

REGULAR ARTICLE

Effect of crop season on the quality and composition of extra virgin olive oils from Greek and Spanish varieties grown in the Oriental region of Morocco

Farid Mansouri^{1,*}, Abdessamad Ben moumen¹, Kamal Belhaj¹, Gaëtan Richard², Marie-Laure Fauconnier², Marianne Sindic³, Hana Serghini Caid¹, Ahmed Elamrani¹

¹Laboratory of Plants and Microorganisms Biology, Faculté des Sciences Oujda, Université Mohammed Premier, BP-717, 60000 Oujda, Morocco, ²General and Organic Chemistry Unit, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium, ³Analysis Quality and Risk Unit, Laboratory of Food Quality and Safety, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

ABSTRACT

This study aims to evaluate the variations in the quality and composition of extra virgin olive oils of Spanish (*Arbequina*, *Arbosana*) and Greek (*Koroneiki*) varieties produced over two crop seasons in the Oriental region of Morocco, taking into consideration the influence of climatic conditions. To this end, several parameters were evaluated, such as quality indices, fatty acids, triacylglycerols, minor compounds (phytosterols, phenolic compounds, tocopherols and pigments) and oxidative stability. The results obtained in this study indicate that the majority of the studied parameters, with the exception of phytosterols, are influenced by climatic conditions of the crop season ($p < 0.05$). Additionally, the majority of the studied parameters were also influenced by the cultivar factor ($p < 0.05$). However, the studied varieties produce an excellent oil quality with a chemical composition respecting the requirements recommended by the International Olive Council. The main variations observed on the analyzed olive oils between the two crop seasons are those of antioxidant parameters, such as phenols and oxidative stability. Triacylglycerols and fatty acids are also influenced by the crop season's climatic conditions. Nonetheless, the behavior of the studied varieties towards climatic conditions is different.

Keywords: Crop season; Extra virgin olive oil; Fatty acid composition; Oxidative stability; Phenolic compounds

INTRODUCTION

Extra virgin olive oil (EVOO) is particularly appreciated for its unique nutritional composition and its good stability compared to other vegetable oils. The benefits of virgin olive oil on human health were associated primarily with its specific fatty acid composition, low saturated fatty acids content and high unsaturated fatty acids proportion, and its high natural antioxidants' content (Owen et al., 2000). The high mediatization of its health benefits has increased its consumption. In fact, world consumption of olive oil has doubled in the last 25 years, from 1,666 in 1990 to 2,916 (1000 tones) in 2015 (IOC, 2016).

In Morocco, the olive tree has become a more modern and even more important crop. Currently, Morocco is the world's fourth largest exporter and producer of olive oil after the

European Union, Tunisia and Turkey (IOC, 2016). The Moroccan olive sector has seen the introduction of new varieties in super-high-density planting system in order to industrialize the new orchards as much as possible with the establishment of modern milling units. Among the olive cultivars adapted to intensive cultivation, three have recently been introduced in Morocco; one of Greek origin: *Koroneiki* and two of Spanish origin: *Arbequina* and *Arbosana*. This planting system was developed with the aim of ensuring an early entry into production, to stabilize the yield (8 to 10 tones/Ha/year), mechanize all operations and minimize harvesting costs (Camposeo et al., 2008; Camposeo and Godini, 2010). This new olive plantation method is therefore intended to reduce production costs and increase production.

The valorization and improvement of olive oil requires the study of its quality and its chemical composition. The

*Corresponding author:

Farid Mansouri, Laboratory of Plants and Microorganisms Biology, Faculté des Sciences Oujda, Université Mohammed Premier, BP-717, 60000 Oujda, Morocco. **Mobile:** +212 664 469 076 **Fax:** +212 536 500 603 **E-mail:** f.mansouri@ump.ac.ma.

