ANOVA was performed using the XLSTAT program (version 2014). Mean separation procedures were conducted using Duncan's multiple range tests with least significant difference (LSD) ($p < 0.05$). A principal component analysis (PCA) was done using XLSTAT, considering variables centered on their means.

3 Results and discussion

3.1 Quality parameters

Free acidity, peroxide value and UV specific extinction were determined for all the considered samples. The quality criteria depend essentially on the olive quality before the extraction and the storage conditions. Several studies¹⁸⁾ reported that the free acidity increase during ripening while peroxide value and K_{232} and K_{270} extinction coefficients decrease slightly during ripening. In order to limit the effect of the ripening stage on the studied chemical parameters, sampling was done at the same stage of maturity. In this case, the values of the quality parameters fell within the ranges established for the highest quality category ‘extra virgin olive oil’.

As shown in Table 1, in all samples the free acidity (0.6–0.8%) was included in the established upper limit of 0.8% for the best commercial quality olive oil, designated extra virgin. Peroxide value (PV) evaluates the hydroperoxides content and offers a measure of the degree of lipid oxidation. Olive oil from soil clay was the sample with the lowest peroxide value, while olive oil from soil sandy had the highest one. In all samples, peroxide values were below the limit of 20 milli-equivalents of active oxygen per kilogram of oil, which is accepted as the limit for the extra virgin

olive oil quality. Results of this study demonstrate that quality parameters are statistically affected by the type of the soil. Contradictory results were previously reported in literature according to this quality parameters in EVOO. Ref.¹⁹⁾ didn't find any difference in the free acidity and in the PV on ‘Chemlali’ olive oils obtained from olives cultivated on soils irrigated with different quality waters. Other authors²⁰⁾ found differences comparing ‘Chemlali’ olive oils belonging to different geographical sites in Tunisia. To evaluate the oxidation level of oil samples the parameters K_{232} and K_{270} have been used. In general, the oxidative status and the stability to the oxidation of an olive oil depend on the oil composition, both in terms of lipids profile and micro-components²¹⁾. The oil composition is demonstrated to be itself affected by several environmental factors, including the soil type²²⁾.

3.2 Total phenolic content

Olive oil is the only vegetable oil which contains appreciable amounts of phenolic compounds acting as antioxidant substances and conferring it a greater oxidative stability during storage²³⁾.

The Folin–Ciocalteu assay was used as method for the estimation of the total phenolic amount in olive oil. This method was chosen as it represents the most common method for total phenols evaluation and it is widely used for analysis of a large variety of natural extracts²⁴⁾. As listed in Table 1, the amount of phenols for the five samples confirmed their extreme variability in extra virgin olive oils originating from the same cultivar. The contents of total phenols of olive oils varied widely according to the soil type ranging from 123 to 284 (mg/kg). Olive oil obtained from soil clay showed the highest value in phenols, whereas olive oil obtained from soil brown recorded the lowest one. Our results are in agreement with the data reported in many studies on olive oils, showing different values of total phenol content depending on various factors, such as cultivar, climate and environmental factors, ripeness, olive processing. The extraction system applied on olives seems to be another variable affecting the phenolic amount in the final product. Usually, oils obtained by centrifugation have

Table 1 Chemical quality parameters of EVOO samples obtained from fruits of trees cultivated in different soil categories (sandy, clay, stony, brown, limestone and gypsum).

	Soil sandy	Soil clay	Soil stony	Soil brown	Soil limestone	EEC limits
Acidity (%C18 :1)	0.8 ^a ± 0.12	0.7 ^b ± 0.06	0.8 ^a ± 0.08	0.6 ^c ± 0.04	0.8 ^a ± 0.01	0.8
PV (MeqO ₂ /K)	13.45 ^a ± 0.07	3.05 ^c ± 0.7	7.14 ^c ± 3.73	4.5 ^d ± 0.00	7.66 ^b ± 4.02	20
K_{232}	1.35 ^a ± 0.16	0.99 ^b ± 0.03	1.14 ^b ± 0.21	0.92 ^c ± 0.01	1.02 ^c ± 0.01	2.5
K_{270}	0.22 ^a ± 0.01	0.07 ^b ± 0.00	0.15 ^{ab} ± 0.09	0.14 ^{ab} ± 0.03	0.17 ^{ab} ± 0.00	0.22
Phenols (mgGAE/kg)	131 ^d ± 9.12	284 ^a ± 91.74	171 ^b ± 66.83	123 ^c ± 15.83	149 ^c ± 5.00	

Data are expressed as mean standard deviation of three determinations.

