Characterization of monovarietal virgin olive oils from introduced cultivars in eastern Morocco

F. Mansouri1*  
A. Benmoumen1  
G. Richard2  
M.L. Fauconnier2  
M. Sindic3  
H. Serghini-Caid1  
A. Elamrani1

1 Laboratoire de Biologie des Plantes et des Microorganismes  
Faculté des Sciences  
Université Mohammed Premier  
Oujda, Maroc

2 Unité de Chimie Générale et Organique  
Gembloux Agro-BioTech  
Université de Liège  
Gembloux, Belgique

3 Laboratoire Qualité et sécurité des produits agroalimentaires  
Unité Analyse, Qualité et Risques  
Gembloux Agro-BioTech  
Université de Liège  
Gembloux, Belgique

(*) CORRESPONDING AUTHOR: Farid Mansouri  
Mobile: +212 664 469076  
Work: +212 536 500601  
Fax: + 212 536 500 603  
Email: mansouri0farid@gmail.com

The aim of this study was to characterize monovarietal virgin olive oils (VOOs) of new high-density planting system of three European cultivars (Arbequina, Arbosana and Koroneiki), recently introduced in eastern Morocco. VOOs’ characterization has been carried out by analyzing several parameters, such as quality indexes, fatty acid contents, minor components, and olive oils’ oxidative stability index (OSI). In this study, we have also conducted a comparison between these monovarietal VOOs and olive oils of autochthones cultivar Picholine marocaine. Significant differences between the analyzed VOOs were highlighted. Koroneiki’s VOO had a high phenols content (493.66 mg/kg) and, consequently, the best oxidative stability (94.83 h); Arbosana’s VOO was distinguished by its abundance of α-tocopherol (460.07 mg/kg) and by an intermediate OSI (64.83 h). In addition, results showed, firstly, that in all the analyzed oils decarboxymethyl ligstroside aglycone and decarboxymethyl oleuropein aglycone were the main phenolic compounds, and, secondly, that VOOs of Koroneiki and Arbosana seem to have similar profiles, with a high content of natural antioxidants and a high oleic/linoleic ratio, thus boasting a better shelf life.

Keywords: virgin olive oil, Arbequina, Arbosana, Koroneiki, Picholine marocaine, fatty acids, phenols, tocopherols, oxidative stability (OSI).

Caratterizzazione degli oli vergini di oliva monovarietali da cultivar introdotte nel Marocco orientale

Lo scopo di questo studio è stato quello di caratterizzare gli oli vergini di oliva monovarietali (VOOs) ottenuti da nuove piantagioni ad alta densità di tre cultivar europee (Arbequina, Arbosana e Koroneiki), recentemente introdotte nel Marocco orientale. La caratterizzazione dei VOOs è stata effettuata analizzando diversi parametri, quali ad esempio indici di qualità, contenuto di acidi grassi, componenti minori, e l’indice di stabilità ossidativa degli oli (OSI).

In questo studio, abbiamo anche condotto un confronto tra questi VOOs monovarietali e l’olio ottenuto da olive di cultivar autoctone quali la Picholine marocaine. Sono state evidenziate differenze significative tra i VOOs analizzati: l’olio di oliva vergine ottenuto dalla Koroneiki aveva un alto contenuto di fenoli (493.66 mg/kg) e di conseguenza la massima stabilità ossidativa (94.83 h); l’ olio di oliva vergine ottenuto da Arbosana si distingueva per la sua ricchezza di α-tocoferolo (460,07 mg/kg) e da un OSI intermedio (64.83 h).

I risultati mostrano inoltre che l’aglicone del decarbossimetil ligrostoside e della decarbossimetil oleuropeina sono stati i principali composti fenolici in tutti gli oli analizzati e che gli VOOs di Koroneiki e Arbosana sembravano avere profili simili con un alto contenuto di antiossidanti naturali e un alto rapporto oleico/linoleico, e di conseguenza hanno mostrato la migliore shelf-life.

Parole chiave: olio extravergine di oliva, Arbequina, Arbosana, Koroneiki, Picholine marocaine, acidi grassi, fenoli, tocoferoli, stabilità ossidativa (OSI).
1. INTRODUCTION

