Full Length Research Paper

The phenolic compounds and compositional quality of Chétoui virgin olive oil: Effect of altitude

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Accepted 7 December, 2011

The differences in the chemical composition of virgin olive oils (VOO) obtained from trees of the Chetoui variety cultivated at different altitudes in Tunisia were evaluated. All samples were harvested using the same controlled procedures and were submitted to a controlled processing in the same laboratory mill. Several analytical parameters such as fatty acid composition, amounts of phenols; o-diphenols and pigments were analyzed. All these parameters showed an important effect on the fatty acids and phenols content of the oils. The total phenol content was positively correlated with the altitude, ranging from 817.33 mg/kg (403 m) to 131.91 mg/kg (10 m). However, the results of the regulated parameters in the potential quality classified all the analyzed oils into the “extra virgin” category.

Key words: Orchard elevation, total antioxidant capacity, phenolic compounds, quality index.

INTRODUCTION

Extra virgin olive oil is the principal source of fat in the Mediterranean diet, with important nutraceutical effects due to its abundance of oleic acid, phenolic compounds and an adequate content of linoleic and linolenic acids, two important essential fatty acids. All these chemicals contribute to lower the risk of coronary heart diseases and cancers (Bandelj et al., 2002). Since the production of olive oil is far below demand, there is a need to increase the cultivation of olive trees, both to produce more oil and to enhance its quality, particularly with regard to components beneficial to the human health, such as natural antioxidants and sterols (Cercaci et al., 2007). These benefits have been related both to its well-balanced fatty acid composition, where oleic acid is the main component and to the presence of minor biomolecules, such as phytosterols, carotenoids, tocopherol and phenols (Medeiros, 2001; Perez-Jimenez et al., 2005).

The phenolic compounds are natural antioxidants present in virgin olive oils (VOO) (Baldioli et al., 1996) but not in refined ones (Montedoro and Cantarelli, 1969). They have a significant importance for their nutritional and technological properties. Their antioxidant function and anti-inflammatory effect (Perona et al., 2006) have been related to the preventive action on certain diseases such as atherosclerosis (Huang and Sumpio, 2008) and cancer (Giovannini et al., 2008). Polyphenols also present an important technological value due to their influence on sensory characteristics (Baccouri et al., 2008; Bendini et al., 2007) and the shelf life of virgin olive oil.

Several attempts have been made to define olive oil origin by means of multivariate analysis of chemical parameters; that is, the principal component analysis (PCA), and the profiles of fatty acid and triacylglycerol have been applied for the geographical classification of Greek oils (Tsimidou and Karakostas, 1993). Using an expert system (SEXIA), Aparicio et al. (1994) have studied data from different chemical analysis to classify Spanish oils...
with respect to their origin and variety. Sacchi et al. (1998),
used high-field proton nuclear magnetic resonance (‘H-
NMR) spectroscopy and PCA to obtained a classification of
Italian olive oils from different regions. Ranalli et al.
(1999) found that phenols, tocoopherol, volatiles and fatty
acids were influenced by the site of production, in
particular by the different climate and soil parameters.
Salvador et al. (2003) assessed the chemical com-
position and quality of Cornicabra virgin olive oils
throughout five crop years in five areas and were able to
show differences in composition due to season and
environmental effects, resulting in a higher quality of the
oil from South and South-West of Toledo with respect to
other regions. In their investigations Temime et al. (2006,
2008), demonstrated that the growing area have a
significant effect on the percent composition of the
volatiles and sterols in the olive oils produced from
Chetoui cultivar harvested and processed in the same
period and with the same technology. Analysis by SPME
of Tunisian Chetoui virgin olive oils from 14 geographic
sites, led to the conclusion that significant differences in
volatile compounds from various origins existed.

The aims of the present work were to study the effects
of different geographic origin, in particular of the different
elevation of the orchards, on the compositional quality
and phenolic compounds of the oil.

MATERIALS AND METHODS
Reagents and standards
Analytical grade solvents and reagents were used to perform
analyses except high-performance liquid chromatography (HPLC)
euents that were of HPLC grade and purchased from Fluka
(Buchs, Switzerland), Sigma-Aldrich Chemical (Stemheim,
Germany) and Merck (Darmstadt, Germany). Bidistilled deionised
water was used and all the reagents were of suitable analytic purity.

