Geographical Discrimination of Virgin Olive Oils from the Tunisian Coasts by Combining Fatty Acids and Phenolic Acids Profiles within a Multivariate Analysis

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Abstract: Virgin olive oils extracted from three principal Tunisian olive cultivars (Chemlali, Chetoui and Zarrazi) and coming from four different regions (Sfax, Beja, Gabes and Medenine) along the Tunisian coasts were analysed. The quality indices as well as fatty acids and phenolic acids content of oil samples were examined using univariate and multivariate statistical analysis. The finding demonstrated that significant differences (p < 0.05) were found in quality indices between the different cultivars and that fatty acid content is the most informative in discriminating olive oils from production sites that are different by geographical and climatic parameters. In fact, southern cultivars (Zarrazi Gabes and Zarrazi Medenine) have the best fatty acid combination according to their oxidative effect. Besides, phenolic acids content was not useful in discriminating olive oil samples and could depend not only on geographic location but also on olive variety and agronomic practices. Nevertheless, Principal Component Analysis allowed us to highlight the Chemlali Beja olive oil for its interesting oxidative stability, fatty acid composition and its richness in phenolic acids content.

Key words: virgin olive oils, geographic effect, fatty acid composition, phenolic acids, Principal Component Analysis

1 Introduction

Over the years, the Mediterranean diet has been promoted as a model for healthy eating; consequently, as a significant part of this diet, the production and consumption of olive oil is becoming more popular¹. Olive oil has components with antioxidant properties, mainly high monounsaturated to polyunsaturated acids, tocopherols, carotenoids, chlorophylls and other phenolic compounds¹–³. The most important classes of phenolic compounds present in olive fruits are phenolic alcohols, secoiridoids, flavonoids, and phenolic acids⁴. However, several studies claim that the quality of the olive oil depends on many factors, such as geographic⁴, harvesting time, agronomic conditions: irrigation regime and fertilization, technological factors such as the extraction system and post-harvest storage conditions⁵.

In Tunisia, the presence of typical cultivars, the peculiar microclimate conditions and precise olive orchard management lead to the production of very valuable olive oils with a distinctive taste. Tunisia is the world’s fourth largest producer and the second largest exporter of olive oil, whose oleoculture depends essentially on two cultivars: Chemlali and Chetoui⁶–⁷. These olive cultivars have increased their growing areas while others, called secondary cultivars, are losing their traditional locations despite their good quality and authenticity. In order to ameliorate the quality of the Tunisian olive oil, many exhaustive studies are in progress of some important olive cultivars. So we can find studies on secondary cultivars like: Chemchali, Gerboui, Zalmati and Oueslati⁸. However, the presence of many different factors makes difficult to characterize olive oils using a small number of chemical compounds or a simple data manipulation, therefore, samples should be characterized by a large number of parameters (chemical profiles such as: major components (fatty acid), minor components and/or sensory descriptors), and data should be analysed by statistical techniques or artificial intelligence algorithms. Consequently, univariate and multivariate statistical methods are applied to numerous variables to obtain significant interpretation⁹. These statistical techniques such as princi-
principal component analysis (PCA) could be applied to the classification of olive oils with respect to their different characteristics such as geographical origin or olive cultivar using various spectroscopic or chemical parameters. In fact, some cultivars of olive oil are recognized as being of higher quality because they derive from well-defined geographical areas, command better prices, and generally are legally protected. Indeed, the aim of protected designations of origin (PDO) is to add value to certain specific high-quality products from a particular origin.

This study investigates the effect of growing region on several quality characteristics of Chemlali, Chetoui and Zarrazi olive oil cultivars based on the analysis of parameters such as peroxide index, coefficients K270 and K322, oxidative stability, minor components, such as chlorophylls, carotenoids and especially on the phenolic acids and fatty acids.

Eventually, this study aimed to investigate the differentiation power of various chemical parameters for a monovarietal olive oil obtained from local olives of a different geographical area.

2 Experimental

2.1 Olive oil samples

The olive oil samples were obtained from fully ripened olives and they belong to various Tunisian cultivars. They were collected as follows: three samples of Chemlali, namely Chemlali Sfax (N: 34.4426°, E: 10.4537°), and Chemlali Beja (N: 36.3118°, E: 09.0620°), two samples of Chetoui, namely Chetoui Sfax and Chetoui Beja, two samples of Zarrazi, namely Zarrazi Gabes (N: 33.3704°, E: 10.1707) and Zarrazi Medenine (N: 33.5253°, E: 10.0553°). After harvesting, the olive fruit samples were immediately transported to the laboratory, where oil was extracted within 24 h using a small oleodoseur extraction unit. The oil was transferred into dark glass bottles, and stored at 4°C until physico-chemical analysis.