Olive oil enjoys a privileged position among edible vegetable oils and is appreciated for its particular aroma, taste, and nutritional values. Olive oil is the key component of the Mediterranean diet, and it attracts the interest of scientists due to the health benefits associated with its consumption. It is recommended for the prevention of cardio circulatory diseases and for its anti-oxidative capacity [1, 2, 3]. Extra virgin olive oil (EVOO) presents biochemical properties and contains biomolecules that make it a functional food [2]. From a chemical point of view, olive oil can be divided into major and minor fractions. The major fraction includes acylglycerols, which represent approximately 98% of olive oil composition. In the minor fraction, different chemical compounds, such as phenols, tocopherols, aliphatic and triterpenic alcohols, sterols, aromatic hydrocarbons, and volatile compounds can be found. Some studies indicate that these phytochemicals, especially phenols, display high free-radical scavenging activity [4, 5]. Also, phenols have important organoleptic and technological values due to their influence on sensory characteristics and the shelf life of olive oil [6, 7, 8]. The most important classes of phenolic compounds in olive fruit include: phenolic acids, phenolic alcohols, lignans, flavonoids and secoiridoid derivatives [9]. Particular taste and oxidative stability (OSI) of an olive oil depend on its content of specific phenolic compounds, e.g. secoiridoids, which are the main agents responsible for the resistance against autoxidation and photodegradation [10]. Moreover, it is widely known that the quality of virgin olive oil (VOO) is influenced by various agronomic factors, such as olive cultivar [11], climatic conditions [12], production process [13], and the degree of maturation and agronomic practices related to the irrigation treatment [14, 15].

In Morocco, olive groves are dominated by Picholine marocaine, a variety spread throughout all olive trees growing in sub regions and covering approximately 96% of Moroccan olive plantations [16]. Picholine marocaine is very resistant to drought, and can serve a double purpose: oil production and conservation as table olive. However, the fruit production is highly changeable. Aiming to improve olive oil production in Morocco, new European olive cultivars (Arbequina, Arbosana, and Koroneiki) have been recently introduced in irrigated areas in the region of Morocco. Likewise, three European cultivars (Arbequina, Arbosana and Koroneiki) have been recently introduced in Morocco to improve olive oil industry by improving the productivity and quality of olive oils produced in this region of Morocco. Therefore, the aim of this work is to compare the biochemical and qualitative characteristics of those European olive oil cultivars that have been newly introduced in this region with the local Picholine marocaine variety. This work was carried out in collaboration with the HDP olive groves owners around Oujda.

2. MATERIAL AND METHODS

2.1 OLIVE OIL SAMPLES

Samples of monovarietal VO Os produced during the 2012/2013 crop season are taken from four varieties grown in eastern Morocco: Arbequina, Arbosana as Spanish varieties; Koroneiki as a Greek olive variety, and Picholine marocaine as an autochthonous variety. The European cultivars were conducted under irrigated an HDP system with a frame of 1,5 m/4 m and a density of 1666 trees/ha. The local cultivar is conducted under rain-fed condition. The irrigation period for the HDP system was 9 months per year, from January to September, with daily irrigation using dripers placed around the trees delivering water flow of 1.2 L/h. The climate is a Mediterranean one, with hot, dry summers and an annual average rainfall ranging from 275.3 to 516.0 mm.

The olive fruits came from groves located in the “Oujda-Angad” region; they were directly processed by a “continuous industrial 2-phase system” at the company “Huiles d’olive de la Méditerranée”. The physicochemical parameters analysis of monovarietal VO Os was carried out within 7 days from production; in the meantime, 500 mL samples were stored in dark bottles with no space at the top, at a temperature of 4°C, for other, further analysis.

2.2 ANALYTICAL METHODS

2.2.1 Physicochemical parameters

Free acidity (g/100 g oleic acid), peroxide value (meq O₂/kg of olive oil) and ultraviolet absorption indexes (K₂₃₀, K₂₇₀ and ΔK) were determined according to the official European methods [18].

2.2.2 Fatty acid analysis

Before analysis, fatty acids were converted into fatty acid methyl esters by shaking a solution of 10 mg oil and 0.2 mL of hexane with 0.5 mL of solution A (A: 55 mL of dry methanol + 20 mL of hexane + 25 mL of BF₃ at 14% weight in methanol), and then placing it in
a 75°C water bath for 90 min. After cooling, 0.5 mL of saturated NaCl solution and 0.2 mL of a 10% H₂SO₄ (v/v) solution were added with agitation. Next, 8 mL of hexane was added, and the mixture shaken vigorously. The tube was then left to rest to allow for phase separation. One µL from the upper layer was injected into the gas chromatograph. This chromatographic separation was carried out using a Hewlett-Packard gas chromatograph (HP 6890 series GC), equipped with a capillary column (VF-WAXmS: 30 m × 250 mm, 0.25 µm) and a flame ionization detector (FID). The carrier gas was helium, at a flow of 1.7 mL min⁻¹.

### 2.2.3 Determination of chlorophylls and carotenoids contents

The chlorophyll fraction was evaluated by absorbance at 670 nm and the carotenoids fraction at 470 nm, according to Minguez-Mosquera et al. [19]. The values of the specific extinction coefficients used were $E_0 = 613$ for pheophytin as a major component and $E_0 = 2000$ for lutein as a major component in the carotenoid fraction and $E_0 = 2500$ for lutein as a major component in the carotenoid fraction.

