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

Mouna Ben Rached¹, Gianni Galaverna², Martina Cirilni², Dalenda Boujneh³, Mokhtar Zarrouk¹ and Mokhtar Guerfel¹,*

¹ Laboratoire de Biotechnologie de l'Olivier, Centre de Biotechnologie de Borj Cédria, B.P. 901, 2050 Hammam-Lif, TUNISIA
² Department of Food Science, University of Parma, Parco Area delle Scienze, 49/a, I-43124 Parma, ITALY
³ Institut de l’Olivier, Station de Sousse, Rue Ibn Khaldoun, B.P. 40, 4061 Sousse, TUNISIA

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, ß-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 diseases. 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 flavors.

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 properties.

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 Greece. 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 prevailing. 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). Furthermore, olive oil classification according to its volatile composition has been considered to be an important tool for the monitoring of adulteration. Several researches correlate the volatile composition of olive oil to varieties, geographical origin, and year of harvest. Other studies were performed to correlate the sterolic fraction to the olive ripening degree, agronomic and climatic conditions, geographical origin and cultivars.

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,
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 until 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 mili-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/2568/91 [16].

2.3 Determination of total phenolic content

Total phenolic content was determined colorimetrically at 765 nm using the Folin–Ciocalteau method [16]. 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–Ciocalteau 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 compounds [17].

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 compounds: 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 this 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...
Virgin olive oil quality of “Chemlali” olive trees

J. Oleo Sci.

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 (Cholesterol, 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 quality criteria depend on the olive quality were determined for all the considered samples. The means.

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 storage.

The Folin–Ciocalteau 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 extracts. 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).

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Acidity (ºC18:1)</th>
<th>PV (MeqO₂/K)</th>
<th>K₂₃₂</th>
<th>K₂₇₀</th>
<th>Phenols (mgGAE/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy</td>
<td>0.57 ± 0.06</td>
<td>13.45 ± 0.07</td>
<td>1.35</td>
<td>0.22</td>
<td>131 ± 9.12</td>
</tr>
<tr>
<td>Clay</td>
<td>0.80 ± 0.12</td>
<td>3.05 ± 0.7</td>
<td>0.99</td>
<td>0.74</td>
<td>284 ± 91.74</td>
</tr>
<tr>
<td>Stony</td>
<td>0.80 ± 0.08</td>
<td>7.14 ± 3.73</td>
<td>1.14</td>
<td>0.15</td>
<td>171 ± 66.83</td>
</tr>
<tr>
<td>Brown</td>
<td>0.60 ± 0.04</td>
<td>4.54 ± 0.00</td>
<td>0.92</td>
<td>0.14</td>
<td>123 ± 15.83</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.80 ± 0.01</td>
<td>7.66 ± 4.02</td>
<td>1.02</td>
<td>0.17</td>
<td>149 ± 5.00</td>
</tr>
</tbody>
</table>

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 phenols. According to 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 identified. EVOO flavor depends on the interaction of hundreds of compounds: aldehydes, alcohols, esters, hydrocarbons, ketones, furans, and others unidentified volatile compounds. 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-flavor.

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 alcohols. Many volatile compounds reported in Table 2 derived from polyunsaturated fatty acids through the lipoygenase 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 studies, 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.

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. The level of esters such as phenyl ethyl acetate was usually found to be lower compared to the amount of aldehydes and alcohols.

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%), phenylethylalcohol (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-pentenal 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-pentenal (1.13%).

Other minor compounds were identified in Chemlali olive oils. Among them, 1-penten-3-ol, methyl seneoicato, 1-octen-3-ol, 1-heptanol, 1-heptanol, decanal, pentyl-cyclohexane, 5-methylfurural, 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 parame-
Virgin olive oil quality of “Chemlali” olive trees

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 conditions.