Received: 14 March 2018; **Accepted:** 21 June 2018

quantitative and qualitative chemical composition of EVOO is affected by many factors such as agronomical and technical factors. They mainly include the genetic factor, pedoclimatic factors related to geographical area, maturation degree, irrigation, crop season and processing methods (Romero et al., 2003; Morelló et al., 2004; Tura et al., 2007; Mansouri et al., 2015). The influence of climatic variations on the olive oil composition is considerable. This factor is a critical variable that could influence and change the composition of olive oil from one crop season to another. Additionally, several works have shown that rainfall and temperature, especially during olive growth and maturation, are the most important environmental factors that can affect oil composition (Romero et al., 2003; Morelló et al., 2006; Agiomyrgianaki et al., 2012). Therefore, the evaluation of the effect of seasonal conditions of the Angad plain on the quality and composition of EVOOs of *Arbequina*, *Arbosana* and *Koroneiki* varieties, introduced in a new environment, may be of particular interest to the producer. The climate of this zone, which is located in the northern part of the Oriental region of Morocco, is characterized by cold winters and long hot and dry summer seasons. Despite the proximity of the Mediterranean Sea, the thermal differences, between winter and summer, distinguish this region from other regions that are characterized by temperate climates. In addition, the rainfall in this area varies from one year to another, but generally with limited and irregular rainfall, hence, the need for irrigation.

This work aims to evaluate the variations in the quality and composition of extra virgin olive oils from Greek (*Koroneiki*) and Spanish (*Arbequina*, *Arbosana*) varieties cultivated in north-eastern Morocco over two crop seasons by taking into consideration the cultivar factor and the influence of climatic conditions in order to gain knowledge about quantitative and qualitative profiles of

the oils of these cultivars obtained by the same extraction process.

MATERIALS AND METHODS

Olive oils sampling

Samples of olive oils are taken from three cultivars grown in north-eastern Morocco: *Koroneiki* as a Greek variety; *Arbosana* and *Arbequina* as Spanish varieties. These three cultivars have been planted since 2007 (total olive growing area: 60 Ha) in a private area (Société Huiles d'Olive de la Méditerranée-Oujda) located in the plain of Angad (Longitude: 001°57'W, Latitude: 34°47'N and Altitude: 458 m) in north-eastern of Morocco in the same pedoclimatic conditions. The plantations of these varieties (99960 plants) are conducted under irrigated super-high-density planting system with a density of 1666 trees/Ha and the distances between trees are 1.5m/4m. The trees were irrigated using drip irrigation system at a depth of 25 cm. The period of irrigation was the same for all three cultivars, from January to September with a daily flow of 1.2 L h⁻¹ during 9 hours. The fertilization treatments in the irrigated system consisted of providing the plantations a contribution of 40-30-50 U Ha⁻¹ of N-P-K.

Samples of olive oils were obtained in two successive crop seasons (2012/2013 and 2013/2014). The climatic data summarized in Table 1 (accumulated rainfall and min and max temperatures) of the Angad plain were measured daily for two years (2012 and 2013). These data were obtained by the station of the National Directorate of Meteorology (Direction de la Météorologie Régionale, Région Nord-Est) at Oujda-Angad Airport (Longitude: 001°56'W, Latitude: 34°47'N and Altitude: 463 m), Morocco. For each season, three samples were taken from each variety,

Table 1: Monthly precipitation and temperature during 2012 and 2013 in north-eastern Morocco (Latitude: 34° 47'N, Longitude: 001° 57'W, Altitude: 458 m)

Month	Average temperature (°C)						Accumulated rainfall (mm)	
	Min		Max		Mean		2012	2013
	2012	2013	2012	2013	2012	2013		
January	0	0.2	21.9	24.6	11.0	12.4	9.5	56.8
February	-2.1	-1.6	22	23.3	10.0	10.9	27.9	31.6
March	0.7	-0.4	30.5	26.2	15.6	12.9	21.1	38.1
April	3.8	3.7	31.4	30.8	17.6	17.3	16	14
May	5.3	4.9	37.2	34.3	21.3	19.6	2	28.4
June	12.8	9.4	41.9	39.2	27.4	24.3	2.2	0.1
July	16.7	14.9	40.7	38.2	28.7	26.6	0	2.2
August	18.1	15.9	45.5	43.7	31.8	29.8	3.9	11.2
September	12.5	14.3	40.9	38.3	26.7	26.3	19.7	15.7
October	8.2	7.1	34.1	37.2	21.2	22.2	44	0
November	5.6	0.4	30.5	28.6	18.1	14.5	111.2	29.5
December	0	0.9	27.2	20.5	13.6	10.7	5.1	62