### 2.2.4 Extraction of phenolic compounds

The liquid/liquid extraction was performed according to the procedure described by Ollivier et al. [20]. 10 g of olive oil was weighed into a centrifuge tube, to which 10 mL of methanol/water (80/20, v/v) was added. The mixture was stirred for 10 min in a vortex apparatus, and the tube was centrifuged at 3800 rpm for 10 min. The methanol layer was then separated from the aqueous layer by filtration through a 0.45 µm PVDF filter to be used for HPLC analysis of phenolic compounds and colorimetric determination of total phenols.

#### 2.2.5 Colorimetric determination of phenol contents

Total phenols were analyzed as described by Ollivier et al. [20]; phenolic contents were determined according to the Folin-Ciocalteu method by using caffeic acid as a standard, and by spectrophotometric absorbance measurement at 750 nm.

#### 2.2.6 Phenolic compound analysis by HPLC

Phenolic compound analysis was performed by HPLC (Agilent Technologies series 1100 system) equipped with an automatic injector, a column oven and a diode array detector (DAD). A Zorbax XDB-C18 column (150 mm × 4.6 mm, 3.5 µm) was used and maintained at 30°C. The injection volume was 10 µL. The mobile phase was a mixture of water (solvent A) and methanol (solvent B), both of them acidified with 0.5% formic acid (v/v). The flow rate was 0.8 mL/min with the following gradient: 5% B at 0 min, 35% B at 7 min, 35% B at 12 min, 50% at 17 min, 60% B at 22 min, 95% B at 25 min, 100% B at 30 min and 5% B at 30.1 min [21]. The chromatograms were taken at 254, 280, 320 and 340 nm. Hydroxytyrosol, tyrosol, vanillic acid, syringic acid, p-coumaric acid, cinamic acid, pinoresinol, luteolin and apigenin were identified and quantified at 280 nm by external standardization with phenolic compounds obtained from Sigma-Aldrich (St-Louis, USA). All calibration curves showed good linearity over the study range ($r^2 > 0.99$). Decarboxymethyl oleuropein aglycone and decarboxymethyl ligstroside aglycone peak identification and quantification, which had no commercial standards, were carried out according to Bakhouch et al., [21] and the response factors determined by Mateos et al. [22].

### Table I - Quality indexes, phenol and pigment contents, and oxidative stability of the studied virgin olive oils produced in eastern Morocco

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Arbequina</th>
<th>Arbosana</th>
<th>Koroneiki</th>
<th>EVOO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free acidity (% C18:1)</td>
<td>0.46 ± 0.03ᵃ</td>
<td>0.53 ± 0.03ᵇ</td>
<td>0.58 ± 0.09ᵃ</td>
<td>0.51 ± 0.3ᵃ</td>
</tr>
<tr>
<td>Peroxide value (meq O₂ kg⁻¹)</td>
<td>8.26 ± 0.49ᵃ</td>
<td>9.10 ± 0.40ᵇ</td>
<td>10.51 ± 0.46ᵇ</td>
<td>8.89 ± 0.73ᵃ</td>
</tr>
<tr>
<td>$K_{270}$</td>
<td>0.08 ± 0.01ᵃ</td>
<td>0.11 ± 0.01ᵇ</td>
<td>0.14 ± 0.01ᵇ</td>
<td>0.13 ± 0.02ᶜ</td>
</tr>
<tr>
<td>$K_{322}$</td>
<td>1.43 ± 0.18ᵇ</td>
<td>1.56 ± 0.01ᵃ</td>
<td>1.63 ± 0.10ᵃ</td>
<td>1.49 ± 0.20ᵃ</td>
</tr>
<tr>
<td>ΔK</td>
<td>0.0020 ± 0.0002ᵃ</td>
<td>0.0010 ± 0.0003ᵃ</td>
<td>0.0040 ± 0.0003ᵇ</td>
<td>0.0020 ± 0.0005ᵇ</td>
</tr>
<tr>
<td>Total phenols (mg/kg)⁺⁺</td>
<td>241.28 ± 6.70ᵃ</td>
<td>411.64 ± 6.70ᵇ</td>
<td>493.66 ± 4.89ᵈ</td>
<td>316.59 ± 10.18ᵇ</td>
</tr>
<tr>
<td>Chlorophylls (mg/kg)</td>
<td>1.86 ± 0.04ᵇ</td>
<td>1.94 ± 0.03ᵇ</td>
<td>3.94 ± 0.01ᵈ</td>
<td>1.69 ± 0.03ᵇ</td>
</tr>
<tr>
<td>Carotenoids (mg/kg)</td>
<td>1.66 ± 0.09ᵇ</td>
<td>1.65 ± 0.01ᵇ</td>
<td>2.17 ± 0.02ᶜ</td>
<td>1.43 ± 0.09ᵃ</td>
</tr>
<tr>
<td>Oxidative stability (h)</td>
<td>50.36 ± 0.45ᵃ</td>
<td>60.17 ± 0.95ᵇ</td>
<td>94.83 ± 0.79ᵈ</td>
<td>44.59 ± 0.49ᵈ</td>
</tr>
</tbody>
</table>

Values are the means of the different four VOO samples (n=3) ± standard deviations. Significant differences in the same row are shown by different letters (a–d) varieties ($p < 0.05$).