2.2 Quality Parameters

The free acidity, the peroxide value and UV absorption characteristics, K270 and K322 were carried out, following the analytical methods described by European Union standard methods. Oxidative stability was determined in terms of hour using Rancimat 679 apparatus (Metrohm, Switzerland). The temperature range of this equipment is 50–220°C with temperature stability less than 0.1°C. Five grams of sample were placed inside the glass reaction vessel for the analysis. Deionized water was the carrier medium. For both columns of the equipment, the reaction temperature and air flow rate were kept constant at 100°C in air flow of 20 L/h.

2.3 Fatty Acid Composition

The fatty acid methyl esters (FAMEs) were prepared as described by European Union standard methods. FAMEs were prepared by shaking vigorously a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potassium hydroxide, and analyzed by gas chromatography (Shimadzu chromatograph equipped with a flame ionization detector (FID) and a fused silica column (30 m length × 0.32 mm i.d. and thickness 0.25 μm) made with 50% cyanopropylmethyl-50% phenylmethyl-polysiloxane). An injection volume of 1 μL was used. The carrier gas was nitrogen with a flow rate of 1 mL/min. The injector and detector temperatures were set at 220°C, whereas the oven temperature was held to 180°C. Ten fatty acids including C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0 and C20:1 were identified from their retention times.

2.4 Determination of chlorophyll and carotenoid contents

Chlorophyll and carotenoid concentrations of the samples were determined colorimetrically as previously described by Minguez-Mosquera et al. in 1991. The olive oil sample (7.5 g) was dissolved in 25 mL of cyclohexane and the absorbance of the samples at 470 and 670 nm, which are associated with carotene and chlorophyll, respectively, were measured with a UV spectrophotometer (Shimadzu UV-1601 Spectrophotometer, Japan).

2.5 Phenolic profiles of olive oils

2.5.1 Phenolic extraction

The phenolic extracts were obtained following the procedure of Ocakoglu et al. in 2009. A sample of olive oil (14 g) was extracted by using 4 × 14 mL of methanol/water (80:20 v/v). Methanol was removed, and then 15 mL of acetonitrile was added to the residue and washed with (3 × 20 mL) of hexane. The resulting acetonitrile solution was evaporated under vacuum and the residue was flushed with nitrogen and dissolved in 1 mL of methanol/water. Final extract was filtered through a 0.45 μm pore-size membrane filter and transferred into a tube. The extract (20 μL) was immediately injected to HPLC. Gallic acid was used as the internal standard.

2.5.2 Total phenol content

The total amount of phenolic compounds was determined using the Folin–Ciocalteau method. In a 20 mL volumetric flask, a volume of 0.5 mL of Folin–Ciocalteau reagent was added to 2 mL of the phenolic extract. After 3 min, 4 mL of a sodium carbonate solution (10%) was added, and the total volume was adjusted with distilled water to 20 mL. After 90 min of incubation in the dark, the solution was centrifuged, and the absorbance was read at 765 nm. The total phenol content was expressed in mg equivalent of gallic acid per kilogram of oil (mg GAE/kg). Three measurements were performed for each sample.
2.5.3 HPLC analysis of phenolic acids

HPLC system, JASCO equipped with a silica column (250 mm length × 4.6 mm, i.d.) was used to analyze phenolic acids. Initial concentrations of the mobile phases were 90% for water/acetic acid and 10% for methanol and the concentrations were adjusted according to the following procedure: firstly, the concentration of methanol was raised to 30% in 10 min and kept for 20 min. Then, the MeOH% was increased to 40% in 10 min, and kept for another 5 min. Followed by raising it up to 50% in 5 min, and maintaining it at this concentration for another 5 min. At last, MeOH was increased to 60, 70 and 100% in 5 min periods. Finally, initial conditions were obtained at the end of 85 min. Chromatograms were obtained at 280 and 320 nm and different phenolic acids were identified by comparing their retention times with those of commercial standards. Phenolic acids were quantified by using their respective 5-point calibration curves and the results were expressed in terms of mg/kg.