The main sterols found in all Chemlali olive oils were

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LRI</th>
<th>LRI lit.</th>
<th>Soil sandy</th>
<th>Soil clay</th>
<th>Soil stony</th>
<th>Soil brown</th>
<th>Soil limestone</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-octane</td>
<td>773</td>
<td>800</td>
<td>0.43±0.01</td>
<td>1.28±0.01</td>
<td>2.47±0.04</td>
<td>5.37±0.04</td>
<td>0.69±0.05</td>
<td>42</td>
</tr>
<tr>
<td>Acetone</td>
<td>807</td>
<td>810</td>
<td>14.56±0.00</td>
<td>10.6±0.14</td>
<td>15.91±0.15</td>
<td>5.55±0.06</td>
<td>14.02±0.06</td>
<td>42</td>
</tr>
<tr>
<td>Ethanol</td>
<td>896</td>
<td>928</td>
<td>–</td>
<td>4.44±0.05</td>
<td>3.84±0.06</td>
<td>–</td>
<td>8.82±0.03</td>
<td>43</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>964</td>
<td>969</td>
<td>22.88±0.00</td>
<td>1.11±0.03</td>
<td>0.64±0.02</td>
<td>14.83±0.04</td>
<td>1.31±0.01</td>
<td>44</td>
</tr>
<tr>
<td>3-ethyl-1,5 octadiene</td>
<td>1019</td>
<td>1017</td>
<td>7.2±0.14</td>
<td>6.47±0.04</td>
<td>3.72±0.03</td>
<td>7.59±0.13</td>
<td>4.31±0.01</td>
<td>44</td>
</tr>
<tr>
<td>Toluene</td>
<td>1037</td>
<td>1026</td>
<td>7.75±0.07</td>
<td>6.97±0.02</td>
<td>3.13±0.18</td>
<td>1.86±0.01</td>
<td>6.07±0.1</td>
<td>44</td>
</tr>
<tr>
<td>Hexanol</td>
<td>1079</td>
<td>1073</td>
<td>2.27±0.04</td>
<td>4.78±0.04</td>
<td>1.76±0.04</td>
<td>5.05±0.06</td>
<td>3.27±0.09</td>
<td>44</td>
</tr>
<tr>
<td>2-pentanol</td>
<td>1115</td>
<td>1091</td>
<td>1.27±0.01</td>
<td>–</td>
<td>–</td>
<td>1.56±0.01</td>
<td>–</td>
<td>42</td>
</tr>
<tr>
<td>1-penten-3-ol</td>
<td>1156</td>
<td>1157</td>
<td>1.38±0.07</td>
<td>1.03±0.11</td>
<td>0.19±0.02</td>
<td>1.25±0.07</td>
<td>–</td>
<td>44</td>
</tr>
<tr>
<td>Methylseneocioate</td>
<td>1168</td>
<td>1184</td>
<td>0.85±0.01</td>
<td>1.46±0.06</td>
<td>1.09±0.04</td>
<td>2.43±0.04</td>
<td>–</td>
<td>45</td>
</tr>
<tr>
<td>D-limonene</td>
<td>1195</td>
<td>1186</td>
<td>0.72±0.03</td>
<td>0.61±0.04</td>
<td>0.35±0.07</td>
<td>1.39±0.05</td>
<td>0.87±0.03</td>
<td>44</td>
</tr>
<tr>
<td>3-methyl-1-butanol</td>
<td>1204</td>
<td>1204</td>
<td>5.04±0.02</td>
<td>1.53±0.11</td>
<td>3.06±0.13</td>
<td>3.31±0.06</td>
<td>2.77±0.1</td>
<td>44</td>
</tr>
<tr>
<td>2-hexenal</td>
<td>1220</td>
<td>1211</td>
<td>–</td>
<td>28.16±0.04</td>
<td>18.18±0.25</td>
<td>–</td>
<td>32.75±0.07</td>
<td>44</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>1248</td>
<td>1248</td>
<td>–</td>
<td>2.07±0.06</td>
<td>1.13±0.03</td>
<td>0.92±0.05</td>
<td>–</td>
<td>44</td>
</tr>
<tr>
<td>y-terpinene</td>
<td>1251</td>
<td>1249</td>
<td>0.86±0.05</td>
<td>4.45±0.01</td>
<td>4.7±0.28</td>
<td>2.45±0.01</td>
<td>–</td>
<td>43</td>
</tr>
<tr>
<td>Styrene</td>
<td>1258</td>
<td>1261</td>
<td>1.12±0.03</td>
<td>1.77±0.19</td>
<td>1.13±0.03</td>
<td>3.05±0.05</td>
<td>2.52±0.03</td>
<td>46</td>
</tr>
<tr>
<td>p-cumene</td>
<td>1282</td>
<td>1282</td>
<td>0.88±0.04</td>
<td>–</td>
<td>0.45±0.07</td>
<td>–</td>
<td>0.26±0.05</td>
<td>47</td>
</tr>
<tr>