*EVOO*: Extra Virgin Olive Oil quality criteria, Values limits set by International Olive Oil Council [24].

**Concentration of polyphenols expressed as milligram of caffeic acid per kilogram of oil (colorimetric method).
2.2.7 Tocopherols analysis

The α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol were evaluated following the AOCS Method Ce 8–89 [23]. A solution of oil in hexane was analyzed by HPLC (Agilent Technologies series 1200 system) equipped with an automatic injector, on a Zorbax StableBond mixed C18 column (150 mm × 3 mm, 3 μm), maintained at 30°C. The injection volume was 10 μL. The mobile phase was hexane/2-propanol (99/1, v/v), eluted at a flow rate of 1 mL/min. An ultraviolet detector was used for absorbance measurements. Tocopherols were identified and quantified at 292 nm by external standardization with tocopherols obtained from Sigma-Aldrich (St-Louis, USA).

2.2.8 Evaluation of oxidative stability

Oxidative stability of olive oils was evaluated by the Rancimat method, and was expressed as the lipid oxidation induction period (hours), measured with a Metrohm Rancimat 743 apparatus using an olive oil sample of 3 g warmed to 100°C, and an air flow of 15 L/h. Analyzed VOOs showed low values for the evaluated physicochemical parameters: acidity ≤ 0.8%; peroxide value ≤ 20 meqO₂/kg; K232 ≤ 0.22; K270 ≤ 2.5, and ΔK < 0.01 (Tab. I). All results were easily within the limits set by the International Olive Oil Council [24] for the extra virgin olive oil category. This proofs of proper olive oil extraction and storage conditions also guarantee the freshness of oil. The low values for those parameters translate to a good quality of those olive oils obtained from fresh and healthy olives harvested at the optimal ripening point, followed by immediate extraction without olives storage. However, according to the olive cultivar, significant differences have been observed for peroxide index and UV absorbance (K270 and ΔK) (p < 0.05). It is known that olives at late ripening stages give olive oils with higher levels of free acidity, as a result of undergoing an increase in enzymatic activity, especially lipolytic enzymes, and are more sensitive to pathogenic infections and mechanical damage [25].

3. RESULTS AND DISCUSSION

3.1 OLIVE OIL’S PHYSICOCHEMICAL INDEXES

Analyzed VOOs showed low values for the evaluated physicochemical parameters: acidity ≤ 0.8%; peroxide value ≤ 20 meqO₂/kg; K232 ≤ 0.22; K270 ≤ 2.5, and ΔK < 0.01 (Tab. I). All results were easily within the limits set by the International Olive Oil Council [24] for the extra virgin olive oil category. This proofs of proper olive oil extraction and storage conditions also guarantee the freshness of oil. The low values for those parameters translate to a good quality of those olive oils obtained from fresh and healthy olives harvested at the optimal ripening point, followed by immediate extraction without olives storage. However, according to the olive cultivar, significant differences have been observed for peroxide index and UV absorbance (K270 and ΔK) (p < 0.05). It is known that olives at late ripening stages give olive oils with higher levels of free acidity, as a result of undergoing an increase in enzymatic activity, especially lipolytic enzymes, and are more sensitive to pathogenic infections and mechanical damage [25].

3.2 FATTY ACID COMPOSITION

The fatty acid composition has previously been used as a parameter for oil classification because of its importance in the description and determination of adulteration [26]. Table II shows fatty acids composi-