Values followed by same letters are not significantly different ($p < 0.05$).

lower phenol content because this process involves the use of hot water that can remove phenols²⁵⁾. According to²⁶⁾, the variability observed could be caused by the oil production techniques and climatic features of the territory where olive trees were cultivated. In this study, as the harvesting period and extraction conditions were similar for all the considered samples, the results indicate that, besides the genetic factor, pedologic conditions have an actual influence on the phenols production.

3.3 Characterization of volatile profile of virgin olive oil by HS-SPME/GC-MS technics

Several studies have been published on the analysis of olive oil volatile profile using HS-SPME extraction and many components have been identified²⁷⁾.

EVOO flavor depends on the interaction of hundreds of compounds: aldehydes, alcohols, esters, hydrocarbons, ketones, furans, and others unidentified volatile compounds²⁸⁾. Compared to several techniques, HS-SPME shows the advantage of being a solvent-free extraction. The substances are concentrated on absorbing fiber and are directly desorbed into the gas chromatograph injector. This technique is very efficient and able to evaluate most of the volatile compounds related to the EVOO flavor and off-flavor²⁹⁾.

35 volatile substances were detected in Chemlali EVOO samples according to HS-SPME/GC-MS analysis (Table 2). The studies related to the characterization of volatile compounds of EVOOs indicated that the major contributors of olive oil aromatic profile were the C6 aldehydes and alcohols³⁰⁾. Many volatile compounds reported in Table 2 derived from polyunsaturated fatty acids through the lipoxygenase pathway, such as hexanal, 2-hexenal, 1-hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenol. 2-hexenal was the major C6 aldehyde compound for olive oil obtained from soil limestone, olive oil from soil clay and olive oil from soil stony whereas for olive oil from soil sandy and olive oil from soil brown, the major compound was 1-hexanol.

According to previous studies³¹⁾, the accumulation of each C6 compound depends on the cultivars. Other compounds present in a relatively high concentration were 2-pentanone (0.64-22.88%), acetone (5.55-14.56%), (*E*)-2-hexenol (0.79-12.54%), ethanol (3.84-8.82%), toluene (1.86-7.75%), 3-ethyl-1,5-octadiene (3.72-7.2%), γ -terpinene (2.45-5.61%) and hexanal (1.76-5.05%). As shown in Table 2, the chemical composition of the volatile fraction of Chemlali olive oils varies widely, depending on the type of soil of the growing area.

All the analysed samples showed a similar volatile profile even if the relative peak intensities resulted quite different, reflecting significant differences in the proportions of volatile constituents in oils of different soil categories (Table 2). Several terpene hydrocarbons (mono- and sesquiterpenes)

were often detected in the volatile composition of EVOO. γ -terpinene (2.45-5.61%), a monoterpene, and α -copaene (0.52-0.67%), a mono-unsaturated sesquiterpene, were the main ones. Other important terpenes were α -farnesene (0.41-0.49%), D-limonene (0.35-1.39%) and p-cumene (0.26-0.88%). These terpenes may be used as markers to differentiate EVOO of different soil textures. Our results are in agreement with the study of ref.²⁷⁾. The level of esters such as phenyl ethyl acetate was usually found to be lower compared to the amount of aldehydes and alcohols¹⁰⁾.

In the headspace of olive oil from soil sandy, the isolated and identified compounds were mainly 1-hexanol (23.79%), 2-pentanone (22.88%), acetone (14.56%), toluene (7.75%), 3-ethyl-1,5-octadiene (7.2%), 3-methyl-1-butanol (5.04%), hexanal (2.27%), phenylethyl alcohol (2.17%). The major constituents of the volatile fraction of OOsC were 2-hexenal (28.16%), acetone (10.6%), (*E*)-2-hexenol (7.95%), toluene (6.97%), 3-ethyl-1,5-octadiene (6.47%), γ -terpinene (5.61%), hexanal (4.78%), 1-hexanol (4.61%) and styrene (1.77%).

