



## Phytosterols composition of virgin olive oils from cultivars introduced in eastern Morocco in comparison to *Picholine Marocaine*

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### Abstract

The aim of this study is to characterize monovarietal virgin olive oils (VOOs) of three European cultivars (*Arbequina*, *Arbosana* and *Koroneiki*), grown under high-density plantation system in eastern Morocco, by comparison of their phytosterols profiles. First, physicochemical properties of these monovarietal VOOs as well as their contents of pigments and phenols were analyzed. Then, VOOs phytosterols profiles were determined by GC-FID, and show that  $\beta$ -sitosterol is the most abundant sterol which represent 75 to 79 % of total phytosterols in analyzed olive oils. On the basis of these results, a comparison between these monovarietal VOOs and olive oil of *Picholine marocaine* (autochthonous olive tree) has been carried out, and shows that majority of analytical parameters presented statistically significant differences ( $p < 0.05$ ). The examined olive varieties produce excellent oils with a chemical composition within the regulatory limits and an appreciable amount of phytosterols. In addition, results showed that, total phytosterols content of olive oil from *Picholine marocaine* is significantly higher (2348.78 mg kg<sup>-1</sup>) than values observed for VOOs of European cultivars, which range from 1595 to 1971mg kg<sup>-1</sup> but, *Koroneiki's* VOO has the highest phenols content (493.66 mg kg<sup>-1</sup>) and the highest pigments content (3.94 and 2.17 mg kg<sup>-1</sup> respectively for chlorophylls for carotenoids). Lastly, according to VOOs' content of minor components (phenols, pigments and phytosterols), the hierarchical cluster analysis shows a good discrimination between olive tree varieties.

**Key words:** Virgin olive oil, *Arbequina*, *Arbosana*, *Koroneiki*, *Picholine marocaine*, Phytosterols, Pigments, Phenols.

### 1. Introduction

Virgin olive oil (VOO) is becoming increasingly more relevant in the diet due to its nutritional value and beneficial effects on human health. In general, these effects are associated with VOO content of phenolic compounds, high amounts of oleic acid, tocopherols and phytosterols [1].

The ratio of unsaponifiable matter in the olive oil is about 1 to 2% [2]. Much of this fraction is represented by phytosterols, which are recognized by their biological effects, such as cancer prevention [3], blood cholesterol control [4], and cytostatic activity [5]. The phytosterols fraction can be categorized into four subclasses: 4,4-desmethylsterols, 4-amethylsterols, 4,4-dimethylsterols and triterpene dialcohols [6]. The quantitative sterolic profiles and quality of olive oil and vegetable oil are affected by several factors [7-9]. Among these factors, the ripening cycle of the fruit and the nature of the cultivar, oil extraction and refining procedures and storage conditions [10, 11] are the main ones. The effects of agronomic and climatic conditions have also been studied [12].

Although Morocco possesses substantial genetic diversity among its olive tree varieties, the distinguishing feature of the varietal structure of Morocco's olive orchards is the predominance of the dual-purpose *Picholine marocaine*. This variety accounts for 90% of the total olive tree orchards [13]. In the hope of ameliorating its performance and quality by renewing olive plantations or by blending, some European cultivars, such as *Arbequina*, *Arbosana* and *Koroneiki*, are introduced in the main olive-growing regions of Morocco (Haouz, Saiss, Orientale), and occurring in irrigated areas with a high-density. Biochemical

characterization of olive oils of these European varieties have been the object of numerous studies in their home regions [14, 15]. However, little is known about the nature and concentrations of minor components of monovarietal olive oils of those Spanish and Greek cultivars that have been recently introduced in eastern Morocco.

Hence, the aim of this investigation is to characterize four monovarietal olive oils for their phytosterol fraction, to provide a further contribution towards the production of oils with different good sensory and chemical composition characteristics, which could be recommended to Moroccan olive growers for large-scale plantations in the future.

## 2. Materials and methods

### 2.1. Plant material

Samples of monovarietal VOOs produced during the 2012 / 2013 crop season are 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 high-density plantation (HDP) system with a frame of 1.5m/4m 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 drippers placed around the trees delivering water flow of 1.2 l/h. The climate is a Mediterranean type with hot and dry summers and an annual average rainfall ranging from 275.3 to 516.0 mm.