### Table II - Fatty acid compositions of the studied virgin olive oils produced in eastern Morocco

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Arbequina</th>
<th>Arbosana</th>
<th>Koroneiki</th>
<th>Picholine marocaine</th>
<th>EVOO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmamic acid</td>
<td>16.96±0.75a</td>
<td>13.72±0.34a</td>
<td>15.70±0.37a</td>
<td>15.93±2.30abc</td>
<td>7.5 - 20.0</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>1.82±0.21a</td>
<td>1.27±0.03a</td>
<td>1.23±0.06a</td>
<td>1.20±0.20ab</td>
<td>0.3 - 3.5</td>
</tr>
<tr>
<td>Heptadecanoic acid</td>
<td>0.11±0.01a</td>
<td>0.15±0.00a</td>
<td>NDa</td>
<td>NDa</td>
<td>≤ 0.3</td>
</tr>
<tr>
<td>Heptadecenoic acid</td>
<td>0.24±0.02a</td>
<td>0.34±0.00a</td>
<td>NDa</td>
<td>NDa</td>
<td>≤ 0.3</td>
</tr>
<tr>
<td>Saturic acid</td>
<td>1.79±0.41a</td>
<td>1.91±0.36a</td>
<td>0.51±0.12a</td>
<td>1.60±0.07b</td>
<td>0.5 - 5.0</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>69.72±1.03a</td>
<td>75.69±0.56a</td>
<td>76.24±0.52a</td>
<td>67.49±2.55ab</td>
<td>55.0 - 83.0</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>8.21±1.04a</td>
<td>5.66±0.05a</td>
<td>5.26±0.07a</td>
<td>12.85±0.02c</td>
<td>3.5 - 21.0</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>0.54±0.01a</td>
<td>0.64±0.02a</td>
<td>0.64±0.01a</td>
<td>0.63±0.01c</td>
<td>≤ 1.0</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>0.32±0.05a</td>
<td>0.37±0.01a</td>
<td>0.24±0.01a</td>
<td>NDa</td>
<td>≤ 0.6</td>
</tr>
<tr>
<td>Gadoleic acid</td>
<td>0.28±0.06a</td>
<td>0.26±0.00a</td>
<td>0.18±0.01b</td>
<td>NDa</td>
<td>≤ 0.4</td>
</tr>
<tr>
<td>ΣSFAs</td>
<td>19.19±0.74a</td>
<td>16.14±0.64a</td>
<td>16.45±0.46a</td>
<td>17.53±2.33ab</td>
<td></td>
</tr>
<tr>
<td>ΣMUFAa</td>
<td>72.06±0.79b</td>
<td>77.56±0.57c</td>
<td>77.65±0.51c</td>
<td>68.69±2.36</td>
<td></td>
</tr>
<tr>
<td>ΣPUFAs</td>
<td>8.75±0.10a</td>
<td>6.30±0.07a</td>
<td>5.90±0.08a</td>
<td>13.78±0.03d</td>
<td></td>
</tr>
<tr>
<td>O/L ratio</td>
<td>8.49±0.21a</td>
<td>13.37±0.05a</td>
<td>14.50±0.25a</td>
<td>5.25±0.21a</td>
<td></td>
</tr>
</tbody>
</table>

Values are the means of the four different VOO samples (n=3) ± standard deviations. Significant differences in the same row are shown by different letters (a–d) varieties (p<0.05). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; O/L, oleic/linoleic ratio; ND, not detected.

*EVOO: Extra Virgin Olive Oil quality criteria, Values limits set by International Olive Oil Council [24].
tions of monovarietal virgin olive oils of the studied varieties. Palmitic, oleic and linoleic acids are the major fatty acids, while palmitoleic, heptadecanoic, heptadecenoic, stearic, linolenic, arachidic and gadoleic acids are present in smaller amounts. The results showed that the distribution of fatty acid composition covered the normal ranges expected for extra virgin olive oil [24]. Significant differences were observed between cultivars \((p < 0.05)\). The most significant difference was observed in the most abundant oleic and linoleic acids, but not in palmitic acid. The relative contents of oleic acid varied from 67.49 to 76.24%. Koroneiki olive oil had the highest content of oleic acid (76.24%) compared to Picholine marocaine, which had the lowest value of this fatty acid (67.49%). Palmitic acid is the major saturated fatty acid in olive oil and its content ranges between 13 and 17%; the highest rate is observed for Arbequina (16.96%), whereas the lowest rate is noticed for the Arbosana variety (13.72%). As far as linoleic acid is concerned, Picholine marocaine olive oil shows the highest mean value (12.85%), whereas the lowest one is observed in Koroneiki (5.26%). The contents of the other fatty acids, including palmitoleic, heptadecanoic, heptadecenoic, stearic, linolenic, arachidic and gadoleic acids, change from one olive variety oil to another, but the amounts are fairly small or even unidentifiable in some varieties. The amounts of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) and the oleic/linoleic acids ratio (O/L; C18:1/C18:2) have been also evaluated (Tab. II). Arbequina olive oil is rather rich in total SFAs (19.19%), but the amounts are fairly small or even unidentifiable in some varieties. The amounts of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) and the oleic/linoleic acids ratio (O/L; C18:1/C18:2) have been also evaluated (Tab. II). Arbequina olive oil is rather rich in total SFAs (19.19%), essentially because of its high content in palmitic acid. Concerning the total MUFAs, and because of its high oleic acid content, Koroneiki olive oil holds the highest percentage (77.65%). Picholine marocaine olive oil is rich in total PUFAs (13.78%) because of its high linoleic acid content, representing the major fatty acid component of this fraction. For European cultivars, the ratio O/L is, respectively, 8.49 for Arbequina and 14.5 for Koroneiki, but this ratio is low for Picholine marocaine (5.25). This O/L ratio can be usefully employed to characterize olive cultivars, and shows a noticeable link with stability [27].