The composition of the volatile fraction of olive oil from soil stony was similar to that from soil clay, particularly from a qualitative point of view, except for (*Z*)-2-pentenol and α -copaene; found only in olive oil from soil clay, while p-cumene was present only in olive oil from soil stony (Table 2). Indeed aroma composition of olive oil from soil stony was mainly identified as 18.18% of 2-hexenal, 16.58% of 1-hexanol, 15.91% of acetone, 12.54% of (*E*)-2-hexenol, 4.45% of γ -terpinene, 3.84% of ethanol, 3.72% of 3-ethyl-1,5-octadiene, 3.13% of toluene, 3.06% of 3-methyl-1-butanol and 2.47% of *n*-octane.

The volatile fraction of oil obtained from soil brown was similar to the others samples and was composed by 1-hexanol (31.88%), 2-pentanone (14.83%), 3-ethyl-1,5-octadiene (7.59%), acetone (5.55%), *n*-octane (5.37%), hexanal (5.05%), γ -terpinene (4.7%), 3-methyl-1-butanol (3.31%), styrene (3.05%), toluene (1.86%), nonanal (1.6%), 2-pentanol (1.56%), phenylethyl alcohol (1.53%), D-limonene (1.39%) and (*Z*)-2-pentenol (1.13%).

Other minor compounds were identified in Chemlali olive oils. Among them, 1-penten-3-ol, methyl senecioate, 1-octen-3-ol, 1-heptanol, 1-heptanol, decanal, pentyl-cyclopropane, 5-methylfurfural, 1-undecanol, 3-methyl-benzaldehyde, phenyl ethyl acetate, pentyl-nonanoate, and benzyl alcohol were identified (Table 2).

The analysis of the volatile fraction of all Chemlali olive oils shows a similar qualitative composition between the various soils with some quantitative variations.

A comparison with literature data on the chemical composition of olive oils is difficult because of the great variability of the volatile compositions. In fact, it has been reported that the concentrations of volatile compounds depend on the enzymatic activity though external param-

ters (climate, soil, harvesting and extraction conditions) may alter the inherent olive oil sensory profile³²⁾. The variation in levels of C6 aldehydes and alcohols for oil samples from different soils implies that pedologic growth conditions may influence the activity of alcohol dehydrogenase (ADH).

3.4 Determination of the sterolic profile

Phytosterols, nutritionally important compounds routinely determined in foods, make up the greatest proportion of the non-saponifiable fraction of lipids. Their composition depends on the agronomic and climatic conditions, fruit or seed quality, oil extraction and refining procedures and storage conditions³³⁾.

The main sterols found in all Chemlali olive oils were b-

Table 2 Composition of volatile fraction of Chemlali olive oils obtained from fruits of trees cultivated in different soil categories (sandy, clay, stony, brown, limestone).

