Preliminary Characterization of monovarietal virgin olive oils produced in eastern area of Morocco

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ABSTRACT

Traditional olive oil production is limited by its high cost, mainly due to labour expenses for harvesting and pruning. New olive cultivars (e.g. *Arbequina, Arbosana, Koroneiki*) with greater adaptability to modern irrigated high-density orchards and producing good quality olive oils are highly demanded by an olive oil industry in continuous change The aim of this study is the characterization of monovarietal virgin olive oils from three cultivars (*Arbequina, Arbosana and Koroneiki*) recently introduced in east of Morocco, and the comparison with traditional local *Picholine Marocaine* olive oil. Several analytical parameters were evaluated; including quality index, Triacylglycerol, fatty acids, phenolic and chlorophyll contents and oxidative stability. Significant differences between the analysed olive oils were detected. Olive oils from *Koroneiki* and *Arbosana* cultivars had higher values of oleic acid (respectively 76.24 and 75.68 %); *Picholine* olive oil, had the lowest one 67.49%. *Koroneiki* olive oil was noteworthy for its higher content of phenolic compounds (459.48 mg/ kg) and a high oxidative stability (93.16 h). We concluded that the recently introduced cultivars are well adapted to the eastern area of Morocco and could be of great interest for producing monovarietal olive oils.

1 Introduction

In Morocco, olive orchards are dominated by the local cultivar *Picholine Marocaine* covering more than 90% of the Moroccan olive groves. The rest is made up of several European introduced cultivars, in particular *Picholine Languedoc*, *Gordal*, *Manzanilla*, *Ascolana Dura*, *Arbequina*, *Arbosana and Koroneiki*. Those plantations are located in irrigated areas where certain cultivars (*Arbequina*, *Arbosana and Koroneiki*) are used for new high-density plantings (HDP) which were seen around Marrakech, Meknes and Oujda.

The advantage of the HDP system lies in the use of mechanical harvesters which are swift and highly efficient. The remarkable harvesting capacity makes it possible to pick large quantities of olives with a perfect degree of ripening on large scale plantations. In some case olive processing can be carried out immediately, since it is becoming increasingly common in HDP plantations to build on-site an olive mill. HDP system allows reduction of production cost and could provide an olive oil of quite good to superior quality (Ait Hmida, 2010)

The many factors affecting the characterisation of virgin olive oils can be clustered into four main groups: environmental (soil, climate), agronomic (irrigation, fertilisation), cultivation (harvesting, ripeness), and technological factors (post-harvest storage and extraction system). The aim of this study is to investigate environmental effect on olive oils from *Arbequina*, *Arbosana* and *Koroneiki* in eastern area of Morocco.

2 Material and Methods

2.1 Olive oil samples

Samples of olive oil, produced during crop season 2012/ 2013, are from four varieties grown in east of Morocco: *Arbequina*, *Arbosana* and *Koroneiki* as European cultivars, and *Picholine* an autochthonous cultivar. The European cultivars were conducted under irrigated HDP with a frame of 1,4m/4m and a density of 1300 trees/ha. The autochthonous cultivar is conducted in rain fed system. The irrigation period for HDP system was 7 month per year (from March 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 330 to 500 mm.

2.2 Analytical methods

2.2.1 Physicochemical parameters

Free acidity (g/100 g oleic acid), peroxide value (meq O_2/kg of olive oil), and ultraviolet absorption indices (K232, K270 and ΔK) were determined according to the commercial standard methods for olive oil "International Olive Oil Council" (IOOC, 2001). The average was calculated by three replications for each sample.

2.2.2 Colorimetric determination of phenols contents

Total phenols was analysed as described by Ollivier et al. (2004); Phenolic content were determined according to Folin–Ciocalteu method using caffeic acid as a standard and by absorbance at λ750 nm.

2.2.3 Determination of chlorophylls and carotenoid contents

The chlorophylls fraction was evaluated by absorbance at λ 670 nm and the carotenoids fraction at λ 470 nm, according to Minguez-Mosquera et al. (1990). The values of the specific extinction coefficients used were E_0=613 for pheophytin as major component in the chlorophyll fraction and E_0=2000 for lutein as major component in the carotenoid fraction. Thus, pigment contents were calculated as follows in the equation 1 and 2.

$$\text{Chlorophyll (mg.kg}^{-1}) = \frac{A_{670} \times 10^6}{613 \times 100 \times d} \quad (1)$$

$$\text{Carotenoid (mg.kg}^{-1}) = \frac{A_{470} \times 10^6}{2000 \times 100 \times d} \quad (2)$$

2.2.4 Fatty acid composition analysis

The fatty acid composition of the oil samples was determined as methyl esters by gas chromatography (HP 6890 series GC), equipped with a capillary column (Supelcowax: 30 m × 250 mm × 0.25 μm) and an FID detector. The carrier gas was nitrogen, at a flow of 1.7 mL/min. Injector and detector temperatures were set at 150 and 250°C, respectively and the oven temperature was set at 210 °C. The injection volume was 1μL.