If compared to VOOS of Arbequina and Arbosana when cultivated in northern Tunisia under irrigated high-density planting system [12] and in their original growing area in Spain [28], Arbequina and Arbosana monovarietal VOOS produced in Morocco showed a lower level of linoleic acid and a rate of oleic acid higher than in its original and northern Tunisia growing areas (Tab. III). However, if compared to their original growing area, Arbequina and Arbosana cultivated in Morocco produced a higher amount of palmitic acid although still lower than that found in Tunisia. The Koroneiki olive oil has a comparable composition of oleic acid (Tab. III), in eastern Morocco as well as in northern Tunisia under irrigated HDP systems [12], and in its original growing area (Greece), even when conducted in rain-fed conditions [29]. Concerning the palmitic and linoleic acids, the Koroneiki variety produced oil with a higher level of palmitic acid and a rate of linoleic acid relatively lower than in its original growing area and in northern Tunisia. Those variations in fatty acid compositions observed for European VOO cultivars between the three growth areas are probably related to environmental conditions experienced during the growth and ripening of the fruit. Those results and observations are in agreement with the findings of other authors [12, 30].

### 3.3 Pigment Contents
The unique color of olive oil is due to pigments like chlorophylls and carotenoids, which are involved in autoxidation and photoxidation mechanisms [19]. As shown in Table I, the pigment contents of monovarietal VOOS ranged, respectively, from 1.69 to 3.94 mg/kg for chlorophylls and from 1.43 to 2.17 mg/kg for carotenoids, with significant differences between cultivars \((p < 0.05)\). Koroneik’s olive oil differs from other VOOS by its higher content of chlorophylls (3.94 mg/kg) and carotenoids (2.17 mg/kg). Picholine marocaine olive oil, a local cultivar, shows low concentrations of chlorophylls and carotenoids; 1.69 and 1.43 mg/kg respectively. These results agree with the findings of other studies [31], which also reported that the presence of various pigments in olive oils depends on factors such as fruit ripeness, olive cultivar, soil and climatic conditions, and extraction and processing procedures.
3.4 TOTAL PHENOLS CONTENT

The phenolic compounds present in VOO are among the reasons of the nutritional importance and shelf life of this oil. This is a desirable characteristic because of the beneficial effects of these components on human health. Phenols correlate with key sensory oil properties, such as the bitterness and sharpness that are associated to olive oil. The classification of olive oil as mild, medium or robust can be linked to the total phenols content. R. Aparicio and Luna G. [29] and M. P. Aguilera, Beltrán G. [27] reported that amount of total phenols usually ranges from 50 to 1000 mg/kg, and depends on several factors, such as variety, agricultural practices, olives ripeness at harvest, type of crushing machine, and oil extraction procedures. In this study, the concentrations of total phenols in analyzed VOOs range between 241.28 and 493.66 mg/kg, and significant differences between cultivars were observed. Phenolic fraction pounds were identified, and significant differences are shown in Figure 1 and Table IV, twelve phenolic compounds vary according to the olive variety. As shown in (HPLC-DAD) method. The HPLC profiles of the phenolic compounds identified in all analyzed VOOs, and hydroxytyrosol and tyrosol were the only two compounds identified in all analyzed VOOs, and hydroxytyrosol contents ranged from 1.51 mg/kg in VOO of Picholine maroccaine to 14.17 mg/kg in VOO of Koroneiki, respectively, while tyrosol contents ranged from 1.49 mg/kg in VOO of Arbequina to 8.04 mg/kg in VOO of Picholine maroccaine. In addition, and with the exception of the Picholine maroccaine variety, tyrosol is present in lesser amounts than hydroxytyrosol in VOOs of European cultivars. This remarkable disparity is in accordance with previous data found in same European cultivars cultivated in Tunisia [12].

Table IV shows also quantitative differences in pinoresinol, the highest content of which was observed in the Arbosana oil (7.08 mg/kg), whereas the lowest was observed for Picholine maroccaine oil (2.93 mg/kg); VOOs of Arbequina and Koroneiki both have intermediate amounts of pinoresinol, with 5.24 and 4.79 mg/kg, respectively. As regards flavonoids such as luteolin and apigenin, considerable amounts were found in the analyzed VOOs. Olive oils of the three European cultivars showed comparable profiles for luteolin, their quantities ranging from 6.54 to 6.89 mg/kg. However, olive oil of Picholine maroccaine showed the lowest flavonoids content, with 2.9 mg/kg for luteolin and 1.74 for apigenin. The major concentration of apigenin was detected in Arbosana olive oil (7.55 mg/kg).