each covering ten trees. The olives were harvested between November 26 and November 29 with a maturity index of 3.8-4.0 according to the method developed by the Agronomic Station of Jaén (Uceda and Hermoso, 1998). The mechanically harvested olives by a straddle harvester were immediately milled by a Peralisi industrial system (Molinova serie ORO, Peralisi Maip SPA, Jesi (Ancona), Italy) consisting of four main steps: crushing, kneading, liquid/solid separation and liquid/liquid separation. After deleafing, cleaning and washing, the olives were crushed by a hammer crusher (FP HP 15 INOX). The obtained paste was mixed for 30 min at $27 \pm 1^\circ\text{C}$ using a malaxing module (MOD 800) and then centrifuged using a centrifugal extractor (SPI 222S). Thereafter, the oil was separated using a vertical centrifuge (Valente). The olive oil samples were then stored in dark glass bottles without headspace in the dark at 4°C prior to analysis.

Acidity, peroxide value and UV indices determinations

Acidity, peroxide value and ultraviolet indices of the oil samples were determined according to the analytical methods (EEC/2568/91) described in the European Commission regulations (EEC, 1991).

Fatty acid analysis

The fatty acids were analyzed after conversion into methyl esters obtained by transesterification of triacylglycerols according to the method described by Ben Moumen et al. (2015a). Methyl esters of the fatty acids are separated, identified and quantified by gas chromatography (HP 6890 series GC system) equipped with a capillary column (Supelco, Bellefonte, PA, USA) Omega wax (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 μm) and coupled to a flame ionization detector (FID). The used carrier gas was helium (99.999%, Air Liquide, Liège, Belgium) at a flow rate of 1.7 ml min^{-1} . The initial oven temperature was 50°C and it was then increased at a rate of $30^\circ\text{C min}^{-1}$ to 150°C followed by an increase of 4°C min^{-1} to 240°C . The injection volume was 1 μl in splitless mode. Standard of fatty acid methyl esters (Supelco, Bellefonte, PA, USA), containing 37 methyl esters of fatty acids, was used to identify the individual peaks.

Triacylglycerol analysis

The determination of triacylglycerol composition was carried out according to the method of Ben Moumen et al. (2015a). A 10 μl aliquot of a 10% (w/v) olive oil solution in acetone were fractionated by a Shimadzu LC-6AD HPLC system (Nakagyo-Ku, Koyoto, Japan) connected to two LC-10AD Shimadzu liquid chromatography pumps and a refractive index detector 10A. The isocratic separation was carried out using an ODS-C18 reverse phase column (250×4.6 mm, 5 μm particle size; Thermo Scientific, Waltham, MA, USA). The mobile phase was composed

of acetone and acetonitrile (63.6/36.4; v/v) at a flow rate of 1 ml min^{-1} . The molecular peaks of the separated triacylglycerols were identified by comparison with the trioleate standard (OOO) obtained from Sigma-Aldrich (St. Louis, MO, USA) and after the analysis of eluates of the various peaks by GC-FID under the same conditions described above.

Phytosterol composition analysis

The phytosterol fraction was extracted according to the method described by Ben Moumen et al. (2015b) in the presence of butilin (Sigma-Aldrich, St. Louis, MO, USA), as an internal standard, at a concentration of 1 mg ml^{-1} . The obtained phytosterols were then silylated in the presence of a mixture (1/1; v/v) of anhydrous pyridine and a silylation reagent (Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane; Supelco, Bellefonte, PA, USA). Phytosterols were analyzed by GC-FID (HP 6890 series GC system) equipped with a capillary column HP 5 ms ($30 \text{ m} \times 0.25 \text{ mm}$, 0.25 μm ; Agilent Technologies, Palo Alto, CA, USA). The used carrier gas was helium with a flow rate of 1 ml min^{-1} . The chromatographic conditions were: injector temperature: 250°C ; detector temperature: 300°C ; Oven programming: initial oven temperature 50°C with a temperature increase rate of $30^\circ\text{C min}^{-1}$ until reaching 275°C . The injection volume was 1 μl in splitless mode. Cholesterol, campesterol, erythrodiol, β -sitosterol and stigmasterol were identified by using a commercial standard (Sigma-Aldrich, St. Louis, MO, USA), and Δ^5 -avenasterol, Δ^7 -avenasterol, clerosterol, 24-methylene cholesterol, Δ^7 -stigmastenol and $\Delta^{5,24}$ -stigmastadienol were identified by comparing relative retention times (β -sitosterol-TMS = 1.00) with those of the European Communities (EEC, 1991).