Compounds	LRI	LRI lit.	Soil sandy	Soil clay	Soil stony	Soil brown	Soil limestone	References
<i>n</i> -octane	773	800	0.43 ^c ± 0.01	1.28 ^c ± 0.01	2.47 ^b ± 0.04	5.37 ^a ± 0.04	0.69 ^d ± 0.05	42)
Acetone	807	810	14.56 ^b ± 0.00	10.6 ^d ± 0.14	15.91 ^a ± 0.15	5.55 ^e ± 0.06	14.02 ^c ± 0.06	42)
Ethanol	896	928	—	4.44 ^b ± 0.05	3.84 ^c ± 0.06	—	8.82 ^a ± 0.03	43)
2-pentanone	964	969	22.88 ^a ± 0.00	1.11 ^d ± 0.03	0.64 ^e ± 0.02	14.83 ^b ± 0.04	1.31 ^e ± 0.01	44)
3-ethyl-1,5 octadiene	1019	1017	7.2 ^b ± 0.14	6.47 ^c ± 0.04	3.72 ^c ± 0.03	7.59 ^a ± 0.13	4.31 ^d ± 0.01	44)
Toluene	1037	1026	7.75 ^a ± 0.07	6.97 ^b ± 0.02	3.13 ^d ± 0.18	1.86 ^e ± 0.01	6.07 ^c ± 0.1	44)
Hexanal	1079	1073	2.27 ^d ± 0.04	4.78 ^b ± 0.04	1.76 ^e ± 0.04	5.05 ^a ± 0.06	3.27 ^c ± 0.09	44)
2-pentanol	1115	1091	1.27 ^b ± 0.01	—	—	1.56 ^a ± 0.01	—	42)
1-penten-3-ol	1156	1157	—	1.38 ^a ± 0.07	1.03 ^b ± 0.11	0.19 ^c ± 0.02	1.25 ^a ± 0.07	44)
Methylsenecioate	1168	1184	—	0.85 ^d ± 0.01	1.46 ^b ± 0.06	1.09 ^c ± 0.04	2.43 ^a ± 0.04	45)
D-limonene	1195	1186	0.72 ^c ± 0.03	0.61 ^c ± 0.04	0.35 ^d ± 0.07	1.39 ^a ± 0.05	0.87 ^b ± 0.03	44)
3-methyl-1-butanol	1204	1204	5.04 ^a ± 0.02	1.53 ^c ± 0.11	3.06 ^c ± 0.13	3.31 ^b ± 0.06	2.77 ^d ± 0.1	44)
2-hexenal	1220	1211	—	28.16 ^b ± 0.04	18.18 ^c ± 0.25	—	32.75 ^a ± 0.07	44)
1-pentanol	1248	1248	—	2.07 ^a ± 0.06	1.13 ^b ± 0.03	0.92 ^c ± 0.05	—	44)
γ-terpinene	1251	1249	—	5.61 ^a ± 0.05	4.45 ^b ± 0.01	4.7 ^b ± 0.28	2.45 ^c ± 0.01	43)
Styrene	1258	1261	1.12 ^d ± 0.03	1.77 ^c ± 0.19	1.13 ^d ± 0.03	3.05 ^a ± 0.05	2.52 ^b ± 0.03	46)
<i>p</i> -cumene	1282	1282	0.88 ^a ± 0.04	—	0.45 ^b ± 0.07	—	0.26 ^c ± 0.05	47)
(<i>Z</i>)-2-pentenol	1319	1314	1.15 ^b ± 0.03	1.08 ^b ± 0.04	—	1.13 ^b ± 0.04	1.34 ^a ± 0.03	44)
1-hexanol	1351	1350	23.79 ^b ± 0.01	4.61 ^d ± 0.27	16.58 ^c ± 0.04	31.88 ^a ± 0.06	3.39 ^e ± 0.04	44)
(<i>Z</i>)-3-hexenol	1383	1378	0.61 ^b ± 0.08	0.51 ^b ± 0.08	0.68 ^b ± 0.11	0.92 ^a ± 0.03	0.69 ^b ± 0.04	44)
Nonanal	1395	1389	1.2 ^{cd} ± 0.15	1.43 ^{bc} ± 0.11	1.09 ^d ± 0.12	1.6 ^b ± 0.01	2.26 ^a ± 0.08	44)
(<i>E</i>)-2-hexenol	1405	1400	0.94 ^c ± 0.04	7.95 ^b ± 0.21	12.54 ^a ± 0.65	0.79 ^c ± 0.05	1.59 ^c ± 0.05	44)
1-octen-3-ol	1450	1420	0.29 ^b ± 0.05	0.35 ^{ab} ± 0.07	0.4 ^{ab} ± 0.06	0.49 ^a ± 0.05	0.35 ^{ab} ± 0.07	42)
1-heptanol	1454	1451	0.39 ^b ± 0.09	0.3 ^b ± 0.14	0.43 ^b ± 0.07	0.9 ^a ± 0.28	0.31 ^b ± 0.06	44)
α-copaene	1490	1482	0.61 ^{ab} ± 0.08	0.67 ^a ± 0.02	—	—	0.52 ^b ± 0.05	44)
Decanal	1511	1500	—	0.33 ^b ± 0.04	0.82 ^a ± 0.05	0.24 ^b ± 0.06	0.25 ^b ± 0.07	43)
pentyl-cyclopropane	1558	—	0.39 ^a ± 0.06	0.4 ^a ± 0.35	0.47 ^a ± 0.04	0.6 ^a ± 0.06	0.5 ^a ± 0.14	
5-methylfurfural	1582	1586	0.72 ^a ± 0.07	0.65 ^a ± 0.13	0.42 ^a ± 0.04	0.45 ^a ± 0.07	0.59 ^a ± 0.19	48)
1-undecanol	1662	1640	0.64 ^a ± 0.1	0.57 ^{ab} ± 0.02	0.41 ^b ± 0.11	0.41 ^b ± 0.04	0.58 ^{ab} ± 0.04	42)
3-methyl-benzaldehyde	1666	—	0.33 ^{bc} ± 0.04	0.25 ^c ± 0.07	0.31 ^{bc} ± 0.06	0.61 ^a ± 0.01	0.4 ^b ± 0.07	
α-farnesene	1760	1745	0.42 ^a ± 0.05	0.41 ^a ± 0.08	0.44 ^a ± 0.06	0.49 ^a ± 0.01	—	44)
phenylethyl acetate	1834	1834	0.82 ^a ± 0.24	0.6 ^{ab} ± 0.06	0.39 ^b ± 0.01	0.5 ^b ± 0.07	0.56 ^{ab} ± 0.01	48)
pentyl-nonanoate	1846	—	0.88 ^a ± 0.17	0.57 ^{bc} ± 0.1	0.42 ^c ± 0.04	0.57 ^{bc} ± 0.06	0.75 ^{ab} ± 0.07	
Benzylalcohol	1895	1896	0.56 ^a ± 0.13	0.4 ^a ± 0.06	0.54 ^a ± 0.06	0.45 ^a ± 0.07	0.42 ^a ± 0.02	48)
phenylethylalcohol	1931	1931	2.17 ^a ± 0.09	1.29 ^d ± 0.06	1.34 ^{cd} ± 0.06	1.53 ^{bc} ± 0.03	1.71 ^b ± 0.13	48)