Iodine index was calculated from fatty acid percentages as described by Maestri and al., (1998)

2.2.5 Chromatographic analysis of triglycerides

The chromatographic HPLC system is a Shimadzu model LC-6AD, CBM 20A controller and refractive index detector RID 10A. HPLC analyses were conducted using C18 reversed- phase column (ODS C18: 250× 5mm, 5μm). The mobile phase consisted of acetone/acetonitile (60/40; V/V)). Elution was carried out at 1ml/min in isocratic conditions. olive oil was dissolved in acetone (9%) and filtered through 0.45μm membranes. The injection volume is 20 μl. All separations are performed at ambient temperature.

2.2.6 Evaluation of oil stability

Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989) and was expressed as the oxidation induction time (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.

2.2.7 Statistical analysis

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation ($\bar{x}$ ± SD). Significant differences between mean ($p < 0.05$) were determined by ANOVA test using SPSS software for windows (SPSS. 19, USA).

3 Results and Discussion

3.1 Olive oil's physicochemical indices

The analysed olive oils (Table1) showed very low values for the regulated physicochemical parameters evaluated (acidity ≤0.8%; peroxide value ≤20meqO_2 kg^{-1}; K270 ≤0.22; K232 ≤2.5 and ΔK ≤0.01). Those quality parameters did not exceed the limits established for the best commercial quality olive oil, designated as "extra virgin olive oil category", by International Olive Oil Council (IOOC, 2011). These results show that the cultivar had no significant influence on these indices, which are rather affected by factors causing damage to the olive fruits. The 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.

It is known that olives at later stages of ripening give oils with higher levels of free acidity since they undergo an increase in enzymatic activity, especially lipolytic enzymes, and are more sensitive to pathogenic infections and mechanical damage (Salvador et al., 2001). Some significant differences in the values of peroxide value and ultraviolet absorbance K270 and ΔK according to varieties were found (p<0.05).

Table 1 Physicochemical quality indices of monovarietal virgin olive oil produced in eastern area of Morocco.

Physicochemical parameters	Introduced cultivars			Autochthonous cultivar	EVOO[a]
	Arbequina	*Arbosana*	*Koroneiki*	*Picholine Marocaine*	
Free fatty acids (% C18:1)	0.46 ± 0.03a	0.53 ± 0.11a	0.58 ± 0.04a	0.51 ± 0.04a	≤ 0.8
Peroxide value (m eqO_2 kg^{-1})	12.55 ± 1.16b	17.54 ± 0.69c	17.08 ± 1.13c	8.89 ± 0.89a	≤ 20
K270	0.08 ± 0.02a	0.11 ± 0.01ab	0.14 ± 0.01b	0.13 ± 0.02b	≤ 0.22
K232	1.43 ± 0.22a	1.56 ± 0.01a	1.63 ± 0.13a	1.49 ± 0.24a	≤ 2.5
ΔK	0.002± 0.0003b	0.001± 0.0003b	0.004± 0.0004c	0.002 ± 0.0006b	≤ 0.01

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).
[a] Extra virgin olive oil, IOOC (2011).

3.2 Fatty acid composition

The fatty acid (FA) composition has previously been used as a parameter for oil classification because of its importance in the description and determination of adulteration (EEC, 2003). Table 2 shows fatty acids composition of monovarietal virgin olive oils of the studied varieties. Palmitic, oleic and linoleic acids are the major fatty acids, while palmitoleic, margaric, margaroleic, 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 virgin olive oil (IOOC, 2011).

Table 2 Fatty acid compositions and iodine index of monovarietal virgin olive oil produced in eastern area of Morocco.