Plant material
The olives were hand-picked from different trees, at the same stage
of maturity (MI = 3), at five different altitudes and the same labo-
atory mill was used to prepare the olive oil samples. Only drupes
not damaged, fresh and healthy were selected. After harvesting, the
olive fruit samples were immediately transported to the laboratory
mill where they were transformed into oil within 24 h. The olives (2.5
to 3 kg) were washed and deleafed, crushed with a hammer crus-
her and the paste mixed at 25°C for 30 min, then centrifuged
without addition of warm water. The oil was transferred into dark
glass bottles, kept in the dark at 4°C and analyzed within a week
(Youssef et al., 2011).

Quality parameters
Determination of physicochemical quality parameters (free acidity,
peroxide value and UV absorption characteristics, K270
(conjugated trienes) and K232 (conjugated dienes), was carried out
following the analytical methods described by Regulation EEC/
2568/91 and EEC/ 1429/92 of European Economic Community

Fatty acid composition
Fatty acid composition, calculated as the percentage of total fatty
acids, was determined by gas chromatography with a Hewlett-
Packard (HP 4890 D) chromatograph (Hewlett-Packard Company,
Wilmington, DE) equipped with a flame ionization detector (FID),
after their conversion to methyl esters, according to regulation EEC

Pigment content
Carotenoids and chlorophylls (mg/kg oil) were determined at 470
and 670 nm, respectively, in cyclohexane, using specific extinction
values, according to the method of Minguez-Mosquera et al. (1991).

Total phenolic content
Total phenol and o-diphenol contents were quantified colori-
metrically (Ranalli et al., 1999). Phenolic compounds were isolated
by triple extraction of a solution of oil (10 g) in hexane (20 ml) with
30 ml of a methanol-water mixture (60:40, v/v). The Folin-Ciocalteau
reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was
added to a suitable aliquot of the combined extracts and the
absorption of the solution at 725 nm was measured. Values are
given as milligrams of caffeic acid per kilogram of oil (Guttfinger,
1981). Ortho-diphenols were also measured colorimetrically at 370
nm after adding 5% (w/v) sodium molybdate in 50% ethanol to the
extract (Guttfinger, 1981). Results are given as milligrams of caffeic
acid per kilogram of oil.

Oil stability
Oxidative stability was evaluated by the Rancimat method
(Gutierrez, 1989). Stability was expressed as the oxidation induc-
tion time (hours), measured with the Rancimat 743 apparatus
(Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g
warmed at 100°C with an air flow of 10 l/h.

Antiradical activity
The olive oil samples were examined for their capacity to scavenge
the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Kalantzakis
et al., 2006). Oil solution 1 ml in ethyl acetate (10%, w/v) was
added to 4 ml of a freshly prepared DPPH solution (10⁻⁶M in ethyl
acetate) in a screw-capped 10 ml test tube. The reaction mixture
was then vigorously shaken for 10 s in a vortex apparatus and the
tube was maintained in the dark for 30 min, until a steady state was
reached. The absorbance of the mixture was measured at 515 nm
against a blank solution. A control sample (no oil) was prepared
daily and measured. A refined olive oil (Minerva S.A. edible Oils,
Shimata, Viota, Greece), devoid of pro-oxidants/antioxidants, was
used for comparison. The radical scavenging activity (RSA) toward
DPPH was expressed as the percent reduction in DPPH
concentration by the constituents of the oils:

% [DPPH] red = 100-(1 [DPPH]0/DPPH]30)

Where, [DPPH]0 and [DPPH]30 were the concentrations of DPPH
in the control sample (t = 0) and in the test mixture after the 30 min
reaction, respectively.

Phenolic compounds
Analysis of phenolic compounds was performed by reverse phase-
Table 1. Means and standard deviations for the regulated physicochemical quality in the olive oil samples analyzed from the Chétoui olive at different altitudes at the same stage of maturation. The results are expressed as mean ± standard deviation of 3 samples.