LRI: linear retention index on a Supelcowax 10 capillary column. Data are expressed as percentages related to the total area of chromatogram. Values followed by same letters are not significantly different ($p < 0.05$).

Table 3 Sterol composition and α -tocopherol (mg/kg) of Chemlali olive oils obtained from fruits of trees cultivated in different soil categories (sandy, clay, stony, brown, limestone).

compound	Soil sandy	Soil clay	Soil stony	Soil brown	Soil limestone
α -tocopherol	39 ^d $\pm$ 0.71	41.1 ^c $\pm$ 0.14	47 ^a $\pm$ 0.71	35 ^c $\pm$ 0.07	45 ^b $\pm$ 0.78
campesterol	31.25 ^c $\pm$ 0.35	12 ^e $\pm$ 0.21	15 ^d $\pm$ 0.99	40 ^b $\pm$ 0.92	49.40 ^a $\pm$ 0.85
stigmasterol	< LOD	7.1 ^a $\pm$ 0.14	< LOD	< LOD	< LOD
beta sistosterol	302.25 ^a $\pm$ 0.35	256 ^c $\pm$ 0.85	259 ^d $\pm$ 0.92	283 ^c $\pm$ 0.57	290 ^b $\pm$ 0.64

Values followed by same letters are not significantly different ($p < 0.05$).

sitosterol, campesterol and stigmasterol. Others peaks, probably corresponding to sterol derivatives, were also found in chromatograms of all samples, but the identification was not possible because their signals were very low.

As shown in **Table 3**, the amounts of individual sterols varied according to the soil category. In the Chemlali variety, the highest phytosterol levels were found for β -sitosterol, characteristic of the virgin olive oil in the pulp of the olive³⁴. These results are in agreement with other studies^{35, 36}.

The amounts of sterols showed a significant difference between samples. This difference in sterolic composition of oils is not only quantitative but also in term of quality. The highest amount of β -sitosterol was observed in olive oil from soil sandy (302 mg/kg), whereas that from soil clay had the lowest one (256 mg/kg). According to ref.³⁷, the content of β -sitosterol generally decreases during ripening. Other authors reported that β -sitosterol is lower when olives are harvested at their optimum³⁸. The health aspects of β -sitosterol have recently been reported in several studies³⁹. Stigmasterol is related to various parameters of the quality of extra virgin olive oil. High levels correlate with acidity and low organoleptic quality⁴⁰. The analysed samples contained very low levels of this sterol, which is indicative that the oil came from healthy fruit³⁸.