2.6 Statistical analysis

All parameters were measured in triplicate for each sample. Analysis of variance (ANOVA) was processed by SPSS statistical package (Version 19.0 SPSS Ltd., Woking, UK). The significance of differences at a 5% level among means was determined by one-way ANOVA, using Tukey’s test. Analysis of variance (ANOVA) was applied in order to evaluate the influence of growing area conditions on Tunisian olive oil cultivars. The principal component analysis (PCA) was applied to separate growing regions according to all the parameters investigated without any rotation. The PCA type was Pearson (n), the biplot type was correlation biplot and the coefficient was automatic. The Pearson correlation matrix and the PCA plots were performed using XLSTAT software for Windows (v.2013.2.03, Addinsoft, NY, USA). A uniform hierarchical cluster analysis (HCA) methodology was also applied on data to investigate the relationship between studied cultivars. HCA was performed using XLSTAT® software for Windows (v.2013.2.03, Addinsoft, NY, USA). Each cluster was determined by the Squared Euclidean distance matrix and the Ward method, generating dendrograms for olive oil samples.

3 Results and Discussion

3.1 Quality indices

Free acidity is considered as an important quality index, which has been used exclusively as a traditional criterion for classifying olive oil. The free acidity of the olive oil samples did not exceed the upper limit of 0.8% established by the COI norm. As shown in Table 1, olive oil samples exhibited quality indices within the ranges established for “extra virgin” olive oil category and show that the geographical origin has significant influence ($p < 0.05$).

The peroxide values evaluate the hydroperoxide content and offer a measure of lipid oxidation. The peroxide values content observed in the virgin olive oil samples investigated ranged between 6.00 to 18.54 meqO₂/kg oil (Table 1). It should be noted that lower peroxide values of 6 and 10.52 were recorded for the Zarrazi Gabes and the Chetoui Sfax cultivars, respectively. All the olive oil samples presented peroxide values within the range of the “extra virgin” olive oil category (Table 1).

UV spectrophotometric characteristics were expressed by measuring the specific extinction coefficients at 232 and 270 nm corresponding to the maximum absorbance of hydroperoxides and secondary products of oxidation. The values of $K_{232}$ and $K_{270}$ were varied between 1.54–2.20 and 0.05–0.12, respectively. As presented in Table 1, $K_{232}$ and $K_{270}$ were ranged in the levels established for the highest quality category “extra virgin” olive oil.

### Table 1: Quality indices, colour pigments and total phenols in Chemlali, Chetoui and Zarrazi olive oils grown in different geographical regions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chemlali Sfax</th>
<th>Chemlali Beja</th>
<th>Chetoui Sfax</th>
<th>Chetoui Beja</th>
<th>Zarrazi Gabes</th>
<th>Zarrazi Medenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free acidity (%)</td>
<td>0.36 ± 0.01$^a$</td>
<td>0.21 ± 0.02$^a$</td>
<td>0.20 ± 0.01$^a$</td>
<td>0.42 ± 0.03$^d$</td>
<td>0.24 ± 0.02$^b$</td>
<td>0.26 ± 0.01$^b$</td>
</tr>
<tr>
<td>PV (meq O₂/kg)</td>
<td>10.52 ± 0.87$^b$</td>
<td>11.57 ± 1.65$^b$</td>
<td>18.54 ± 1.22$^c$</td>
<td>16.57 ± 0.98$^c$</td>
<td>6.00 ± 0.41$^a$</td>
<td>17.24 ± 1.21$^c$</td>
</tr>
<tr>
<td>$K_{232}$</td>
<td>1.77 ± 0.04$^d$</td>
<td>2.25 ± 0.05$^b$</td>
<td>2.20 ± 0.06$^b$</td>
<td>1.85 ± 0.00$^b$</td>
<td>1.54 ± 0.01$^a$</td>
<td>1.75 ± 0.05$^b$</td>
</tr>
<tr>
<td>$K_{270}$</td>
<td>0.12 ± 0.02$^a$</td>
<td>0.12 ± 0.01$^b$</td>
<td>0.05 ± 0.00$^a$</td>
<td>0.06 ± 0.01$^a$</td>
<td>0.11 ± 0.03$^b$</td>
<td>0.11 ± 0.02$^b$</td>
</tr>
<tr>
<td>Oxidative stability (h)</td>
<td>4.27 ± 0.21$^a$</td>
<td>4.78 ± 1.82$^a$</td>
<td>4.34 ± 1.23$^a$</td>
<td>7.67 ± 2.41$^b$</td>
<td>11.26 ± 1.56$^c$</td>
<td>12.37 ± 0.24$^d$</td>
</tr>
<tr>
<td>Chlorophyll (ppm)</td>
<td>5.95 ± 0.12$^c$</td>
<td>4.85 ± 0.11$^b$</td>
<td>3.01 ± 0.08$^a$</td>
<td>4.94 ± 0.06$^b$</td>
<td>3.05 ± 0.09$^a$</td>
<td>5.82 ± 0.11$^c$</td>
</tr>
<tr>
<td>Carotenoids (ppm)</td>
<td>10.05 ± 0.21$^a$</td>
<td>9.94 ± 0.12$^a$</td>
<td>6.11 ± 0.06$^a$</td>
<td>8.07 ± 0.06$^b$</td>
<td>7.95 ± 0.08$^b$</td>
<td>10.03 ± 0.12$^c$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD
Values with different superscript letters (a-c) within a row are significantly different ($p < 0.05$) by Tukey’s test multiple comparison post hoc test.
Oxidative stability of all the olive oils used in this study is presented in Table 1. It was observed that olive oils belonging to Zarrazi cultivar (Gabies and Medenine regions) have the highest average (10.14 and 12.73 hours respectively). Statistically, olive oils from Chemlali and Chetoui have lower (p < 0.05) oxidative stability compared to samples of Zarrazi cultivar (Table 1). The oxidative stability value fluctuations could be due to the compositional parameters (concentration of minor compounds and fatty acid profile) as well as post-harvest conditions.