<table>
<thead>
<tr>
<th>Component</th>
<th>Altitude</th>
<th>EEC regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>403 m</td>
<td>379 m</td>
</tr>
<tr>
<td>Free acidity (%C18:1)</td>
<td>0.25 ± 0.10</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>Peroxide value (meq O2 kg⁻¹)</td>
<td>2.92 ± 0.36</td>
<td>5.16 ± 1.25</td>
</tr>
<tr>
<td>UV absorption (K270)</td>
<td>0.18 ± 0.03</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>UV absorption (K232)</td>
<td>1.69 ± 0.16</td>
<td>1.41 ± 0.35</td>
</tr>
<tr>
<td>Chlorophylls mg kg⁻¹</td>
<td>4.51 ± 0.39</td>
<td>3.63 ± 0.08</td>
</tr>
<tr>
<td>Carotenoids mg kg⁻¹</td>
<td>2.15 ± 0.30</td>
<td>1.66 ± 0.09</td>
</tr>
<tr>
<td>Total phenols mg kg⁻¹</td>
<td>1128.27 ± 13.2</td>
<td>828.24 ± 58.97</td>
</tr>
<tr>
<td>o-diphenols mg kg⁻¹</td>
<td>282.82 ± 40.95</td>
<td>91.90 ± 11.95</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>73.25 ± 5.81</td>
<td>42.02 ± 0.57</td>
</tr>
<tr>
<td>Radical scavenging activity (%)</td>
<td>94.94 ± 1.24</td>
<td>85.17 ± 0.99</td>
</tr>
</tbody>
</table>

Mean ± SD, significant differences within the same row are shown by different letters (P < 0.05). EEC, European Economic Community.

RESULTS AND DISCUSSION

Quality parameters

All the oils (Table 1) showed values well below the maximum admitted for the regulated physicochemical parameters: (acidity ≤ 0.8; peroxide value ≤ 20 meq O2/Kg; K270 ≤ 0.22; K232 ≤ 2.5). This value permits all of them to be included in the “extra virgin” category, as stated by the European Economic Community Regulation, 2003. Was the decrease significant (P < 0.05).

Fatty acid composition

Total fatty acids composition is an important aspect of the quantitative evaluation of olive oils. It is also used to assure their authenticity and to discover frauds, such as mixtures with other vegetable oils (Christopoulos et al., 2004). Among fatty acids, palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acids were identified (Table 2). Palmitic, stearic, oleic and linoleic acids were quantified as the major ones. Palmitoleic, linolenic and arachidic acids were also determined, but in smaller amounts, in all the samples. The study of acidic composition of Chétou olive oils of various origins allowed distinguishing differences in the levels of the main fatty acids, which result sharply influenced the altitude (Table 2).

Palmitic acid showed inconstant variations among locations, whereas the levels of oleic acid decreased gradually.

The gradient elution program used was: 95% (A) / 5% (B) in 0 min, then an aliquot of 20 μl of the final solution was injected into a JASCO HPLC system equipped with a double plunger pump and a diode array UV detector. A Lichrospher 100 RP-18 column (250 mm x 4 mm id, particle size 5 μm, Merck) was used. The mobile phase consisted of a mixture of water/phosphoric acid (99:5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The gradient elution program used was: 95% (A) / 5% (B) in 0 min, 70% (A) / 30% (B) in 25 min, 62% (A) / 38% (B) in 40 min, 45% (A) / 45% (B) in 45 min, 52.5% (A) / 47.5% (B) in 5 min and 100% (B) in 5 min. Less than 100% (B) was maintained for 5 min and the run was stopped. Quantification of total phenols was carried out at 280 nm using p-hydroxyphenyl acetic acid as internal standard and the quantification of flavones and ferulic acid was performed at 335 nm using o-coumaric acid as internal standard. Results were expressed in mg kg⁻¹ oil. All the determinations were performed in triplicate.

Statistics

All parameters were determined in triplicate for each sample. Analysis of variance (ANOVA) was processed with the SPSS statistical package (Version 12.0 for Windows, SPSS Inc. Chicago, Illinois, 2003). The significance of differences at a 5% level among means was determined by one way ANOVA, using the Tukey’s test. ANOVA was applied to evaluate the influence of growing area conditions on Chétoui olive oil. PCA and hierarchical cluster analysis (HCA) were carried out using XLStat-Pro7.5 (2007) for Windows (Addinsoft, New York, USA).
passing from higher to lower altitudes. On the contrary, linoleic acid levels showed an opposite trend. The ratio unsaturated/saturated fatty acids for the oils obtained at lower altitudes ranged between one and three. This ratio increased at the higher elevations (three to five) (Table 2). This increase was probably related to the fact that lower temperatures favour the oil unsaturation. Low temperatures reduce the rate of chemical reactions and microbial activity that may result in the loss of quality of the olive fruits and consequently, also of quality of the extracted oil. Similar results were reported by Osman et al. (1994) for different cultivars.