Extraction of phenolic compounds

Phenolic compounds extraction was carried out according to the method described by Ben Moumen et al. (2015a) using a methanol/water mixture (80/20; v/v) as extraction solvent.

Colorimetric determination of total phenols

The total phenol content was determined by the Folin-Ciocalteu method at 750 nm using a UV spectrophotometer (RAYLEIGH UV1800, UV-Visible) according to the procedure described by Ben Moumen et al. (2015a) using caffeic acid (Sigma-Aldrich, St. Louis, MO, USA) as external standard ($r^2 > 0.98$).

Analysis of phenolic compounds by HPLC

Phenolic compounds' separation was carried out on a Zorbax XDB-C18 column ($150 \text{ mm} \times 4.6 \text{ mm}$, 3.5 μm particle size; Agilent Technologies, Palo Alto, CA, USA) by HPLC (Agilent Technology series 1100 system, Palo Alto,

CA, USA) equipped with a diode array detector. The used solvent system was a gradient of A (water + 0.5% formic acid) and B (methanol + 0.5% formic acid) according to the method described by Bakhouché et al. (2013). The flow rate was 1 ml min⁻¹. Detection was made at 254, 280, 320 and 340 nm and the identification of phenolic compounds was carried out at 280 nm. Hydroxytyrosol, tyrosol, vanillin, apigenin, luteolin and vanillic, *p*-coumaric and cinnamic acids were identified and quantified by external standardization ($r^2 > 0.99$) with commercial standards (Sigma-Aldrich, St. Louis, MO, USA). The identification and quantification of decarboxymethyl ligstroside aglycone and decarboxymethyl oleuropein aglycone were performed by comparing their retention time with those published by Bakhouché et al. (2013) and the use of response factors determined by Mateos et al. (2001).

α -Tocopherol analysis

α -Tocopherol was determined according to the AOCS method Ce 8-89 (AOCS, 1989) on an HPLC (Agilent technology series 1200 system, Palo Alto, CA, USA) equipped with UV detector. The separation was carried out on a column of silica Uptisphere 120Å NH₂ (150 mm × 3 mm, 5 µm particle size; Interchim, Montluçon, France) which was eluted with a mobile phase composed of n-hexane/2-propanol (99/1; v/v) at a flow rate of 1 ml min⁻¹. The identification was performed using commercial tocopherol standards (a, b, g and d-tocopherol, Sigma-Aldrich, St. Louis, USA) at 292 nm. The a-tocopherol concentration was then calculated from the external calibration curve ($r^2 > 0.99$).

Determination of carotenoids and chlorophylls contents

Carotenoids and chlorophylls contents were calculated by absorbance at 470 and 670 nm, respectively, using a UV spectrophotometer (RAYLEIGH UV1800; UV-Visible)

according to the method described by Minguez-Mosquera et al. (1990).

Evaluation of olive oil Stability

The oxidative stability of olive oil was evaluated by the Rancimat test using Metrom Rancimat 743 (Metrom Co., Basel, Switzerland) and expressed in induction time (hours). The operating conditions are as follows: 3±0.01 g of olive oil sample were subjected to thermal degradation at 100±1.6 °C by bubbling a stream of air at a rate of 15 L h⁻¹. The oxidation products (mainly volatile organic acids) from the oil samples were collected in a measuring cell containing 60 ml of distilled water.

Statistical analysis

The results presented in this work are the averages of the analyses carried out in triplicate with the corresponding standard deviations for each season (for each parameter: 3 determinations × 3 samples = 9). One-way ANOVA statistical analysis and Duncan's Post-hoc test were used to determine the significant differences between the varieties. The significant differences between the results of the two crop seasons were determined by the t-Student test. The significant difference threshold was set at 5%. The applications of these statistical analyses were carried out using IBM Statistical Package for the Social Sciences (version 20; IBM SPSS) for Windows.