The olive fruits came from orchards located in "Oujda-Angad" region are harvested at the optimum ripening index (RI = 3.8) and immediately, processed by a continuous industrial 2-phase system « Perialisi », at the Company "Huiles d'olive de la Méditerranée". Olives of the studied varieties were cleaned of leaves, washed with water and crushed with a hammer crusher. The resulting paste was malaxed for 30 min at 27°C and then centrifuged twice, respectively horizontal and vertical centrifuges.

The physicochemical parameters of monovarietal VOOs were carried out within 7 days after production; but in the meantime, samples of 500 mL were stored in dark bottles without leaving space in the head, at a temperature of 4°C, for others analysis. The moisture contents of olive oil samples are lower than 1%.

### 2.2. Quality index

The determination of free acidity, peroxide value and specific absorbance at 232 and 270 nm ( $K_{232}$ ,  $K_{270}$  and  $\Delta K$ ) were determined according to the European Communities official methods (EEC) [16].

### 2.3. Determination of chlorophyll and carotenoid compounds

Chlorophyll and carotenoid compounds were determined at 670 and 470 nm, respectively, in cyclohexane using the specific extinction values, were  $E_0=613$  for pheophytin as a major component in the chlorophyll fraction and  $E_0=2000$  for lutein as a major component in the carotenoid fraction [17].

### 2.4. Determination of the total phenolic content

The phenolic compounds were extracted according to the method described by Ollivier *et al.*[18]. A 10 mL aliquot of a methanol/water solution (80/20; V/V) was added to 10 g of olive oil in a centrifuge tube. After 10 min of vigorous mixing, the tubes were centrifuged for 15 min at 3800 rpm. The hydro-methanolic phase was recovered and transferred to a 5 mL volumetric flask. This operation was repeated two times and the volume was brought to 25 mL using the methanol/water solution (80/20; V/V).

Total phenols were determined according to Folin-Ciocalteu method. A 2 ml aliquot of each solution was placed in a test tube, and 1 ml of Folin-Ciocalteu reagent (Sigma Aldrich, St Louis, MO, USA), 5 ml of distilled water and 5 ml of a 10% solution of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added. The solutions were shaken immediately and were thoroughly mixed and then were maintained in darkness for 30 min. The absorbance of each solution at 750 nm relative to that of a blank was determined. A calibration curve was obtained using four solutions of caffeic acid (Sigma Aldrich, St Louis, Mo, USA) at concentrations of 0.01–0.20 mg ml<sup>-1</sup> [18].

### 2.4. Phytosterols analysis

The analysis of phytosterols was conducted according to the method described by Vanderplanck *et al.* [19], with some modifications. One gram of olive oil was added to butilin (1mg/ml), used as internal standard, and

was saponified with a potassium hydroxide methanolic solution (2 M). After 1 hour of boiling, water was added and the extraction of the unsaponifiable fraction was carried out with diethyl ether. Following purification with water and drying over anhydric sodium sulfate, diethyl ether was evaporated. The unsaponifiable fraction was dissolved in chloroform, and approximately 600 µl were loaded on a basic silica plate chromatography. The sterol fraction was separated by elution with a mixture of chloroform, diethyl ether and ammonia water (90:10:0.5; V/V). The corresponding band was visualized under UV light after being sprayed with a 2',7'-dichlorofluoresce in 0.2% ethanolic solution, than scraped off with a spatula, and extracted with chloroform. After the extract was evaporated under a gentle stream of nitrogen, phytosterols were converted to trimethylsilyl ethers by the addition of 100 µl of a (1:1; V/V) mixture of anhydrous pyridine and silylation reagent [trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) 99:1; V/V (Supelco, Bellefonte, USA)], left for 30 min at 90°C. The reagents were evaporated under nitrogen. The mixture was analyzed by gas chromatography using a chromatograph Hewlett-Packard (HP 6890 series GC) equipped with a capillary column (HP 5 ms: 30 m × 0.32 mm, 0.25 µm film thickness) and a flame ionisation detector. The injector was operated in splitless mode. The operating conditions were as follow: carrier gas: Helium at 1 ml min<sup>-1</sup>; column temperature: 275 °C; injector and detector temperature: 250 and 300 °C, respectively; injection volume: 5 µl. β-sitosterol, cholesterol, stigmasterol, campesterol, erythridiol and uvaol were identified by using a commercial standard obtained from sigma Aldrich (St Louis, Mo, USA), and clerosterol, Δ-5-avenasterol, Δ-5,24-Stigmatsadienol, Δ-7-Stigmastanol and Δ-7-Avenasterol were identified by comparing the relative retention times (β-sitosterol – TMS = 1.00) with those of olive oil reference (EEC, Annexes V and VI) [20]. Quantification was performed by the internal standard (butilin) method.