Fatty acid	Introduced cultivars			autochthonous cultivar	EVOO[a]
	Arbequina	*Arbosana*	*Koroneiki*	*Picholine Marocain*	
C16:0 (%)	17.65 ± 3.64a	13.71 ± 0.43a	15.7 ± 0.37a	15.93 ± 2.82a	7,5 - 20,0
C16:1 (%)	1.89 ± 0.37b	1.27 ± 0.04a	1.23 ± 0.06a	1.2 ± 0.24a	0,3 - 3,5
C17:0 (%)	0.11 ± 0.02a	0.14 ± 0.01b	ND	ND	≤ 0,3
C17:1 (%)	0.27 ± 0.04a	0.34 ± 0.01b	ND	ND	≤ 0,3
C18:0 (%)	1.86 ± 0.56b	1.91 ± 0.44b	0.51 ± 0.13a	1.6 ± 0.09b	0,5 - 5,0
C18:1 (%)	69.05 ± 4.14a	75.68 ± 0.7b	76.24 ± 0.52b	67.49 ± 3.13a	55,0 - 83,0
C18:2 (%)	8.13 ± 0.1c	5.66 ± 0.07b	5.26 ± 0.07a	12.85 ±0.03d	3,5 - 21,0
C18:3n3 (%)	0.54 ± 0.02a	0.64 ± 0.02b	0.64 ± 0.01b	0.93 ± 0.01c	≤ 1,0
C20:0 (%)	0.26 ± 0.1a	0.37 ± 0.01b	0.24 ± 0.01a	ND	≤ 0,6
C20:1 (%)	0.21 ± 0.07ab	0.26 ± 0.01b	0.18 ± 0.01a	ND	≤ 0,4
□SFAs	19.88 ± 3.86a	16.13 ± 0.79a	16.45 ± 0.45a	17.53 ± 2.85a	
□MUFAs	71.42 ± 3.8a	77.55 ± 0.72b	77.65 ± 0.51b	68.69 ± 2.89a	
□PUFAs	8.67 ± 0.12c	6.3 ± 0.08b	5.9 ± 0.08a	13.87 ± 0.04d	
O/L ratio	8.49 ± 0.46b	13.37 ± 0.06c	14.5 ± 0.25d	5.25 ± 0.25a	
Iodine Index	80.19 ± 3.5a	81.33 ± 081a	81.07 ± 0.36a	87.74 ± 2.5b	75-94

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;. C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, margaric acid; C17:1, margaroleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid and C20:1, gadoleic acid.
[a] Extra virgin olive oil, IOOC (2011).

Significant differences were observed between cultivars (p<0.05). The main difference is observed for the most abundant oleic and linoleic acids: but not for palmitic acid. The relative contents of oleic acid varied from 67.5% to 76.24%. *Koroneiki's* olive oil had the highest content of oleic acid (76.24%) compared to

Picholine , 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 13.71 and 17.65%, according to cultivars. The highest percentage is observed for *Arbequina* (17.65%), whereas the lowest value is for *Arbosana* (13.71%). Concerning linoleic acid, *Picholine Marocaine* olive oil has the highest mean value (12.9%) whereas the lowest one is for *Koroneiki* (5.26%). The contents of the other fatty acids, including palmitoleic (C16:1), margaric acid (C17:0), margaroleic (C17:1), stearic (C18:0), linolenic (C18:3), arachidic (C20:0) and gadoleic (C20:1) acids, change from one olive oil to another, but the amounts are quite small or unidentifiable in some varieties. The amounts of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated FA (PUFAs) and the oleic /linoleic acids ratio (O/L: C18:1/C18:2) have been also evaluated. *Arbequina* olive oil is quite rich in total SFAs (19.88%), essentially because of its high content in palmitic acid, which represents the major acid of the SFAs fraction. Concerning the total MUFAs, *Koroneiki* olive oil contains the highest percentage (77.65%) because of its high content in oleic acid. *Picholine* olive oil is rather rich in total PUFAs (13.78%) because of its high contents in linoleic acid, representing the major fatty acid component of that fraction. For European cultivars the ratio O/L are respectively 8.49 for *Arbequina* and 14.5 for Koroneiki, but this ratio (O/L=5.25) is low for *Picholine*, as autochthonous cultivar. This ratio can be useful to characterize olive cultivars and to have a marked relationship with stability. These results were confirmed by the low values of iodine index 80.19% and 81.07% for *Arbequina* and *Koroneiki* cultivars, respectively (Table 2).

Variations in FA composition observed in olive oils analysed (Table 2) are probably related to both genetic factors and environmental conditions during the development and maturity of the fruit. These results are in agreement with the findings of other authors (Morello et al., 2004).

As compared to olive oils of these European varieties when they are cultivated in original growing area in Spain (Tous and Romero, 1992, 2000), *Arbosana* , grown in East of Morocco, produced olive oil with a rate of oleic acid relatively higher than in Spain (Table 3), whereas, *Arbequina* cultivated in Morocco produced lower amount of oleic acid as compared to its original growing area.