<td>(Z)-2-pentenol</td>
<td>1319</td>
<td>1314</td>
<td>1.15±0.03</td>
<td>1.08±0.04</td>
<td>–</td>
<td>1.13±0.04</td>
<td>1.34±0.03</td>
<td>44</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>1351</td>
<td>1350</td>
<td>23.79±0.01</td>
<td>4.61±0.27</td>
<td>16.58±0.04</td>
<td>31.88±0.06</td>
<td>3.39±0.04</td>
<td>44</td>
</tr>
<tr>
<td>(Z)-3-hexenal</td>
<td>1383</td>
<td>1378</td>
<td>0.61±0.08</td>
<td>0.51±0.08</td>
<td>0.68±0.11</td>
<td>0.92±0.03</td>
<td>0.69±0.04</td>
<td>44</td>
</tr>
<tr>
<td>Nonanal</td>
<td>1395</td>
<td>1389</td>
<td>1.22±0.15</td>
<td>1.43±0.11</td>
<td>1.09±0.12</td>
<td>1.6±0.01</td>
<td>2.26±0.08</td>
<td>44</td>
</tr>
<tr>
<td>(E)-2-hexenal</td>
<td>1405</td>
<td>1400</td>
<td>0.94±0.04</td>
<td>7.95±0.21</td>
<td>12.54±0.65</td>
<td>0.79±0.05</td>
<td>1.59±0.05</td>
<td>44</td>
</tr>
<tr>
<td>1-octen-3-ol</td>
<td>1450</td>
<td>1420</td>
<td>0.29±0.05</td>
<td>0.35±0.07</td>
<td>0.4±0.06</td>
<td>0.49±0.05</td>
<td>0.35±0.07</td>
<td>42</td>
</tr>
<tr>
<td>1-heptanol</td>
<td>1454</td>
<td>1451</td>
<td>0.39±0.09</td>
<td>0.3±0.14</td>
<td>0.43±0.07</td>
<td>0.9±0.28</td>
<td>0.31±0.06</td>
<td>44</td>
</tr>
<tr>
<td>α-copacene</td>
<td>1490</td>
<td>1482</td>
<td>0.61±0.08</td>
<td>0.67±0.02</td>
<td>–</td>
<td>–</td>
<td>0.52±0.05</td>
<td>44</td>
</tr>
<tr>
<td>Decanal</td>
<td>1511</td>
<td>1500</td>
<td>0.33±0.04</td>
<td>0.82±0.05</td>
<td>0.24±0.06</td>
<td>0.25±0.07</td>
<td>–</td>
<td>43</td>
</tr>
<tr>
<td>pentyl-cyclop propane</td>
<td>1558</td>
<td>–</td>
<td>0.39±0.06</td>
<td>0.4±0.35</td>
<td>0.47±0.04</td>
<td>0.6±0.06</td>
<td>0.5±0.14</td>
<td>–</td>
</tr>
<tr>
<td>5-methylfurural</td>
<td>1582</td>
<td>1586</td>
<td>0.72±0.07</td>
<td>0.65±0.13</td>
<td>0.42±0.04</td>
<td>0.45±0.07</td>
<td>0.59±0.19</td>
<td>48</td>
</tr>
<tr>
<td>1-undecanol</td>
<td>1662</td>
<td>1640</td>
<td>0.64±0.1</td>
<td>0.57±0.02</td>
<td>0.41±0.11</td>
<td>0.41±0.04</td>
<td>0.58±0.04</td>
<td>42</td>
</tr>
<tr>
<td>3-methyl-benzaldehyde</td>
<td>1666</td>
<td>–</td>
<td>0.33±0.04</td>
<td>0.25±0.07</td>
<td>0.31±0.06</td>
<td>0.61±0.01</td>
<td>0.4±0.07</td>
<td>42</td>
</tr>
<tr>
<td>α-farnesene</td>
<td>1760</td>
<td>1745</td>
<td>0.42±0.05</td>
<td>0.41±0.08</td>
<td>0.44±0.06</td>
<td>0.49±0.01</td>
<td>–</td>
<td>44</td>
</tr>
<tr>
<td>phenethyl acetate</td>
<td>1834</td>
<td>1834</td>
<td>0.82±0.24</td>
<td>0.6±0.06</td>
<td>0.39±0.01</td>
<td>0.5±0.07</td>
<td>0.56±0.01</td>
<td>48</td>
</tr>
<tr>
<td>pentyl-nonanoate</td>
<td>1846</td>
<td>–</td>
<td>0.88±0.17</td>
<td>0.59±0.1</td>
<td>0.42±0.04</td>
<td>0.57±0.06</td>
<td>0.75±0.07</td>
<td>42</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>1895</td>
<td>1896</td>
<td>0.56±0.13</td>
<td>0.4±0.06</td>
<td>0.54±0.06</td>
<td>0.45±0.07</td>
<td>0.42±0.02</td>
<td>48</td>
</tr>
<tr>
<td>phenethylalcohol</td>
<td>1931</td>
<td>1931</td>
<td>2.17±0.09</td>
<td>1.29±0.06</td>
<td>1.34±0.06</td>
<td>1.53±0.03</td>
<td>1.71±0.13</td>
<td>48</td>
</tr>
</tbody>
</table>