### 2.5. Statistical analysis

Values of different parameters were expressed as the mean ± standard deviation (± SD). Significant differences between mean ( $P < 0.05$ ) were determined by ANOVA test using SPSS software for windows (SPSS 20, USA). Furthermore, all the obtained data were submitted to a classification by hierarchical cluster analysis (HCA) using the XLSTAT software for windows, version 2013.5.06 (Addinsoft).

## 3. Results and discussion

### 3.1. Determination of oil quality

All the oils analyzed showed low values for the regulated physicochemical analytical parameters evaluated (acidity ≤ 0.8%; peroxide value ≤ 20 meq O<sub>2</sub> kg<sup>-1</sup>; K<sub>270</sub> ≤ 0.22; K<sub>232</sub> ≤ 2.5 and ΔK ≤ 0.01) (Table 1), with all of them falling within the extra virgin olive oil category, as stated International Olive Oil Council [21].

**Table 1:** Free acidity, peroxide value and UV absorbance of the studied virgin olive oils produced in oriental region of Morocco.

Physicochemical parameters	Varieties				EVOO*
	Introduced cultivars			Autochthonous cultivar	
	<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	<i>Picholine marocaine</i>	
Free acidity (% C18:1)	0.46±0.03 <sup>a</sup>	0.53±0.03 <sup>a</sup>	0.58±0.09 <sup>a</sup>	0.51±0.3 <sup>a</sup>	≤ 0.8
Peroxide value (meq O <sub>2</sub> kg <sup>-1</sup> )	8.26±0.49 <sup>a</sup>	9.10±0.40 <sup>a</sup>	10.51±0.46 <sup>b</sup>	8.89±0.73 <sup>a</sup>	≤ 20
K <sub>270</sub>	0.08±0.01 <sup>a</sup>	0.11±0.01 <sup>ab</sup>	0.14±0.01 <sup>b</sup>	0.13±0.02 <sup>bc</sup>	≤ 0.22
K <sub>232</sub>	1.43±0.18 <sup>a</sup>	1.56±0.01 <sup>a</sup>	1.63±0.10 <sup>a</sup>	1.49±0.20 <sup>a</sup>	≤ 2.5
ΔK	0.0020±0.0002 <sup>ab</sup>	0.0010±0.0003 <sup>a</sup>	0.0040±0.0003 <sup>c</sup>	0.0020±0.0005 <sup>b</sup>	≤ 0.01

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

\*Extra virgin olive oil quality criteria, Values limits set by International Olive Oil Council [21].

Note that low values for those quality parameters can be translated into a higher quality of the oil obtained from fresh and healthy olives, harvested at the optimal ripening point, followed by immediate extraction without proceeding to olive storage [22]. Although some significant differences ( $P < 0.05$ ) in peroxide values and ultraviolet absorbance (K<sub>232</sub> and ΔK) were found, they were not useful for discriminating between oil samples. These results are consistent with the findings of who reported that cultivar had no significant influence on these analytical parameters [23, 24]. In addition, the effects of irrigation on oil quality indices are

quite controversial. In fact, Greven et al. [25] and Dag et al. [26] reported that the olive oils coming from the irrigated plants showed values of free acidity significantly higher than those found in the olive oils obtained from the non-irrigated trees. Conversely, Palese et al [27] and Berenguer et al. [28] concluded that these quality parameters were influenced by fruit and past manipulation than by the irrigation practices.