Table 3 Comparison of major FA in olive oil between cultivars planted in eastern of Morocco and their original sites.

	Varieties					
	Arbequina		*Arbosana*		*Koroneiki*	
	In Morocco	In Spain	In Morocco	In Spain	In Morocco	In Greek
C16:0 (%)	17.65 ± 3.64a	13.3[a]	13.71± 0.43a	13.4[a]	15.7 ± 0.37a	10.36[b]
C18:1 (%)	69.05 ± 4.14a	70.5[a]	75.68 ± 0.7b	74.3[a]	76.24 ± 0.52b	76.22[b]
C18:2 (%)	8.13 ± 0.1c	11.7[a]	5.66 ± 0.07b	7.6[a]	5.26 ± 0.07a	8.34[b]
Total phenols (mg kg^{-1})	209.1 ± 6,25a	128[a]	260.85 ± 8.27a	278[a]	459.48 ± 11.6c	583[b]

Data are expressed by mean values ± S.D. of three independent experiments.
[a] Irrigated high-density system.
[b] Rain fed cultural system.

Arbequina and *Arbosana* monovarietal olive oils produced in Morocco showed a relatively low level of linoleic acid and a rate of palmitic acid relatively higher than in its original growing area (Tous and Romero, 1992, 2000). *Koroneiki* Greek variety has the same composition of oleic acid in eastern of Morocco as well as in its original growing area (Greece) even conducted in rain fed conditions (Koutsaftakis et al., 2000). Concerning the palmitic and linoleic acids, *Koroneiki* variety produced oil with higher level of palmitic acid and a rate of linoleic acid relatively lower than in its original growing area (Koutsaftakis et al., 2000).

3.3 Triacylglycerol composition

Because of the specificity of the triacylglycerol composition in different kinds of fats and oils, it is increasingly used in food industry to confirm authenticity. The mean values of triacylglycerols (TAGs) for the four oils are shown in Table 4. The oils are characterized by three primary TAGs: triolein (OOO), dioleopalmitin (POO) and dioleolinolein (LOO), and six secondary TAGs: LPO, LOL, SOO, LPL, POP and POLn. For the studied olive oils, OOO. POO constitute the most representative TAGs, which percentages vary greatly as previously described for fatty acids. Thus, the highest OOO percentage is for *Koroneiki* (49%), while the lowest one is for *Arbequina* oil (37.53%).The presence of a high 1,2,3-trioleylglycerol (OOO) level in olive oil constitutes a favorable authenticity indicator. In the same way, a wide range of POO can also be noticed, from 19.8% for *Picholine* oil to 27.59% for *Arbosana* oil. Again, statistical differences ($p < 0.05$) were found between cultivars in terms of TAG content, and these results agree with those found for fatty acid composition.

Table 4 Triacylglycerol compositions of monovarietal olive oil produced in eastern of Morocco.

Triacylglycerols (%)	Introduced cultivars			Autochthonous cultivar
	Arbequina	*Arbosana*	*Koroneiki*	*Picholine Marocain*
LOL	2.24 ± 0.44b	1.09 ± 0.11a	1.11 ± 0.04a	2.45 ± 0.08b
LPL	1.76 ± 0.27b	1.4 ± 0.17a	1.39 ± 0.07a	1.81 ± 0.03b
POLn	0.23 ± 0.04a	0.62 ± 0.09b	0.54 ± 0.03b	0.78 ± 0.07c
LOO	14.87 ± 0.4c	10.29 ± 0.02b	9.87 ± 0.02a	17.28 ± 0.01d
LPO	7.89 ± 0.17d	4.98 ± 0.17b	4.36 ± 0.08a	5.94 ± 0.02c
OOO	37.53 ± 0.09a	44.91 ± 0.42b	49.08 ± 0.21d	47.82 ± 0.33c
POO	25.97 ± 0.48b	27.59 ± 0.04c	26.35 ± 0.15b	19.76 ± 0.23a
POP	1.29 ± 0.22c	0.72 ± 0.12b	0.27 ± 0.04a	0.18 ± 0.02a
SOO	4.3 ± 0.11a	4.11 ± 0.03a	3.52 ± 0.39b	2.04 ± 0.02c

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

3.4 Total phenols content

The phenolic compounds present in virgin olive oil are one of the bases 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. The amounts of total phenols show significant differences ($p < 0.05$) among the different varieties (Table 5). These compounds showed changes in the studied oils according to the cultivar. In fact, *Koroneiki* oil showed the highest phenols contents (459.48 mg kg^{-1}) whereas *Arbequina* oil presented the lowest values (209.1 mg kg^{-1}); *Arbosana* and *Picholine Marocaine* oils had intermediate values (260.85 and 316.59 mg kg^{-1}, respectively). As reported by different authors, the amount of total phenols normally ranges between 50 and 1000 mg kg^{-1}, depending on various factors such as cultivar, climate, location, degree of maturation, type of crushing machine and oil extraction procedures (Aguilera et al., 2005).