RESULTS AND DISCUSSION

Quality parameters

The results listed in Table 2 show that olive oils of the studied varieties, in the two crop seasons (2012/2013 and 2013/2014), have quality indices (acidity, peroxide value and UV indices) which comply with the limits established by the

Table 2: Standard quality parameters of monovarietal extra virgin olive oils obtained in two crop seasons

Quality parameters	Crop season	Olive varieties			Sig. level
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	
Free acidity (%C18:1)	2012/2013	0.46±0.03 ^{ab}	0.53±0.03 ^{bb}	0.58±0.09 ^{bb}	*
	2013/2014	0.24±0.02 ^{ba}	0.21±0.04 ^{aa}	0.29±0.04 ^{ca}	***
	Sig. level	***	***	***	
Peroxide value (meq O ₂ kg-1)	2012/2013	8.26±0.49 ^{ab}	9.10±0.40 ^{bb}	10.51±0.46 ^{cb}	***
	2013/2014	6.32±0.32 ^{aa}	7.50±0.79 ^{ba}	9.02±1.15 ^{ca}	***
	Sig. level	***	***	***	
K ₂₇₀	2012/2013	0.08±0.01 ^{aa}	0.11±0.01 ^{ba}	0.14±0.01 ^{ca}	***
	2013/2014	0.10±0.00 ^{aa}	0.12±0.00 ^{ba}	0.15±0.01 ^{ca}	***
	Sig. level	NS	NS	NS	
K ₂₃₂	2012/2013	1.43±0.18 ^{aa}	1.56±0.01 ^{ba}	1.63±0.10 ^{bb}	*
	2013/2014	1.66±0.06 ^{bb}	1.73±0.03 ^{cb}	1.50±0.03 ^{aa}	***
	Sig. level	***	***	**	

Values are the means±standard deviations (n=9). For each parameter, significant differences in the same line (comparison among cultivars) are indicated by different superscript letters and significant differences in the same column (comparison between crop seasons) are indicated by different lowercase letters. Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS=not significant

International Olive Council (COI, 2013) for extra virgin olive oil category (acidity $\leq 0.8\%$, peroxide value ≤ 20 meq O_2 kg^{-1} , $K_{270} \leq 0.22$, $K_{232} \leq 2.5$). K_{270} values remained almost constant, with some non-significant differences between the two crop seasons. On the other hand, the acidity and peroxide value of all 2013/2014 samples are significantly lower than those observed in 2012/2013. These variations between the two crop seasons are statistically significant ($p < 0.001$). Therefore, one could say that these variations are due to the seasonal variations of the climatic conditions of this region. In fact, the climatic conditions of the two crop season showed a clear difference in rainfall and especially at the level of minimum and maximum temperatures registered in the October-November period. Similar results have also been reported on the oil of *Arbequina* variety grown in Spain (Romero et al., 2003; Morelló et al., 2006). Additionally, the significant effect of the varietal factor was observed for all of the quality parameters ($p < 0.05$). According to several studies (Rotondi and Magli, 2004; Khlil et al., 2017), the varietal factor seems to have no effect on these quality indices. These indices are mainly affected by the factors that cause damage to olive fruits.

Composition of fatty acids and triacylglycerols

The results of fatty acids analysis of the studied varieties, during two crop seasons, are summarized in Table 3. With the exception of heptadecenoic acid, which is considered by some authors as a variety character (Piscopo et al., 2016), the examination of these results shows that the fatty acid composition of the analyzed EVOOs is in accordance with the specifications required by the commercial standard established by the International Olive Council for high-quality oils for the two crop seasons. Also, there is a clear influence of the varietal factor ($p < 0.05$). For the two crop seasons, EVOOs of *Arbosana* and *Koroneiki* varieties have relatively different fatty acid profiles compared to *Arbequina* oil. In comparison with *Arbequina* oil during the 2012/2013 and 2013/2014 crop seasons, *Arbosana* and *Koroneiki* oils are characterized by higher percentages of oleic acid (*Koroneiki*: 76.24 and 77.15%, *Arbosana*: 75.69 and 73.10%, respectively), lower levels of palmitic acid (*Koroneiki*: 15.70 and 12.08%, *Arbosana*: 13.72 and 14.84%, respectively) and linoleic acid (*Koroneiki*: 5.26 and 6.26%, *Arbosana*: 5.66 and 6.40%, respectively). *Arbequina* oil showed a different fatty acid profile from the two previous cultivars with higher levels of palmitic acid (16.96 and 16.42%, respectively), higher percentages of polyunsaturated fatty acids (8.75% and 13.14%, respectively) and the lowest C18:1/C18:2 ratio (8.49 and 5.22, respectively), due to its low oleic acid content (69.72 and 65.68%, respectively) and its high content of linoleic acid (8.21 and 12.59%, respectively).