3.2 Chlorophyll and carotenoid contents

Chlorophylls and carotenoids are the main pigments in virgin olive oils which impact the characteristic color of the oil. Univariate statistical analysis showed that there are significant differences (p < 0.05) between chlorophyll content belonging to the same cultivar (Table 1). Chemlali Sfax olive oil samples had the highest average of chlorophyll content (5.95 mg/Kg), while Chetoui Sfax had the lowest one (3.01 mg/Kg). Carotenoid pigments displayed concentrations between 6.11 and 10.05 mg/kg (Table 1). In fact, significant differences (p < 0.05) were observed between carotenoid content in Chetoui and Zarrazi olive oils (Table 1).

3.3 Fatty acid composition

The specific fatty acid composition in olive oil varies depends on various factors such as olive cultivar, climate, latitude, and stage of maturity. The composition of fatty acids influences the stability, nutritional value and is characteristic of olive oils. Besides, the nutritional benefits are primarily related to the fatty acid composition, mainly due to both the high content of oleic acid and the balanced ratio of saturated and polyunsaturated fatty acids.

The mean FAME composition of samples from the analysed cultivars was shown in Table 2. The major fatty acids were oleic, linoleic, palmitic and stearic acids. Furthermore, a significant difference (p > 0.05) was observed between oleic acid in Chetoui, Chemlali and Zarrazi olive oils (Table 2). Equally, the oleic acid mean values for the Zarrazi cultivar had the highest average (Table 2). According to the Tukey test there was no statistically significant difference (p > 0.05) between the oleic acid content of olive oils belonging to Sfax and Beja (Table 2). Another important polyunsaturated fatty acid (PUFA), linoleic acid, was found in the range of 10.14 to 25.12% for Zarrazi Medenine.