**Chlorophylls and carotenoids**

Chlorophylls and carotenoids are very common pigments that give colour to vegetables and several fruits (Del Giovine and Fabietti, 2005) and play key roles in photosynthesis. Neither chlorophylls nor carotenoids can be synthesized by animals, though they need to obtain these molecules with food that will be chemically modified during assimilation. Several reports have demonstrated the healthy role of many plant pigments (Mayne et al., 1996), that is, the potential health benefit of antioxidants intake of a diet rich in carotenoids for the prevention of cardiovascular and degenerative diseases (Landrum and Bone, 2001).

Oils obtained from olives cultivated at higher altitudes showed higher chlorophyll content compared with those from lower altitudes. Chlorophyll content varied between 3.1 and 4.5 mg/kg, according to the location. Elevation influenced not only the chlorophyll content but also that of carotenoids that followed the same trend, ranging from 2.15 mg/kg (403 m) to 1.28 mg/kg (10 m) (Table 1).

The chlorophyll content could contribute to the photo-oxidative deterioration of the oil, increasing its peroxide value (Interesse et al 1971; Carlsson et al., 1976). The loss of chlorophylls corresponds to the forma-tion of other pigments, such as anthocyanins, responsible for the purple colour of the olives, or the products of oxidation of phenolic compounds (oleuropein), respon-sible for the blue colour (Fedeli, 1977).

**Oxidative stability**

Stability to oxidation is an important property of olive oil, which is improved by synergistic interactions between the various antioxidants present in the oil itself, and also depends on the lipid composition. A direct correlation between stability and altitude of cultivation was observed, in particular the olive oil from the higher locations exhibited the highest stability (73.25 h vs. 16.78 h) (Table 1), was significantly different (P < 0.05). This charac-teristic could be attributed in part to the lower levels of rainfall in these regions, as reported by Tovar et al. (2002), which hypothesized that oxidative stability is significantly affected by the water regime that in turn determines the phenols content of the olive fruit. On the other hand, genetic factor and the ensuing high levels of mono unsaturated fatty acids (MUFA) could also be causative factors of these findings. Furthermore, the greater stability of the oil from higher elevations could be probably related to the fact that low temperatures favour the oil unsaturation. Table 4 shows this relationship for “Chetoui” oils. The correlation obtained for samples over-all showed a good linear relationship between total phenols (r = 0.74), α-diphenols (r = 0.90) and oxidative stability measured by Rancimat.

**Antiradical activity**

Further experimentation on the radical scavenging

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Table 2. Changes in fatty acid composition of olive oil obtained from plant cultivated at different altitudes. The results are expressed as mean ± standard deviation of 3 samples.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>403 m</th>
<th>379 m</th>
<th>354 m</th>
<th>21 m</th>
<th>10 m</th>
<th>ECC regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>12.55±0.16</td>
<td>9.02±0.18</td>
<td>11.32±0.18</td>
<td>11.64±0.04</td>
<td>13.04±0.16</td>
<td>7.5-20.0</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.31±0.00</td>
<td>0.25±0.09</td>
<td>0.32±0.08</td>
<td>0.32±0.07</td>
<td>0.38±0.05</td>
<td>0.3-3.50</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.28±0.17</td>
<td>2.94±0.04</td>
<td>2.96±0.16</td>
<td>3.14±0.01</td>
<td>3.59±0.08</td>
<td>0.5-5.0</td>
</tr>
<tr>
<td>C18:1</td>
<td>67.85±0.43</td>
<td>68.73±0.59</td>
<td>63.16±1.28</td>
<td>58.73±0.08</td>
<td>52.92±0.79</td>
<td>55.0-83</td>
</tr>
<tr>
<td>C18:2</td>
<td>15.01±0.34</td>
<td>18.13±0.02</td>
<td>19.84±0.69</td>
<td>25.78±0.07</td>
<td>28.81±0.60</td>
<td>3.5-21.0</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.55±0.02</td>
<td>0.67±0.03</td>
<td>0.76±0.09</td>
<td>0.83±0.02</td>
<td>0.76±0.01</td>
<td>≤1.0</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.40±0.05</td>
<td>0.47±0.01</td>
<td>0.44±0.02</td>
<td>0.28±0.33</td>
<td>0.48±0.01</td>
<td>≤0.6</td>
</tr>
<tr>
<td>SFA</td>
<td>16.23±0.12</td>
<td>12.43±0.08</td>
<td>14.72±0.12</td>
<td>15.06±0.13</td>
<td>17.11±0.08</td>
<td>-</td>
</tr>
<tr>
<td>MUFA</td>
<td>68.16±0.21</td>
<td>68.98±0.63</td>
<td>63.49±0.68</td>
<td>57.05±0.07</td>
<td>53.30±0.42</td>
<td>-</td>
</tr>
<tr>
<td>PUFA</td>
<td>15.56±0.18</td>
<td>18.8±0.02</td>
<td>20.61±0.34</td>
<td>27.06±0.17</td>
<td>29.57±0.30</td>
<td>-</td>
</tr>
<tr>
<td>MUFA/PUFA</td>
<td>4.38</td>
<td>3.66</td>
<td>3.08</td>
<td>2.10</td>
<td>1.80</td>
<td>-</td>
</tr>
</tbody>
</table>