### Table IV - Phenolic compounds composition of the studied virgin olive oils produced in eastern Morocco

<table>
<thead>
<tr>
<th>Peak</th>
<th>Phenolic compounds (mg/kg)</th>
<th>Arbequina</th>
<th>Arbosana</th>
<th>Koroneiki</th>
<th>Picholine maroccaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydroxytyrosol</td>
<td>1.94±0.03</td>
<td>9.75±0.19</td>
<td>14.17±0.16</td>
<td>1.51±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Tyrosol</td>
<td>1.49±0.03</td>
<td>4.66±0.08</td>
<td>6.97±0.17</td>
<td>8.04±0.04</td>
</tr>
<tr>
<td>3</td>
<td>Vanillic acid</td>
<td>0.41±0.00</td>
<td>0.25±0.00</td>
<td>0.43±0.00</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>4</td>
<td>Syringic acid</td>
<td>0.37±0.02</td>
<td>0.78±0.01</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>5</td>
<td>Vanillin</td>
<td>0.22±0.00</td>
<td>0.29±0.00</td>
<td>0.18±0.03</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>6</td>
<td>p-Coumaric acid</td>
<td>0.13±0.00</td>
<td>0.07±0.00</td>
<td>0.12±0.01</td>
<td>0.14±0.00</td>
</tr>
<tr>
<td>7</td>
<td>Decarboxymethyl oleuropein aglycone</td>
<td>85.37±1.43</td>
<td>126.53±2.00</td>
<td>146.72±1.79</td>
<td>26.35±0.27</td>
</tr>
<tr>
<td>8</td>
<td>Pinoresin</td>
<td>5.24±0.09</td>
<td>7.08±0.04</td>
<td>4.79±0.07</td>
<td>2.93±0.08</td>
</tr>
<tr>
<td>9</td>
<td>Decarboxymethyl ligstroside aglycone</td>
<td>108.33±1.82</td>
<td>157.16±0.50</td>
<td>165.56±1.84</td>
<td>75.41±1.39</td>
</tr>
<tr>
<td>10</td>
<td>Cinamic acid</td>
<td>0.12±0.00</td>
<td>0.17±0.02</td>
<td>0.55±0.01</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td>11</td>
<td>Luteolin</td>
<td>6.89±0.11</td>
<td>6.71±0.03</td>
<td>6.54±0.39</td>
<td>2.9±0.15</td>
</tr>
<tr>
<td>12</td>
<td>Apigenin</td>
<td>3.33±0.06</td>
<td>7.55±0.05</td>
<td>3.55±0.05</td>
<td>1.74±0.09</td>
</tr>
</tbody>
</table>

Values are the means of the four different VOO samples (n=3) ± standard deviations. Significant differences in the same row are shown by different letters (a-d) (p < 0.05). tr, traces.
Phenolic acids were detected in analyzed VOOs, albeit at very low amounts. Syringic acid ranged from mere traces in Koroneiki's and Picholine oils to a maximum of 0.78 mg/kg for Arbosana oil. Contents of the other identified phenolic acids (cinamic, vanillic and p-coumaric acids) ranged from 0.07 mg/kg, determined for p-coumaric acid in Arbosana's oil, to 0.55 mg/kg for cinamic acid in Koroneiki VOO.

3.6 TOCOPHEROLS
Tocopherols are a class of organic compounds consisting of various methylated phenols. Vitamin E is a general term employed for the designation of tocopherols (α, β, γ and δ-homologues). Tocopherols, with phenolic compounds, contribute to the antioxidant properties of olive oils. Their profile and composition are often used as criteria of olive oil purity. In this study, α, β, and γ−tocopherols were quantified and high concentrations of α-tocopherol were observed for all the olive oils (Tab. V). Relative contents (calculated from results in Tab. V) vary from 95-98% for α-tocopherol to 0.56-1.34% and 0.61-3.25% for β- and γ-tocopherols, respectively. As the main form, α-tocopherol contents for Arbosana, Koroneiki, Arbequina and Picholine marocaine VOOs are, respectively: 460.07, 344.58, 322.36 and 243.43 mg/kg. As already observed by several authors [32, 33, 34], the amount of tocopherols in VOOs was noticed to be remarkably variety-dependent, with α-homologue being the main tocopherol found in olive oils.

3.7 OXIDATIVE STABILITY
The oxidative stability and sensory quality of VOOs stem from a prominent and well-balanced chemical composition. The oxidative stability is mainly related to the presence of minor components such as tocopherols, and particularly phenolic compounds. In the present study, the oxidative stability index (OSI) of the VOOs extracted from Arbequina, Arbosana, Koroneiki and Picholine was assessed as induction period by Rancimat in order to predict the shelf-life of olive oils. For this purpose, Rancimat tests were carried out; the results (Tab. I) showed significant differences according to the cultivar. The highest OSI was observed for Koroneiki olive oil (94.83 h), whereas lower values of OSI (50.36 and 44.55 h) were observed for Arbequina and Picholine olive oils respectively.