### Table 2 Fatty acids in Chemlali, Chetoui and Zarrazi olive oils grown in different geographical regions.

<table>
<thead>
<tr>
<th></th>
<th>Chemlali Sfax</th>
<th>Chemlali Beja</th>
<th>Chetoui Sfax</th>
<th>Chetoui Beja</th>
<th>Zarrazi Gabes</th>
<th>Zarrazi Medenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>15.81 ± 0.13a</td>
<td>9.25 ± 0.08b</td>
<td>8.08 ± 0.04c</td>
<td>10.96 ± 0.11b</td>
<td>8.44 ± 0.06a</td>
<td>8.73 ± 0.10a</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.61 ± 0.06b</td>
<td>0.44 ± 0.00a</td>
<td>0.27 ± 0.01c</td>
<td>0.61 ± 0.02b</td>
<td>0.17 ± 0.00c</td>
<td>0.13 ± 0.01c</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.03 ± 0.00a</td>
<td>0.04 ± 0.00b</td>
<td>0.03 ± 0.00a</td>
<td>0.02 ± 0.00c</td>
<td>0.02 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
</tr>
<tr>
<td>C17:1</td>
<td>0.06 ± 0.00c</td>
<td>0.06 ± 0.00b</td>
<td>0.04 ± 0.00b</td>
<td>0.06 ± 0.00c</td>
<td>0.03 ± 0.00b</td>
<td>0.04 ± 0.00b</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.77 ± 0.02a</td>
<td>2.15 ± 0.05b</td>
<td>3.55 ± 0.06c</td>
<td>1.69 ± 0.02a</td>
<td>2.95 ± 0.02a</td>
<td>2.52 ± 0.04a</td>
</tr>
<tr>
<td>C18:1</td>
<td>61.92 ± 0.66a</td>
<td>74.19 ± 0.54b</td>
<td>61.47 ± 0.34c</td>
<td>73.62 ± 0.23a</td>
<td>75.45 ± 0.44a</td>
<td>77.16 ± 0.93a</td>
</tr>
<tr>
<td>C18:2</td>
<td>15.77 ± 0.55b</td>
<td>12.61 ± 0.42c</td>
<td>25.12 ± 1.21c</td>
<td>12.12 ± 0.04c</td>
<td>11.72 ± 0.98c</td>
<td>10.14 ± 0.03c</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.56 ± 0.01b</td>
<td>0.50 ± 0.03c</td>
<td>0.59 ± 0.00a</td>
<td>0.44 ± 0.01a</td>
<td>0.47 ± 0.01c</td>
<td>0.48 ± 0.00b</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.29 ± 0.01c</td>
<td>0.35 ± 0.00b</td>
<td>0.40 ± 0.01c</td>
<td>0.27 ± 0.00c</td>
<td>0.34 ± 0.00c</td>
<td>0.37 ± 0.01c</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.13 ± 0.00a</td>
<td>0.35 ± 0.01b</td>
<td>0.43 ± 0.02c</td>
<td>0.16 ± 0.00c</td>
<td>0.34 ± 0.00c</td>
<td>0.39 ± 0.01b</td>
</tr>
<tr>
<td>C18:1/ C18:2</td>
<td>3.92 ± 0.22b</td>
<td>5.88 ± 0.34c</td>
<td>2.44 ± 0.11a</td>
<td>6.07 ± 0.39a</td>
<td>6.43 ± 0.11a</td>
<td>7.61 ± 0.07a</td>
</tr>
<tr>
<td>∑SFA (%)</td>
<td>17.91 ± 0.44b</td>
<td>11.81 ± 0.11a</td>
<td>12.07 ± 0.09a</td>
<td>12.97 ± 0.14a</td>
<td>11.79 ± 0.04a</td>
<td>11.62 ± 0.05a</td>
</tr>
<tr>
<td>∑MUFA (%)</td>
<td>65.74 ± 0.64b</td>
<td>75.06 ± 0.45b</td>
<td>62.19 ± 0.22a</td>
<td>74.45 ± 0.12b</td>
<td>76.01 ± 0.23b</td>
<td>77.73 ± 0.21b</td>
</tr>
<tr>
<td>∑PUFA (%)</td>
<td>16.33 ± 0.23a</td>
<td>13.11 ± 0.05b</td>
<td>25.71 ± 0.12a</td>
<td>12.56 ± 0.22a</td>
<td>12.22 ± 0.09a</td>
<td>10.62 ± 0.12a</td>
</tr>
<tr>
<td>∑MUFA (%) / ∑PUFA (%)</td>
<td>4.02 ± 0.41b</td>
<td>5.72 ± 0.34b</td>
<td>2.41 ± 0.22a</td>
<td>5.92 ± 0.26b</td>
<td>6.23 ± 0.11b</td>
<td>7.31 ± 0.19b</td>
</tr>
</tbody>
</table>

C16:0: Palmitic acid; C16:1: Palmitoleic acid; C17:0: Margaric acid; C17:1: Heptadecenoic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; C18:3: Linolenic acid; C20:0: Arachidic acid; C20:1: Gondoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Values are expressed as mean ± SD. Values with different superscript letters (a-c) within a row are significantly different (p < 0.05) by Tukey’s test multiple comparison post hoc test.
and Chetoui Sfax, respectively (Table 2). The ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA) varied from 2.41 to 7.31 for Zarrazi Medenine and Chetoui Sfax, respectively (Table 2). The differences observed between locations for the fatty acid composition may be explained by the difference in altitude and temperature between the chosen regions. In fact, Beja is a Mediterranean coastal region located in the extreme North West of Tunisia whereas Sfax is a central coastal region while Gabes and Medenine are two South coastal regions. This result is consistent with the finding of Servili et al. in 2004 demonstrating that fatty acid composition could be related to the geographical location, the characteristics of the olive grove zones and the salinity of soil. Likewise, our results were similar with those of Ben Temime et al. in 2006 who suggested the importance of the environment parameter in defining the oil quality of the Tunisian Chetoui cultivar. In fact, they pointed out that virgin olive oils produced by Chetoui olive cultivar from Beja provenance were particularly rich in oleic acid and contain relatively low levels of linoleic acid compared to the Chetoui oils originated from other studied localities.