C16:0, palmitic; C16:1, palmitoleic; C18:0, stearic; C18:1, oleic; C18:2, linoleic; C18:3, linolenic; C20:0, arachidic; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ECC, European Economic Community.
activity (RSA) of these VOO permitted the verification of the characteristics discussed earlier (Table 1). Radical scavenging activity of the Chetoui olive oils varied according to altitude (54.80 to 94.94%). Chetoui oils produced at higher altitudes showed the highest scavenging ability, with an average value of 94.94%. Conversely, at lower altitudes, the average value dropped down to 54.80%. This activity is necessarily linked to the much lower oxidative stability and oleic acid content. Our results show significant variation of the radical scavenging activity of the Chétoui oils due to the effect of cultivar-environment (altitude) interaction. Overall, the correlation showed a positive linear relationship between radical scavenging capacity and oxidative stability measured by Rancimat. ($r = 0.67$). These results are similar to those reported by several authors for other olive oil varieties (Usenik et al., 2008).

### Quantification of phenolic components

The olive oils from different altitudes did not show significant qualitative differences in the HPLC profile of their phenolic fraction. However, significant quantitative differences were observed for many of the phenols (Table 3) was ($P<0.05$). The most representative components were the dialdehydic form of elenolic acid linked to hydroxytyrosol and the dialdehydic form of elenolic acid linked to tyrosol. A higher concentration of these two chemicals was observed in the olive oil obtained from plants cultivated at 403 m, with 17.61 and 26.94 mg kg$^{-1}$, respectively.

Among secoiridoids, the dialdehydic form of ligrostide aglycone was identified in relatively high amounts, followed by the dialdehydic form of oleuropein aglycone. Both the secoiridoids were produced in greater quantities by plants growing at higher altitude: 114.17 vs. 726.69 mg kg$^{-1}$ for ligrostide aglycone and 3.24 vs. 42.60 mg kg$^{-1}$ for oleuropein aglycone (Table 3). According to ANOVA tests, these differences were significant. Other simple phenols, such as vanillic, vanillin, ferulic acid and $p$-coumaric acid, were detected in very low amounts.

Concerning lignans, the pinoresinol content varied between 0.14 (403 m) and 0.42 mg/kg (379 m), whereas acetoxypinoresinol was detected in larger quantities, ranging between 0.34 (10 m) and 2.14 mg/kg (379 m). Pinoresinol and acetoxypinoresinol were recently described in the olive oil of other cultivars (Brener, 2000; Owen, 2000). According to Owen et al. (2000), they form the main components of the phenolic fraction of the olive seeds, while they are completely absent in the flesh, leaves and branches. Therefore their presence in the oil is due to the breaking of the pits when the olives are crushed.

The extra virgin olive oil obtained from Chétoui olives collected at different altitudes showed quite high total phenols content, exceeding sometimes 800 mg kg$^{-1}$ (Folin-Ciocalteu method). The total phenol content was positively correlated with the altitude, ranging from 817.33 mg/kg (403 m) to 131.91 mg/kg (10 m) (Table 1). These results are in good agreement with those reported for the Cornicabra variety (Salvador et al., 2001). Similar mean values were also reported for Burlat and Van Cherrys by Goncalves et al. (2007) and Gao and Mazza (1995), while lower values for Burlat were reported by Usenik et al. (2008).