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).

J. Oleo Sci.
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 oils is not only quantitative but also in term of quality. The difference 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 olive. These results are in agreement with other studies.

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., the content of β-sitosterol generally decreases during ripening. Other authors reported that β-sitosterol is lower when olives are harvested at their optimum. The health aspects of β-sitosterol have recently been reported in several studies. Stigmasterol is related to various parameters of the quality of extra virgin olive oil. High levels correlate with acidity and low organoleptic quality.

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 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 Spain and from Portugal.

Several authors highlighted that the presence and distribution of the various sterol forms depend on many factors such as olive ripening degree, agronomic and climatic conditions, harvesting techniques, oil extraction and storage conditions, refining process as well as cultivars and geographical origin.

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.

### 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).

<table>
<thead>
<tr>
<th>compound</th>
<th>Soil sandy</th>
<th>Soil clay</th>
<th>Soil stony</th>
<th>Soil brown</th>
<th>Soil limestone</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>39 ± 0.71</td>
<td>41.1 ± 0.14</td>
<td>47 ± 0.71</td>
<td>35 ± 0.07</td>
<td>45 ± 0.78</td>
</tr>
<tr>
<td>campesterol</td>
<td>31.25 ± 0.35</td>
<td>12 ± 0.21</td>
<td>15 ± 0.99</td>
<td>40 ± 0.92</td>
<td>49.40 ± 0.85</td>
</tr>
<tr>
<td>stigmasterol</td>
<td>&lt; LOD</td>
<td>7.1 ± 0.14</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>beta sitosterol</td>
<td>302.25 ± 0.35</td>
<td>256 ± 0.85</td>
<td>259 ± 0.92</td>
<td>283 ± 0.57</td>
<td>290 ± 0.64</td>
</tr>
</tbody>
</table>

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

### 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 tocopherol is 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 oil from soil sandy contained the lowest level of α-tocopherol.

Virgin olive oil quality of “Chemlali” olive trees

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, K232, K270, Z-3-hexenol, 2-pentanol, D-limonene, styrene, and soil limestone. This group was characterised by high levels of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem. Toxicol. 38, 647-659 (2000).

3.7 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, K232, K270, Z-3-hexenol, 2-pentanol, D-limonene, styrene, and soil limestone. This group was characterised by high levels of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem. Toxicol. 38, 647-659 (2000).

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, K232, K270, Z-3-hexenol, 2-pentanol, D-limonene, styrene, and soil limestone. This group was characterised by high levels of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem. Toxicol. 38, 647-659 (2000).

References
11) Lukic, M.; Lukic, I.; Krapac, M.; Sladonja, B.; Pilizota, V. Sterols and triterpene diols in olive oil as indicators of variety and degree of ripening. Food Chem. 136, 251-258.


Rivera-del Alamo, R.M.; Fregapane, G.; Aranda, F.; Go-
Virgin olive oil quality of “Chemlali” olive trees


42) NIST, NIST2008 mass spectral library.