### 3.4 Phenolic composition

#### 3.4.1 Total phenol content

The amount of phenolic compounds in virgin olive oil is an important factor when evaluating the oil quality, given that the natural phenols improve its resistance to oxidation, and to a certain extent, are responsible for its sharp bitter taste. As shown in Table 1, the values obtained by colorimetric determination of total phenol content in oil extracts ranged between 187.86 mg GAE/kg (Chemlali Beja) and 266.88 mg GAE/kg (Chemlali Sfax). Furthermore, no significant differences (p > 0.05) were observed between total phenol content in Chetoui olive oils (Table 3). These results were in accordance with some reports by different authors demonstrating that the amount of phenolic compounds in olive oil varied from 50 to 1000 mg/kg depending on the location, the variety and the oil extraction process. Phenolic acids contribute, to a great extent, to the biological value and antioxidant properties of olive oil. Phenolic acids are able to inhibit the propagation of the autooxidation reactions of unsaturated fatty acids. Accordingly, phenolic acids are of great importance to the nutritional, sensory and shelf life characteristics of virgin olive oil. They have significant effects on the taste, bitterness, astringency, pungency and flavour stability of olive oil.

#### 3.4.2 Phenolic acids profile

Phenolic acids contribute, to a great extent, to the biological value and antioxidant properties of olive oil. Phenolic acids are able to inhibit the propagation of the autooxidation reactions of unsaturated fatty acids. Accordingly, phenolic acids are of great importance to the nutritional, sensory and shelf life characteristics of virgin olive oil. They have significant effects on the taste, bitterness, astringency, pungency and flavour stability of olive oil.

| Table 3 | Total phenols content (TPC) and phenolic acids (PA) in Chemlali, Chetoui and Zarrazi olive oils grown in different geographical regions. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Chemlali Sfax   | Chemlali Beja   | Chetoui Sfax    | Chetoui Beja    | Zarrazi Gabes   | Zarrazi Medenine |
| TPC (ppm)       | 266.68 ± 5.76   | 187.86 ± 2.98   | 220.18 ± 8.88   | 217.55 ± 7.65   | 188.63 ± 4.67   | 219.15 ± 8.97    |
| Hpha            | 4.51 ± 0.04     | 16.92 ± 0.21    | 11.31 ± 0.12    | 7.96 ± 0.09     | 11.18 ± 0.17    | 8.29 ± 0.12      |
| PCA             | 5.91 ± 0.13     | 17.12 ± 0.65    | 0.98 ± 0.00     | 7.16 ± 0.03     | 9.29 ± 0.12     | 1.15 ± 0.01      |
| Ocoa            | 4.42 ± 0.11     | 7.46 ± 0.09     | 5.34 ± 0.13     | 7.14 ± 0.02     | 8.02 ± 0.15     | 9.11 ± 0.19      |
| Peoa            | 2.32 ± 0.22     | 0.11 ± 0.00     | 0.91 ± 0.00     | 0.21 ± 0.00     | 0.87 ± 0.01     | 0.1 ± 0.00       |
| Fa              | 4.51 ± 0.23     | 11.21 ± 0.22    | 4.73 ± 0.11     | 0.11 ± 0.00     | 0.09 ± 0.00     | 8.22 ± 0.19      |
| HCCA            | 5.61 ± 0.19     | 9.64 ± 0.05     | 6.16 ± 0.02     | 9.45 ± 0.12     | 0.16 ± 0.00     | 9.01 ± 0.08      |
| Hdba            | 3.02 ± 0.12     | 0.02 ± 0.00     | 0.09 ± 0.00     | 3.18 ± 0.18     | 8.21 ± 0.03     | 0.11 ± 0.02      |
| Sya             | 5.55 ± 0.25     | 0.31 ± 0.02     | 0.33 ± 0.02     | 4.34 ± 0.02     | 0.28 ± 0.02     | 11.26 ± 0.32     |
| Va              | 4.53 ± 0.15     | 9.33 ± 0.12     | 0.41 ± 0.04     | 6.24 ± 0.31     | 6.01 ± 0.02     | 0.44 ± 0.06      |
| Cina            | 4.11 ± 0.21     | 8.97 ± 0.19     | 6.35 ± 0.11     | 4.87 ± 0.21     | 6.68 ± 0.04     | 6.63 ± 0.12      |
| ∑ PA (ppm)      | 44.49 ± 0.43    | 72.12 ± 0.21    | 36.61 ± 0.32    | 50.66 ± 0.33    | 50.79 ± 0.19    | 54.32 ± 0.23     |
| ∑ PA / TPC × 100 | 16.68 ± 0.09    | 38.39 ± 0.24    | 16.63 ± 0.16    | 23.29 ± 0.05    | 26.93 ± 0.28    | 24.79 ± 0.11     |