All the olive oil samples analysed showed low campesterol content, with a global range from (12 mg/kg) to (49.40 mg/kg) in oil from soil clay and oil from soil limestone, respectively. Campesterol content was below the limit established by EU Regulations (4%) in all of the oils studied, indicating a particularity of this olive oil variety. Significant differences were observed in the campesterol content in relation to the soil category. Chemlali olive cultivar showed low amounts of campesterol when compared with other studied cultivars, from Spain³⁶ and from Portugal³⁵.

Several authors highlighted that the presence and distribution of the various sterol forms depend on many factors such as olive ripening degree³⁷, agronomic and climatic conditions¹², harvesting techniques, oil extraction and storage conditions, refining process³⁸ as well as cultivars and geographical origin^{11, 13, 14}.

Compositional analysis of the sterol fraction of olive oil can be used to assess the degree of purity of the oil and the

absence of other plant oils. This determination also permits characterization of the type of olive oil in question: extra virgin, virgin, refined, etc.⁴¹.

3.5 α -tocopherols

Alpha tocopherol (vitamin E), which is also part of the unsaponifiable fraction of the olive oil, is obtained together with the sterol fraction. The contents of this tocopherols shown in **Table 3**. There were clear differences between analysed oils. amounts of olive oil from soil stony contained the highest level of α -tocopherol (47 mg/kg) whereas olive

Fig. 1 Principal component analysis applied to all samples: 1: *n*-octane; 2: acetone; 3: ethanol; 4: 2-pentanone; 5: 3-ethyl-1,5 octadiene; 6: toluene; 7: hexanal; 8: 2-pentanol; 9: 1-penten-3-ol; 10: methylsenecioate; 11: D-limonene; 12: 3-methyl-1-butanol; 13: 2-hexenal; 14: 1-pentanol; 15: γ -terpinene; 16: styrene; 17: *p*-cumene; 18: (*Z*)-2-pentenol; 19: 1-hexanol; 20: (*Z*)-3-hexenol; 21: nonanal; 22: (*E*)-2-hexenol; 23: 1-octen-3-ol; 24: 1-heptanol; 25: α -copaene; 26: decanal; 27: pentyl-cyclopropane; 28: 5-methylfurfural; 29: 1-undecanol; 30: 3-methyl-benzaldehyde; 31: α -farnesene; 32: phenylethyl acetate; 33: pentyl-nonanoate; 34: benzylalcohol; 35: phenylethylalcohol; S2: campesterol; S3: stigmasterol; S4: β -sitosterol.

Fig. 2 Principal component analysis: a score plot of Chemlali samples from different soils.

oil from soil brown contained the lowest one (35 mg/kg).

3.6 Principal component analysis

The application of the principal component analysis algorithm (PCA) showed three distinct groups (Figs. 1 and 2). Forty five variables were selected for the PCA explaining 61.52% of the total variance (F1:33.21%; F2:28.31%). The first group was composed by Chemlali olive oil from soil brown, known by their high levels of some volatile compounds (*n*-octane, 2-pentanol, D-limonene, styrene, 1-hexanol, (*Z*)-3-hexenol, 1-octen-3-ol, 1-heptanol, 3-methyl-benzaldehyde). The second group was composed by Chemlali olive oil from soil sandy, characterised by a good correlation with PV, K_{232} , K_{270} , β -sitosterol and some volatile compounds (2-pentanone, 3-methyl-1-butanol, *p*-cumene, phenylethyl acetate, pentyl-nonanoate, phenylethylalcohol). Finally, the third group was composed by three samples, Chemlali olive oil from soil stony, soil clay and soil limestone. This group was characterised by high levels of total phenols, α -tocopherol, stigmasterol and some volatile compounds (ethanol, methylsenecioate, 1-pentanol, styrene, nonanal, (*E*)-2-hexenol, decanal).

4 Conclusion

The application of SPME to the analysis of Chemlali extra virgin olive oil showed significant differences in the proportions of the volatile compounds from oils of various soil types. A significant difference in the composition of the sterolic fraction was also detected. As the harvesting period and extraction conditions were similar for all the studied samples, the results indicate that, besides the genetic factor, pedologic conditions influence the volatiles, the sterols composition and α -tocopherol of the olive oils. Thus, the soil type of olive orchard is found to be a key

factor for the quality of olive oils.

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