### 3.2. Chlorophyll and carotenoid contents

In olive oil, lutein and pheophytin are the main compounds of carotenoids and chlorophylls, respectively. Moreover, both chlorophylls and carotenoids are also involved in autoxidation and photooxidation mechanisms [17]. These pigments, in olive oils, act as prooxidants in presence of light and as antioxidants in darkness [29]. The amounts of chlorophylls and carotenoids in all olive oils samples show significant differences ( $P < 0.05$ ) among the different varieties (Table 2). The highest contents of chlorophylls and carotenoids were observed in *Koroneiki* oil with 3.94 and 2.17 mg kg<sup>-1</sup>, respectively; the lowest amounts were recorded in *Picholine marocaine* oil (1.69 and 1.43 mg kg<sup>-1</sup>, respectively). As reported by different authors, the presence of the pigment in the oil depends on several factors, such as the olive cultivar, soil and climatic conditions, fruit ripeness and the processing procedures [30].

**Table 2:** Phenol and pigment (chlorophylls & carotenoids) contents of the studied virgin olive oils produced in oriental region of Morocco.

(mg kg <sup>-1</sup> )	Varieties			
	Introduced cultivars			Autochthonous cultivar
	<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	<i>Picholine marocaine</i>
Total phenols*	241.28±6.70 <sup>a</sup>	411.64±6.70 <sup>a</sup>	493.66± 4.89 <sup>d</sup>	316.59±10.18 <sup>c</sup>
Chlorophylls	1.86±0.04 <sup>b</sup>	1.94±0.03 <sup>c</sup>	3.94±0.01 <sup>d</sup>	1.69±0.03 <sup>a</sup>
Carotenoids	1.66±0.09 <sup>b</sup>	1.65±0.01 <sup>b</sup>	2.17±0.02 <sup>c</sup>	1.43±0.09 <sup>a</sup>

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

\*Concentration of polyphenols expressed as milligram per kilogram of oil caffeic acid equivalent (colorimetric method).

### 3.3. Total phenols content

The phenolic compounds present in virgin olive oil samples are one of the bases of nutritional importance and shelf life of this oil [31]. Table 2 presents the phenolic content of each sample of VOOs. Significant differences ( $P < 0.05$ ) between cultivars are observed in the total phenol contents. The maximum phenols concentration was detected in *Koroneiki* oil, with 493.66 mg kg<sup>-1</sup>. While *Arbequina* oil showed the lowest value (241.28 mg kg<sup>-1</sup>). These results are in agreement with the findings of Aguilera et al. [32], which reported that the amount of total phenols normally ranges between 50 and 1000 mg kg<sup>-1</sup>, depending on various factors such as cultivar, climate, location, degree of maturation, type of crushing machine and oil extraction procedures. As mentioned above, the effects of irrigation on total phenols are quite controversial. In fact, a negative correlation between phenol content in olive oil and soil water availability, depending on accumulated rainfall or irrigation, has been observed by many studies [27, 33]. Whereas, in another study, the phenol content assessed in the olive oil obtained from irrigated plants resulted significantly higher than the measured in the olive oil from non-irrigated trees [25].

### 3.4. Phytosterols

The composition of the sterol fraction of olive oil is a very useful parameter for detecting adulterations or to check authenticity, since it can be considered as a fingerprint [34]. The amounts of total phytosterols show significant differences ( $P < 0.05$ ) among the different varieties (Table 3). It's higher than the minimum established by International Olive Oil Council [21] for extra virgin olive oil category ( $\geq 1000$  mg kg<sup>-1</sup>) in all samples. The highest content of these components was detected in *Picholine marocaine* oil, with 2348.78 mg kg<sup>-1</sup>; the lowest amount was recorded in *Koroneiki* oil (1595.89 mg kg<sup>-1</sup>). This was probably due to the effects of irrigation on phytosterol content in olive oil. In fact, Stefanoudaki et al. [12] reported that the phytosterol content in the olive oil from water stressed plants were significantly higher than those found in the oil obtained from irrigated trees.

Table 3 shows the phytosterol composition of monovarietal virgin olive oils of the studied varieties. The four extra virgin olive oils shows a phytosterol composition in compliance within the established limits, which

ranges depend on the varieties ( $P < 0.05$ ).  $\beta$ -sitosterol and  $\Delta$ -5-avenasterol are the major phytosterols in all samples (Fig 1). These two phytosterols are negatively correlated. The relative contents of  $\beta$ -sitosterol and  $\Delta$ -5-avenasterol are within the range of 75–83% and 9–16%, respectively. *Picholine marocaine* oil show the highest value for  $\beta$ -sitosterol (82.75%) and the lowest one for  $\Delta$ -5-avenasterol (9.53%), while *Korneiki* is characterized by the lowest percentage of  $\beta$ -sitosterol (75.65%) and the highest one of  $\Delta$ -5-avenasterol (15.97%). Other researchers reported that  $\beta$ -sitosterol is minimal and  $\Delta$ -5-avenasterol is maximal when olives are harvested at their optimum [10, 35].