Hpha : 4-hydroxyphenylacetic acid ; PCAA : protocatechuic acid ; Ocoa : o-coumaric acid ; Peoa : p-coumaric acid ; Fa : Ferulic acid ; HCCA : 4-hydroxy cinnamic acid ; Hdba : 4 hydroxy benzoic acid ; Sya : Syringic acid ; Va : Vanillic acid ; Cina : Cinnamic acid.

Values are expressed as mean ± SD
Values with different superscript letters (a-c) within a row are significantly different (p < 0.05) by Tukey’s test multiple comparison post hoc test.

The most significant phenolic acids (p < 0.05) for olive oils from the Chemlali, Chetoui and Zarrazi are: 4-hydroxyphenylacetic acid (Hpha), protocatechuic acid (PCAA), o.coumaric acid (Ocoa), p.coumaric acid (Pcoa), ferulic acid (Fa), 4-hydroxy cinnamic acid (HCCA), 4 hydroxy benzoic acid (Hdha), syringic acid (Sya), vanillic acid (Va) and cinnamic acid (Cina) (Table 3). Interestingly, as shown in Table 3, Chemlali Beja had the high levels of Hpha (16.92 ppm), PCAA (17.12 ppm), Fa (11.21 ppm), HCCA (9.64 ppm), Va (9.33 ppm) and Cina (8.97), equally phenolic acids enclosed 38.39% of the total phenol content. Zarrazi Medenine had high levels of Ocoa and Sya, respectively at 9.11 and 11.26 ppm (Table 3).

3.5 Differentiation using the fatty acid profile

In order to examine the geographical location effect on the fatty acid profile, a multivariate data set of 10 fatty acid variables was used. Oxidative stability parameter was also introduced to the data set trying to find clearer correlations between fatty acid composition and oxidative stability of the studied samples. Principal Component Analysis (PCA) was conducted to get a general overview of the data distribution, thus a new set of latent factors or principal components (PCs) was generated.

The first principal component (PC1) had the highest eigenvalue of 8.37, and accounted for 44.07% of the variability in the data set. The second, third, fourth and fifth PCs (PC2, PC3, PC4 and PC5) have eigenvalues of 5.97, 2.17, 1.83 and 0.63, and explained 31.43%, 11.45%, 9.66% and 3.36% of the variance in the data, respectively. Subsequently, plotting the scores of the samples in the sub-space of the first two PCs (Fig. 1A) (75.51% of the total variance of the data) showed a clear grouping based on the geographical origin.

The variables that contributed positively to PC1 are oxidative stability, C18:1, C18:1/C18:2, ΣMUFA and ΣMUFA/ΣPUFA while C18:2, C18:3 and ΣPUFA contributed negatively to the first PC. In the other hand, the second PC (31.43% of variance) was positively correlated to C16:0, C16:1, C17:0 and ΣSFA and negatively correlated with C18:0, C20:0 and C20:1.

As shown in Fig. 1A, three distinctive groups could be distinguished independently of the olive cultivar (Chemlali, Chetoui or Zarrazi). Indeed, the first group encompassed two cultivars: Chemlali Sfax and Chetoui Sfax which are characterized by their high contents on saturated and polyunsaturated fatty acids, respectively. The second group included Zarrazi Gabes and Zarrazi Medenine cultivars which have the highest oxidative stability and the highest content of oleic acid, MUFA and the ΣMUFA/ΣPUFA ratio. The last group comprised two cultivars: Chemlali Jerba and Chemlali Sfax which are characterized by a balanced content of SFA, MUFA and PUFA.
similarity was observed in the HCA dendrogram between Zarrazi Gabes and Zarrazi Medenine. It should be noted that Gabes and Medenine are very close region located in the South of Tunisia and that climatic and geographic parameters are very similar for both.

Accordingly, these results showed that fatty acid composition is highly related to the geographical origin of the oil sample, in fact Gabes and Medenine cultivars have the best fatty acid composition and the highest oxidative stability while Sfax cultivars have the lowest stability and the worst composition in terms of fatty acids. These results are in agreement with those observed by Gargouri et al. in 201322 and Servili et al. in 200415. The authors related the dissimilarity in fatty acids percentages to the differences in temperature, altitude or other climatic variables such as soil characteristics and salinity of the olive grove zones.