Table V - Tocopherols composition of the studied virgin olive oils produced in eastern Morocco

<table>
<thead>
<tr>
<th>Tocopherols (mg/kg)</th>
<th>Varieties</th>
<th>Introduced cultivars</th>
<th>Autochthonous cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arbequina</td>
<td>Arbosana</td>
<td>Koroneiki</td>
</tr>
<tr>
<td>α−Tocopherol</td>
<td>322.36±11.05a</td>
<td>460.07±12.38a</td>
<td>344.56±9.42a</td>
</tr>
<tr>
<td>β−Tocopherol</td>
<td>1.83±0.16a</td>
<td>4.73±0.22c</td>
<td>4.79±0.19b</td>
</tr>
<tr>
<td>γ−Tocopherol</td>
<td>2.1±0.54a</td>
<td>6.32±0.22b</td>
<td>8.69±0.82c</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>326.30±10.77a</td>
<td>471.13±12.29b</td>
<td>358.06±10.42c</td>
</tr>
</tbody>
</table>

Values are the means of the four different VOO samples (n=3) ± standard deviations. Significant differences in the same row are shown by different letters (a-d) (p < 0.05).
Several studies concerning the contribution of olive oil constituents to VOO stability concluded that virgin olive oil, due to its triacylglycerol composition low in polyunsaturated fatty acids, and due to the presence of a group of phenolic antioxidants composed mainly of phenols and tocopherols, virgin olive oil presents a high resistance to oxidative deterioration. In this regard, linear regressions based on VOO’s content of phenols and on their O/L ratio have shown a good correlation with the stability to oxidation of VOOs [35, 36, 37]. Our results are in accordance with the published data, and confirm that high O/L ratio and olive oil’s richness of phenols are the major contributors and indicators of the high oxidative stability of the analyzed VOOs. Statistical data analysis exhibits positive correlations (Tab. VI) between, VOOs’ stability to oxidation, their content of total phenols ($r^2 = 0.915, p < 0.05$), and their O/L ratio ($r^2 = 0.918, p < 0.05$). Furthermore, individual secoiridoid derivatives (DLA and DOA), which are the most important phenolic compounds in all analyzed olive oils (Tab. IV), have shown in this study (Tab. VI) good correlations with VOO stability (DLA $r^2 = 0.909, p < 0.05$, and DOA $r^2 = 0.893, p < 0.05$). However, tocopherols content correlated poorly with VOO stability ($r^2 = 0.515, p < 0.05$). Thus, we concluded that olive oils of Koroneiki and Arbosana, rich in DOA and DLA, were characterized by their high OSI, and therefore by their good stability to oxidation and longer shelf lives. The same observation, i.e. that olive oils with high secoiridoid derivatives content show a higher oxidative stability, has been also noticed by other authors [38].

### 3.8 HCA ANALYSIS

A statistical analysis classification of the data from the studied area was performed using the HCA model. The result of this discriminative analysis was generally satisfactory. In fact, the dendrogram obtained from HCA analysis (Fig. 2) indicates that, at a rescaled distance of 166, the cultivars are distributed into three major clusters. Cluster 1 exclusively includes the Koroneiki cultivar, which is distinguished from the others for its high mean values of total phenols, phenolic alcohols, secoiridoid derivatives, pigments, and oxidative stability. The Arbosana variety, characterized by high rates of tocopherols and lignans, forms cluster 2. Finally, Arbequina and Picholine marocaine VOOs constitute Cluster 3. At a rescaled distance of 348, the cultivars analyzed distribute into two major clusters: one cluster groups the Koroneiki and Arbosana cultivars, while the second cluster includes the Arbequina and Picholine marocaine cultivars.

### 4. CONCLUSION

The olive oils analyzed in the present study displayed interesting qualitative characteristics underlining their classification as extra virgin olive oils. The VOOs contain a pool of minor compounds, particularly phenolic compounds and α–tocopherol; their powerful antioxidant activity contributes to their resistance to oxidation. A positive correlation between the oxidative stability and the high content in minor compounds, particularly phenols, is well established. Significant differences between analyzed olive oils of European cultivars were highlighted when the same were compared with each other, with the VOO of Picholine marocaine, and with their VOOs as produced in their respective sites of origin. The observed differences seem to relate mainly to olive cultivars and to climatic conditions. Thus, among VOOs of European cultivars newly introduced in eastern Morocco, Koroneiki olive oil showed the highest content of phenols and the best resistance to oxidation, followed by Arbosana’s olive oil. However, Arbequina and Picholine olive oils showed low resistance to oxidation. The prediction of the shelf life of olive oils is a desirable goal to the food industry, since in the olive oil trade this is considered a criterion of quality. Compared to the local variety Picholine marocaine, Koroneiki and Arbosana cultivars seem to produce the best olive oils with a good shelf life. This is a confirmation of the adaptability and effectiveness of these varieties to high-density planting systems in eastern Morocco.

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