The major fatty acids' composition of EVOOs from Spanish varieties (*Arbequina* and *Arbosana*) introduced in

north-eastern Morocco is different from those observed in these same varieties cultivated in their original site (Hermoso et al., 2011) and in Tunisia (Allalout et al., 2009). *Arbequina* and *Arbosana*, when grown in north-eastern Morocco, produce oils with lower levels of linoleic acid compared to those of their site of origin (11.10 and 7.90%, respectively) and in Tunisia (12.93 and 12.09%, respectively). However, the oils produced in Morocco have oleic acid proportions close to those obtained in Spain (69.40 and 73.00%, respectively) and much higher than those obtained in Tunisia (58.82 and 64.79%, respectively). The *Koroneiki* variety olive oil has a comparable oleic acid composition between north-eastern Morocco and northern Tunisia (75.53%), where the cultivar is grown under irrigated high-density planting system (Allalout et al., 2009), and in its original growing area (76.22%), even when the variety is conducted in rainfed cultural system (Koutsaftakis et al., 2000).

As shown in Table 3, a significant effect of crop season was observed on the fatty acid composition of the analyzed EVOOs. The major fatty acids, such as palmitic, oleic and linoleic acids, are the most influenced. With the exception of palmitic acid in *Arbequina* oil ($p > 0.05$), the percentages of these fatty acids showed significant differences between the two crop seasons. The oleic acid content was higher in EVOOs from the 2012/2013 crop season than in 2013/2014 for *Arbequina* and *Arbosana* varieties ($p < 0.001$). Keeping in mind the influence of other factors on olive oil's fatty acid composition, mainly the genetic factor and the olives' harvesting time (Poiana and Mincione, 2004), the differences observed between the two crop seasons can also be related to the impact of environmental factors on fatty acid biosynthesis. In fact, previous studies have shown that temperature and water intake are the main factors that affect the rate and metabolism of fatty acids in olives (Harwood, 1984; Romero et al., 2003). While other authors have noted that certain fatty acids, like oleic and linoleic acids and the ratio between them (C18:1/C18:2), as well as the synthesis of certain triacylglycerols are affected by water supply and the climatic conditions of the summer period corresponding to the final stage of the formation of olive fruits (Angerosa et al., 1996; Stefanoudaki et al., 2001). In a recent study, García-Inza et al. (2014) found that increasing temperature reduces the level of oleic acid and increases the content of palmitic, palmitoleic, linoleic and linolenic acids in olive oil. As a result, a link was observed between the variation in the proportion of these fatty acids and the maximum temperature of the June-August period between the two crop seasons (Table 1). Fatty acids biosynthesis begins in plastids by mainly producing palmitic and stearic acids under the action of two enzymes, acetyl COA carboxylase and fatty acid synthase (Harwood, 1996). The former insures palmitic acid synthesis, while the latter

Table 3: Fatty acid composition of monovarietal extra virgin olive oils obtained in two crop seasons