Table 5 Minor component contents and oxidative stability of monovarietal olive oil produced in east of Morocco

	Introduced cultivars			Autochthonous cultivar
	Arbequina	*Arbosana*	*Koroneiki*	*Picholine Marocaine*
Total phenols (mg kg^{-1})[a]	209.1 ± 6,25a	260.85 ± 8.27a	459.48 ± 11.6d	316.59 ± 12.47c
Chlorophylls (mg kg^{-1})	1.86 ± 0.05b	1.94 ± 0.04c	3.94 ± 0.01d	1.69 ± 0.04a
Carotenes (mg kg^{-1})	1.66 ± 0.11b	1.65 ± 0.01b	2.17 ± 0.02c	1.43 ± 0.11a
Oxidative stability (h)	49.69 ± 1.06b	60.17 ± 1.16c	93.16 ± 1.67d	43.55 ± 0.59a

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) ($p < 0.05$).

[a] Concentration of polyphenols expressed as milligram of caffeic acid per kilogram of oil (colorimetric method).

As shown in Table 3, *Arbosana* variety, when grown in eastern of Morocco, produced lower amount of total phenols as well as in Spain under HDP system (Tous and Romero, 2000) whereas, *Arbequina* cultivated in Morocco produced higher amount of total phenols as compared to its original growing area (Tous and Romero, 1992). Moreover, *Koroneik*i variety, showed a lower amount of total phenols when grown in eastern of Morocco (Table 5) as compared to original site (Aparicio and Luna, 2002) but this difference may be due to irrigation in HDP system in Morocco. No data are provided under such olive-farming system in Greece.

3.5 Pigment contents

Chlorophylls and carotenoids are the main pigments in vegetable oils. In olive oils, the main carotenoids and chlorophylls are lutein and pheophytin, respectively. Furthermore, both chlorophylls and carotenoids are also involved in autoxidation and photo oxidation mechanisms (Minguez-Mosquera et al., 1991). As shown in Table 5, Chlorophylls and carotenoids ranged, respectively from 1.69 to 3.94 mg kg^{-1} and from 1.43 to 2.17 mg kg^{-1} for all the studied cultivars. These results show that significant differences between cultivars ($p < 0.05$) were also observed in pigment contents. Furthermore, *Koroneiki* cultivar was distinguished from the other European cultivars for its higher level of chlorophylls and carotenes (3.94 and 2.17 mg kg^{-1}, for chlorophylls and carotenes, respectively). Autochthonous cultivar *Picholine Marocaine* olive oil shows low concentrations of chlorophylls and carotenes 1.69 and 1.43 mg kg^{-1} respectively. These results are in agreement with the findings of Psomiadou and Tsimidou (2001), which reported that 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.

3.6 Oxidative Stability

The oxidative stability (table 5) of the olive oils from the studied varieties, evaluated by Rancimat tests, shows significant differences according to the cultivar ($p < 0.05$). Among the studied European oils, the highest OS value was observed for *Koroneiki* oil (93.16 h) whereas the low OS value was for *Arbequina* oil (49.69 h). *Picholine Marocaine* oil has the lowest OS value (43.55 h). The fatty acids of virgin olive oil are mainly monosaturated, this fact and the presence of pigments and phenolic compounds make virgin olive oil more stable than other edible oils (Salvador et al., 2001). Recently, some authors studied the levels of contribution of different olive oil constituents on oxidative stability and they reported that phenols appear to have the highest influence (Haddada et al., 2008).

4 Conclusions

Arbequina and *Arbosana as Spanish cultivars*, when grown in Morocco under irrigated HDP system, produced oils with some differences from those obtained in their traditional growing area. However *Koroneiki*, a Greek *variety* cultivated in the same conditions as the previous varieties, produced an olive oil which has the same composition as in Greece.

Acknowledgements

The research was supported by the Morocco Belgian cooperation, Wallonia Brussels International project 2.9 and the Ministry of Education, Higher Education and Scientific Research in Morocco.

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