**Table 3:** Phytosterol composition of the virgin olive oils samples (Results are expressed as mg kg<sup>-1</sup> and percentage of total phytosterols) produced in oriental region of Morocco.

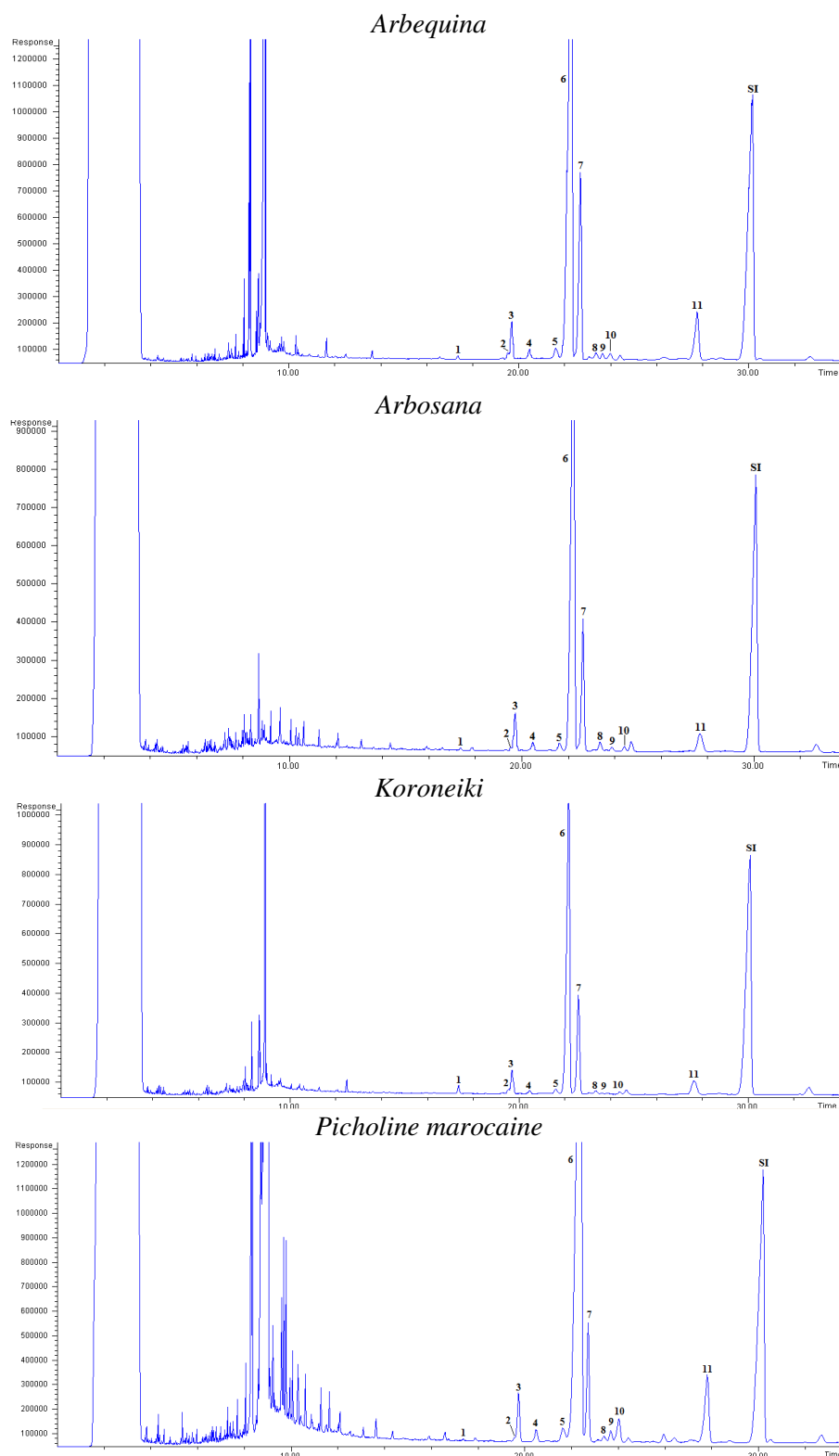
Phytosterols		Varieties				EVOO**
		Introduced cultivars			Autochthonous cultivar	
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	<i>Picholine marocaine</i>	
Concentration (mg kg <sup>-1</sup> )	Cholesterol	3.48±0.43 <sup>c</sup>	2.32±0.12 <sup>ab</sup>	2.58±0.11 <sup>b</sup>	1.99±0.07 <sup>a</sup>	
	24-Methylencholesterol	7.58±0.52 <sup>c</sup>	3.54±0.26 <sup>a</sup>	6.79±0.37 <sup>b</sup>	4.21±0.15 <sup>a</sup>	
	Campesterol	57.01±3.07 <sup>b</sup>	66.36±1.65 <sup>c</sup>	40.13±1.09 <sup>a</sup>	71.04±0.38 <sup>d</sup>	
	Stigmasterol	14.81±0.30 <sup>b</sup>	15.03±0.34 <sup>b</sup>	6.50±0.20 <sup>a</sup>	17.61±0.33 <sup>c</sup>	
	Clerosterol	23.10±2.44 <sup>b</sup>	20.04±1.30 <sup>ab</sup>	15.64±6.17 <sup>a</sup>	25.37±2.13 <sup>b</sup>	
	$\beta$ -Sitosterol	1553.36±15.06 <sup>b</sup>	1501.96±54.89 <sup>b</sup>	1207.76±63.65 <sup>a</sup>	1943.68±25.25 <sup>c</sup>	
	$\Delta$ -5-Avenasterol	247.23±28.72 <sup>ab</sup>	261.98±2.90 <sup>b</sup>	254.60±3.27 <sup>b</sup>	223.72±8.23 <sup>a</sup>	
	$\Delta$ -5,24-Stigmastadienol	12.86±0.62 <sup>c</sup>	21.49±1.30 <sup>d</sup>	9.13±0.30 <sup>b</sup>	7.48±0.14 <sup>a</sup>	
	$\Delta$ -7-Stigmastanol	7.76±0.17 <sup>c</sup>	5.24±0.28 <sup>a</sup>	6.76±0.74 <sup>b</sup>	10.64±0.55 <sup>d</sup>	
	$\Delta$ -7-Avenasterol	18.67±1.32 <sup>d</sup>	13.37±0.37 <sup>c</sup>	11.42±0.40 <sup>b</sup>	4.52±0.29 <sup>a</sup>	
	Erythrodiol	25.28±0.68 <sup>a</sup>	29.80±1.01 <sup>ab</sup>	34.57±6.08 <sup>bc</sup>	38.51±1.30 <sup>c</sup>	