Fatty acids (%)	Crop season	Olive varieties			Sig. level
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	
Palmitic acid	2012/2013	16.96±0.75 ^{ca}	13.72±0.34 ^{aa}	15.70±0.37 ^{bb}	***
	2013/2014	16.42±0.01 ^{ca}	14.84±0.00 ^{bb}	12.08±0.39 ^{aa}	***
	Sig. level	NS	***	***	
Palmitoleic acid	2012/2013	1.82±0.21 ^{ba}	1.27±0.03 ^{aa}	1.23±0.06 ^{ab}	***
	2013/2014	1.71±0.00 ^{ca}	1.35±0.01 ^{bb}	0.66±0.09 ^{aa}	***
	Sig. level	NS	***	***	
Heptadecanoic acid	2012/2013	0.11±0.01 ^{ab}	0.15±0.00 ^{ba}	ND	***
	2013/2014	0.10±0.00 ^{ba}	0.17±0.00 ^{cb}	0.04±0.01 ^a	***
	Sig. level	*	***		
Heptadecenoic acid	2012/2013	0.24±0.02 ^{aa}	0.34±0.00 ^{ba}	ND	***
	2013/2014	0.22±0.00 ^{ba}	0.36±0.00 ^{cb}	0.08±0.01 ^a	***
	Sig. level	NS	**		
Stearic acid	2012/2013	1.79±0.41 ^{ba}	1.91±0.36 ^{ba}	0.51±0.12 ^{aa}	***
	2013/2014	1.88±0.00 ^{aa}	2.21±0.00 ^{ba}	2.27±0.32 ^{bb}	***
	Sig. level	NS	NS	***	
Oleic acid	2012/2013	69.72±1.03 ^{ab}	75.69±0.56 ^{bb}	76.24±0.52 ^{ba}	***
	2013/2014	65.68±0.01 ^{aa}	73.10±0.01 ^{ba}	77.15±0.18 ^{cb}	***
	Sig. level	***	***	**	
Linoleic acid	2012/2013	8.21±0.10 ^{ca}	5.66±0.06 ^{ba}	5.26±0.07 ^{aa}	***
	2013/2014	12.59±0.01 ^{cb}	6.40±0.00 ^{bb}	6.26±0.11 ^{ab}	***
	Sig. level	***	***	***	
α-Linolenic acid	2012/2013	0.54±0.01 ^{aa}	0.64±0.02 ^{ba}	0.64±0.01 ^{ba}	***
	2013/2014	0.56±0.01 ^{aa}	0.65±0.00 ^{ba}	0.63±0.05 ^{ba}	***
	Sig. level	NS	NS	NS	
Arachidic acid	2012/2013	0.32±0.05 ^{ba}	0.37±0.01 ^{ba}	0.24±0.01 ^{aa}	**
	2013/2014	0.40±0.00 ^{ab}	0.44±0.00 ^{bb}	0.40±0.07 ^{ab}	*
	Sig. level	*	***	***	
Gadoleic acid	2012/2013	0.28±0.06 ^{ba}	0.26±0.00 ^{ba}	0.18±0.01 ^{aa}	***
	2013/2014	0.29±0.00 ^{bb}	0.30±0.00 ^{bb}	0.26±0.05 ^{ab}	*
	Sig. level	*	***	***	
ΣSFA	2012/2013	19.19±0.74 ^{ba}	16.14±0.64 ^{aa}	16.45±0.46 ^{ab}	**
	2013/2014	18.80±0.01 ^{ca}	17.66±0.00 ^{bb}	14.79±0.02 ^{aa}	***
	Sig. level	NS	**	***	
ΣMUFA	2012/2013	72.06±0.79 ^{ab}	77.56±0.57 ^{bb}	77.65±0.51 ^{ba}	***
	2013/2014	67.90±0.01 ^{aa}	75.11±0.00 ^{ba}	78.15±0.12 ^{cb}	***
	Sig. level	***	***	*	
ΣPUFA	2012/2013	8.75±0.10 ^{ca}	6.30±0.07 ^{ba}	5.90±0.08 ^{aa}	***
	2013/2014	13.14±0.01 ^{cb}	7.05±0.00 ^{bb}	6.89±0.09 ^{ab}	***
	Sig. level	***	***	***	
C18:1/C18:2	2012/2013	8.49±0.21 ^{ab}	13.37±0.05 ^{bb}	14.50±0.25 ^{cb}	***
	2013/2014	5.22±0.00 ^{aa}	11.41±0.00 ^{ba}	12.33±0.21 ^{ca}	***
	Sig. level	***	***	***	

Values are the means±standard deviations (n=9). For each parameter, significant differences in the same line (comparison among cultivars) are indicated by different superscript letters and significant differences in the same column (comparison between crop seasons) are indicated by different lowercase letters. Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS=not significant. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids

catalyzes palmitic acid elongation to produce stearic acid. This step is important because it determines the proportion of fatty acids with 16 and 18 carbon atoms in the oil (Salas et al., 2000). For *Arbosana* and *Arbequina* cultivars, the highest proportion of oleic acid was in olive oils from the 2012/2013 crop season and the lowest palmitic acid rate was in olive oils from the 2012/2013 crop season for *Arbosana* variety. The opposite variation was observed for the *Koroneiki* cultivar.