As it was hypothesised, it is possible that the lower temperatures of the higher elevation sites trigger the mechanism for producing more phenolics. Similarly, cold stress induced the accumulation of phenolic compounds in apples (Thomai et al., 1998), tomato and watermelon  

### Table 3. Phenols amount (mg/kg) in virgin olive oil from Chetoui variety at different altitudes. The results are expressed as mean ± standard deviation of 3 samples.

<table>
<thead>
<tr>
<th>Component</th>
<th>403 m</th>
<th>379 m</th>
<th>354 m</th>
<th>21 m</th>
<th>10 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxytyrosol</td>
<td>17.61 ± 0.03</td>
<td>13.28 ± 0.04</td>
<td>13.18 ± 0.06</td>
<td>12.71 ± 0.05</td>
<td>2.92 ± 0.09</td>
</tr>
<tr>
<td>Tyrosol</td>
<td>26.94 ± 0.56</td>
<td>24.25 ± 0.10</td>
<td>23.83 ± 0.05</td>
<td>16.28 ± 0.12</td>
<td>9.32 ± 0.05</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0.19 ± 0.03</td>
<td>0.06 ± 0.00</td>
<td>0.05 ± 0.002</td>
<td>0.42 ± 0.01</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.31 ± 0.01</td>
<td>0.36 ± 0.03</td>
<td>0.35 ± 0.05</td>
<td>0.26 ± 0.01</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td>$p$-coumaric acid</td>
<td>0.53 ± 0.03</td>
<td>0.50 ± 0.01</td>
<td>0.34 ± 0.04</td>
<td>0.19 ± 0.01</td>
<td>0.25 ± 0.10</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.38 ± 0.06</td>
<td>0.33 ± 0.01</td>
<td>0.47 ± 0.01</td>
<td>0.15 ± 0.06</td>
<td>0.25 ± 0.06</td>
</tr>
<tr>
<td>Dialdehydic form of oleuropein aglycone</td>
<td>42.60 ± 1.35</td>
<td>29.40 ± 0.34</td>
<td>28.49 ± 0.30</td>
<td>27.39 ± 0.47</td>
<td>3.24 ± 0.20</td>
</tr>
<tr>
<td>Dialdehydic form of ligrostide aglycone</td>
<td>726.69 ± 5.60</td>
<td>344.72 ± 4.87</td>
<td>327.16 ± 1.30</td>
<td>279.24 ± 5.50</td>
<td>114.17 ± 6.01</td>
</tr>
<tr>
<td>Pinoresinol</td>
<td>0.14 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.36 ± 0.01</td>
<td>0.22 ± 0.00</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>Acetoxyypinoresinol</td>
<td>1.94 ± 0.54</td>
<td>2.14 ± 0.12</td>
<td>1.81 ± 0.04</td>
<td>0.98 ± 0.03</td>
<td>0.34 ± 0.23</td>
</tr>
<tr>
<td>Total phenols</td>
<td>817.33</td>
<td>415.46</td>
<td>396.04</td>
<td>337.84</td>
<td>131.91</td>
</tr>
</tbody>
</table>

**Note:** Mean ± SD, significant differences within the same row are shown by different letters ($P < 0.05$).
Table 4. Relationship between content in some phenolic compounds, radical scavenging activity and oxidative stability of the VOO samples obtained from Chetoui variety cultivated at different altitudes. The results are expressed as mean ± standard deviation of 3 samples.

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Scavenging activity</th>
<th>Oxidative stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH. Tyrosol</td>
<td>0.80</td>
<td>0.89</td>
</tr>
<tr>
<td>Tyrosol</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Vanillic</td>
<td>0.81</td>
<td>-0.73</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.60</td>
<td>-0.68</td>
</tr>
<tr>
<td>p. coumaric</td>
<td>0.71</td>
<td>0.70</td>
</tr>
<tr>
<td>Ferulic</td>
<td>0.83</td>
<td>0.67</td>
</tr>
<tr>
<td>Dialdehydic form of oleuropein aglycon</td>
<td>0.81</td>
<td>0.90</td>
</tr>
<tr>
<td>Dialdehydic form of ligstroside aglycon</td>
<td>0.80</td>
<td>0.91</td>
</tr>
<tr>
<td>Pinoresinol</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>Acetoxyioresinol</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>Total phenol</td>
<td>0.92</td>
<td>0.86</td>
</tr>
<tr>
<td>o-diphenols</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td>MUFA/PUFA</td>
<td>0.88</td>
<td>0.84</td>
</tr>
</tbody>
</table>

plants (Rivero et al., 2001).