	Uvaol	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	
	Total phytosterols	1971.13±12.55 <sup>b</sup>	1941.14±54.24 <sup>b</sup>	1595.89±62.19 <sup>a</sup>	2348.78±18.92 <sup>c</sup>	≥ 1000
Relative amount (%)	Cholesterol	0.18±0.02 <sup>c</sup>	0.12±0.00 <sup>b</sup>	0.16±0.01 <sup>c</sup>	0.08±0.00 <sup>a</sup>	≤ 0.5
	24-Methylencholesterol	0.38±0.03 <sup>b</sup>	0.18±0.01 <sup>a</sup>	0.43±0.01 <sup>c</sup>	0.18±0.01 <sup>a</sup>	
	Campesterol	2.89±0.17 <sup>b</sup>	3.42±0.17 <sup>c</sup>	2.52±0.17 <sup>a</sup>	3.02±0.03 <sup>b</sup>	≤ 4
	Stigmasterol	0.75±0.02 <sup>b</sup>	0.77±0.01 <sup>b</sup>	0.41±0.01 <sup>a</sup>	0.75±0.01 <sup>b</sup>	≤ Campesterol
	Clerosterol	1.17±0.13 <sup>a</sup>	1.03±0.04 <sup>a</sup>	0.97±0.35 <sup>a</sup>	1.08±0.09 <sup>a</sup>	
	$\beta$ -Sitosterol	78.81±1.10 <sup>b</sup>	77.36±0.71 <sup>b</sup>	75.65±1.11 <sup>a</sup>	82.75±0.46 <sup>c</sup>	
	$\Delta$ -5-Avenasterol	12.54±1.39 <sup>b</sup>	13.51±0.50 <sup>b</sup>	15.97±0.69 <sup>c</sup>	9.53±0.42 <sup>a</sup>	
	$\Delta$ -5,24-Stigmastadienol	0.65±0.03 <sup>c</sup>	1.11±0.04 <sup>d</sup>	0.57±0.02 <sup>b</sup>	0.32±0.01 <sup>a</sup>	
	$\Delta$ -7-Stigmastanol	0.39±0.01 <sup>b</sup>	0.27±0.02 <sup>a</sup>	0.42±0.03 <sup>b</sup>	0.45±0.02 <sup>c</sup>	≤ 0.5
	$\Delta$ -7-Avenasterol	0.95±0.07 <sup>c</sup>	0.69±0.03 <sup>b</sup>	0.72±0.05 <sup>b</sup>	0.19±0.01 <sup>a</sup>	
	Erythrodiol	1.28±0.04 <sup>a</sup>	1.54±0.06 <sup>a</sup>	2.18±0.46 <sup>b</sup>	1.64±0.05 <sup>a</sup>	
	Erythrodiol + Uvaol	1.28±0.04 <sup>a</sup>	1.54±0.06 <sup>a</sup>	2.18±0.46 <sup>b</sup>	1.64±0.05 <sup>a</sup>	≤ 4.5
	Apparent $\beta$ -sitosterol*	93.17±0.34 <sup>a</sup>	93.01±0.29 <sup>a</sup>	93.17±0.65 <sup>a</sup>	93.68±0.04 <sup>a</sup>	≥ 93