Moreover, it was shown in PCA analysis that there was a significant difference in the content of oleic acid and linoleic acid between Chetoui Sfax and Chetoui Beja. This result is incompatible with the Ivanov rule, i.e., “the amount of linoleic acid rises when the temperature decreases, contrary to oleic acid”25. Furthermore, the PCA results showed also significant correlation ($p<0.05$) of oxidative stability with oleic acid C18:1 ($r = 0.767$) and with the ratio C18:1/C18:2 ($r = 0.811$). These findings are in agreement with previous work of Zribi et al. in 201426 and Jabeur et al. in 201427.

3.6 Differentiation using phenolic acids profile

Principal component analysis was applied to all olive oil samples trying to classify them according to their phenolic acids content. Thus another data set was generated including total phenol content (TPC) along with phenolic acids composition. The first principal component (PC1) had the highest eigenvalue of 6.38, and accounted for 49.12% of the variability in the data set. The second, third, fourth and fifth PCs (PC2, PC3, PC4 and PC5) had eigenvalues of 2.96, 1.74, 1.27 and 0.63, and showed 22.76%, 13.43%, 9.76% and 4.9% of the variance, respectively. According to the biplot of the scores in the sub-spaces PC1 vs. PC2 (Fig. 2A) (displaying 71.89% of the total variance), no clear grouping based on the geographical origin of samples was observable. In fact, PC1 was positively correlated with Cina, Hpha, $\Sigma$PA, $\Sigma$PA/TPC and PCA. The latters were also significantly correlated with Chemlali Beja olive oil suggesting that it was the most loaded oil in term of phenolic acids and the poorest in total phenol content. In fact, the negative side of the axis PC1 correlates with TPC and Pcoa, and surprisingly three cultivars were grouped on this part: Chemlali Sfax, Chetoui Sfax and Chetoui Jerba signifying that although these cultivars were rich in total phenol, they have low amount of phenolic acids content. The PC2 axis correlates positively with Hdba, the significantly most important phenolic acid for the Zarrazi Gabes cultivar, and correlates negatively with Hcca and Sya. The latters were the most significantly important phenolic acids for Zarrazi Medenine cultivars.

The dataset obtained were submitted to Hierarchical Cluster Analysis dendrogram using matrix of squared euclidean distances (HCA) in order to get clearer information about a plausible classification of the samples according to their phenolic composition. In fact, Figure 2B showed four distinct clusters based on the threshold value (equal to 979), with a high similarity between Chetoui Sfax and Chetoui Beja and Zarrazi Medenine, whereas Chemlali Sfax, Chemlali Beja and Zarrazi Gabes presented high dissimilarity in their composition. In this case, slight difference could be observed between the HCA clusters and the groups formed in PCA. Accordingly, the phenolic content of the olive oil could depend not only on the cultivar or the

![Biplot representation on the factor-plane (PC1-PC2) showing vector distribution of 10 phenolic acids within score plot of the oil samples with the analysed compounds.](image1)

![HCA dendrogram showing clustering of olive oils samples considering phenolic acids content.](image2)
geographical location, but also on the climatic and environmental conditions, agronomic practices and the technological process used in oil extraction. Similar results were found by Ocakoglu et al. in 2009 and Cerretani et al. in 2006, the authors demonstrated that the phenolic composition was not useful in discriminating the olive oil samples. However, contradictory results were found by Ben Brahim et al. in 2017 and Bajoub et al. in 2017. Their results suggested that phenolic acids data could constitute advisable and reliable information to trace the origin of virgin olive oils from different geographical provenance.

Conclusion

With the aim of studying differences among Tunisian olive oils belonging to different geographical locations, we explored quality indices as well as fatty acids composition and phenolic acids content of different virgin olive oil samples. The potential for improved geographical classification based on the combination of ANOVA analysis and PCA / HCA approaches was investigated. The results showed that fatty acid content was significantly ($p<0.05$) influenced by both olive cultivar and geographic region. As a general statement, it can be said that Southern cultivars (Zarrazi Gabes and Zarrazi Medenine) have the best fatty acid combination according to their oxidative effect followed by Chemlali and Chetoui Northern cultivars (Beja) and then by central (Sfax) region cultivars. The findings showed also that phenolic acids content displayed significant qualitative and quantitative differences among the cultivars and their geographical origins. We highlighted that Chemlali Beja oil stood out for its highly oxidative stability and its richness in phenolic acids content.

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