Oxidative stability of the oils was positively correlated to hydroxytyrosol ($r = 0.89$), tyrosol ($r = 0.91$), p-coumaric acid ($r = 0.7$), ferulic acid ($r = 0.67$), vanillin acid ($r = 0.83$), dialdehydic form of oleuropein aglycon ($r = 0.9$) and dialdehydic form of ligstroside aglycon ($r = 0.91$), and negatively correlated to vanillic acid ($r = 0.73$) and vanillin ($r = 0.68$) (Table 4). The results of the antioxidant tests of the phenolics suggest that the Chétoui olive oil part of the antioxidative effectiveness of these chemicals on the oil itself can be explained by their radical scavenging activity, as also observed by Morello et al. (2005) with the DPPH test. Radical scavenging activity was positively correlated to tyrosol ($r = 0.91$), hydroxytyrosol ($r = 0.8$), vanillic acid ($r = 0.81$), ferulic acid ($r = 0.83$), dialdehydic form of ligstroside aglycone ($r = 0.81$), dialdehydic form of oleuropein aglycone ($r = 0.8$) and p-coumaric acid ($r = 0.71$). On the contrary, radical scavenging activity was negatively correlated to vanillic acid ($r = 0.6$) (Table 4).

Discriminant analysis

The application of the principal component analysis algorithm to data and clustering of olive oil samples using all collected data showed three distinctive groups (Figures 1 and 2). By the observation of eigenvalues, it can be concluded that two factors were sufficient to account for 82.44% of the total variance (Factor 1: 63.87%; Factor 2: 18.57%). The first group was composed by Chetou oils obtained from plants cultivated at the lowest altitudes, the second one by plants grown at average altitudes (about 300 m) and the third one composed by the single sample growing at the highest altitude (>400 m), (Figure 2). These results imply a great difference in terms of fatty acid composition, phenols compounds, o-diphenols content and oxidative stability of the oils characterizing these varieties. The PCA algorithm (Figure 1) explains this classification. Indeed, the first group (lower altitude) was correlated with C18:2, poly unsaturated fatty acids (PUFA), C16:1, C18:3, vanillic and vanillin, saturated fatty acid (SFA), PV and K232. The second group (average altitude) was correlated with caffeic, C18:1 and MUFA. The last group (highest altitude) was correlated with C18:1, tyrosol, hydroxytyrosol, carotenoids, total phenols, o-diphenols, oxidative stability, MUFA/PUFA and p-coumaric acid. These conclusions are in good agreement with the results presented in (Tables 1, 2 and 3).

Conclusion

Summarizing, the elevation of the cultivation site has a marked influence on the antioxidant content, with higher elevation orchards producing olives with larger contents of antioxidant compounds with respect to lower elevation ones. These results indicate that the cultivar and the environmental conditions influence the quantitation production of phenolic compounds. Consequently, phenolic compounds may be used as altitude indicators for Chetoui oils.

ACKNOWLEDGEMENTS

This work had been done as part of a National Research...
Figure 1. Principal component analysis algorithm. Hydroxytyrosol, tyrosol, \( p \)-coumaric acid, ferulic acid, dialdehydic form of oleuropein aglycon, dialdehydic form of ligstroside aglycon, Pinoresinol, Acetoxypinoresinol, vanillic acid, vanillin acid, acidity, K232, K270, chlorophylls, oxidative stability, radical scavenging activity, fatty acid, SFA and MUFA/PUFA.

Figure 2. Clustering of olive oil samples using all collected data. 1, 403 m; 2, 10 m; 3, 354 m; 4, 379 m; 5, 21 m.
Project. We thank the Ministry of Higher Education, Scientific Research and Technology for financially supporting this program. This work was supported by The Tunisian Ministry of High Education, Scientific Research and Technology. The authors gratefully acknowledge the personnel of the Laboratories.

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affect their occurrence in the oil. J. A. Chromatogr. 1054: 113-127.