Values are the means of the four different VOO samples ± standard deviations. Significant differences in a same row are showed by different letters (a-d) ( $P < 0.05$ ). ND, Component not detected.

\*Apparent  $\beta$ -sitosterol =  $\beta$ -sitosterol +  $\Delta$ -5-avenasterol + clerosterol +  $\Delta$ -5,24-stigmastadienol.

\*\*Extra virgin olive oil quality criteria, Values limits set by International Olive Oil Council [21].

The other main phytosterols identified in these extra virgin olive oils are stigmasterol and campesterol. Their contents vary from one cultivar to another. Stigmasterol is present in all samples in lesser amounts than campesterol, which indicates that all oil samples have been obtained from healthy fruits, naturally ripened on the plant [10]. Also the Campesterol never exceed the upper limit established by International Olive Oil Council (4%). The campesterol content for *Picholine marocaine* and *Arbequina* was significantly higher than the values of the other cultivars (66.36 and 71.04 mg kg<sup>-1</sup>, respectively).



**Figure 1:** GC-FID chromatograms of phytosterol components detected in studied virgin olive oil samples. (1) Cholesterol; (2) 24-Methylencholesterol; (3) Campesterol; (4) Stigmasterol; (5) Clerosterol; (6)  $\beta$ -Sitosterol; (7)  $\Delta$ -5-Avenasterol; (8)  $\Delta$ -5,24-Stigmastadienol; (9)  $\Delta$ -7-Stigmastanol; (10)  $\Delta$ -7-Avenasterol; (11) Erythrodiol; (SI) Internal standard (Butilin).

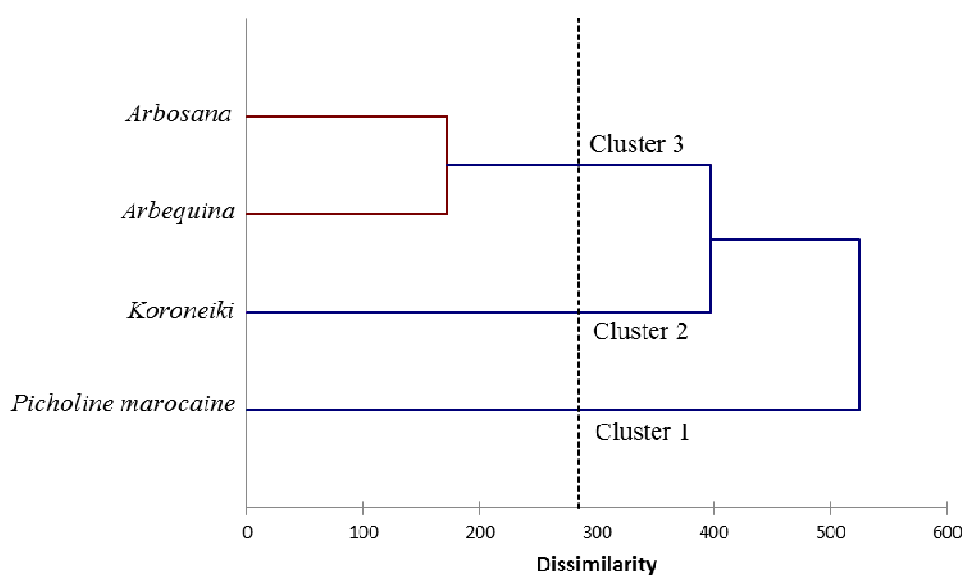
Others phytosterols, such as cholesterol, 24-methylenecholesterol, clerosterol,  $\Delta$ -5,24-stigmastadienol,  $\Delta$ -7-stigmastanol and  $\Delta$ -7-avenasterol were also determined at low amounts. However, the amount of cholesterol for *Picholine marocaine* oil (0.08 %) is significantly lower than the values of the other varieties. The highest

24-methylenecholesterol content was found in the *Koroneiki* sample (0.43 %). Whereas, the highest clerosterol and  $\Delta$ -7-avenasterol contents were found in *Arbequina* olive oil (1.17 and 0.95 %, respectively). Concerning apparent  $\beta$ -sitosterol, all of the monovarietal oils contain more than 93 %, the minimum value established by the International Olive Oil Council for extra virgin olive oil category. This parameter expressed by the sum of the contents of  $\beta$ -sitosterol and the other sterols formed by the degradation of  $\beta$ -sitosterol ( $\Delta$ -5,24- stigmastadienol, clerosterol and  $\Delta$ -5-avenasterol) [21].

On the other hand, for the triterpenic dialcohols (erythrodiol and uvaol) in the total sterol fraction, only erythrodiol was detected and quantified. The amounts of erythrodiol were within the established limit for the extra virgin olive oil category in all samples, higher values would indicate blending with olive-pomace oil [21]. *Koroneiki*'s VOO had the highest level of erythrodiol (2.18 %) compared to *Picholine marocaine*, which had the intermediate level of this compound (1.64 %).

### 3.5. Hierarchical cluster analysis

HCA is an unsupervised technique that uses the information obtains from the measured variables to reveal the natural clusters existing between the studied samples [36]. The formation of groups is based on the similarities between the samples. Fig 2 shows the results from HCA. The dendrogram obtained indicates that at a rescaled distance of 284, the cultivars are distributed into three major clusters. Cluster 1 exclusively includes the *Picholine marocaine* cultivar, which is distinguished from the others for its high mean values of total phytosterols,  $\beta$ -sitosterol and low content of pigments. *Koroneiki* variety, which is characterized by high rates of total phenols and low content of total phytosterols, form cluster 2. Finally, Cluster 3 is constituted by *Arbequina* and *Arbosana* VOOs. At a rescaled distance of 525, the cultivars analyzed are distributed into two major clusters: one cluster groups *Arbequina*, *Arbosana* and *Koroneiki* cultivars, while the second cluster includes autochthones cultivar.



**Figure 2:** Dendrogram of analytical virgin olive oil variables obtained from different studied cultivars using Euclidean distance.

### Conclusion

The analysis of VOOs from four varieties cultivated in East of Morocco showed that all the quality parameters fall within the limits established for the extra virgin olive oil category. The European cultivars evaluated, when grown in east of Morocco, can produce good olive oils with different characteristics in terms of phenols, pigments and phytosterols. In fact, the European cultivars had the lowest levels of phytosterols compared to *Picholine marocaine*, which had the highest level in these compounds. Whereas, except for *Arbequina*, the European cultivars showed the highest phenols and pigments contents, in comparison to autochthones cultivar. This is a confirmation of the adaptability to the environmental conditions, especially the semi-arid climate of East of Morocco, and effectiveness of the high-density planting system in east of Morocco.

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