Another remarkable and significant change ($p < 0.001$) was observed in the level of linoleic acid between the two crop season for all the analyzed samples (Table 3). The highest levels of this fatty acid were observed in the olive oil samples of the 2013/2014 crop season. These variations could be linked to the difference of the seasonal temperatures of summer (June-August), which was warmer in the 2012/2013 crop season than the 2013/2014 crop season (Table 1). The climatic conditions seem to cause a

change in the degree of unsaturation of fatty acids, mainly linoleic acid. In fact, after the desaturation of stearic acid by the action of Δ -9 stearyl desaturase, the oleic acid is converted into linoleic acid by the consecutive actions of Δ -12 and Δ -15 desaturases (Hernández et al., 2011). These two enzymes are regulated by different environmental factors, mainly temperature, which control the action of these enzymes to maintain the fluidity of biological membranes (Los and Murata, 1998; Hernández et al., 2011).

Similarly, the statistical analysis revealed an effect of climatic variations on the molecular composition of triacylglycerols for all the analyzed EVOOs (Table 4). As specified for fatty acids, the variation in the proportions of the major triacylglycerols between the two crop seasons is not the same in the three studied cultivars, but it is important to note that the majority of molecular groups revealed statistically significant differences. Based on the results in Table 4, we found that all EVOOs from the 2013/2014 crop season are characterized by a significant decrease ($p < 0.001$) in the triolein percentage (OOO) compared to the oil samples from 2012/2013 crop season. On the other hand, a significant increase was observed in

the linoleyldiolein (LOO) level in EVOOs of *Arbequina* and *Koroneiki* varieties ($p < 0.01$), while *Arbosana* did not show a significant difference ($p > 0.05$). Similarly, palmyldiolein (POO) did not show a significant variation in the proportion between the two crop seasons for the *Koroneiki* variety ($p > 0.05$), while *Arbequina* and *Arbosana* oils are characterized by a significant increase ($p < 0.01$) in the POO proportion during the 2013/2014 crop season. This different behavior, towards climatic conditions, could be explained by the genetic factor.

The highest OOO levels during the 2012/2013 and 2013/2014 crop seasons were observed in *Koroneiki* oil (49.08 and 48.02%, respectively), followed by *Arbosana* oil (44.91 and 40.40, respectively) and *Arbequina* oil (37.53 and 31.51%, respectively). The latter's oil is distinguished by the highest LOO contents (14.87 and 16.31%, respectively), while *Arbosana* oil is distinguished from other varieties by its high POO proportions during the two crop season (27.59 and 29.80%, respectively). These intervarietal variations in the proportion of these triacylglycerols are statistically significant ($p < 0.05$). Taking into account the influence of other factors on olive oil's triacylglycerol

Table 4: Triacylglycerol composition of monovarietal extra virgin olive oils obtained in two crop seasons

Triacylglycerols (%)	Crop season	Olive varieties			Sig. level
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	
LOL	2012/2013	2.24±0.36 ^{ba}	1.09±0.09 ^{aa}	1.11±0.04 ^{aa}	***
	2013/2014	3.11±0.07 ^{cb}	1.04±0.30 ^{aa}	1.34±0.08 ^{bb}	***
	Sig. level	***	NS	***	
LPL	2012/2013	1.76±0.22 ^{bb}	1.40±0.13 ^{aa}	1.39±0.05 ^{aa}	***
	2013/2014	1.52±0.17 ^{ba}	1.37±0.25 ^{aba}	1.34±0.19 ^{aa}	*
	Sig. level	**	NS	NS	
POLn	2012/2013	1.23±0.04 ^{ca}	0.62±0.08 ^{ba}	0.54±0.03 ^{aa}	***
	2013/2014	1.30±0.07 ^{bb}	0.78±0.06 ^{ab}	0.78±0.05 ^{ab}	***
	Sig. level	**	***	***	
LOO	2012/2013	14.87±0.32 ^{ca}	10.29±0.01 ^{ba}	9.87±0.02 ^{aa}	***
	2013/2014	16.31±0.15 ^{bb}	10.32±1.33 ^{aa}	10.54±0.36 ^{ab}	***
	Sig. level	***	NS	**	
LPO	2012/2013	7.89±0.14 ^{ca}	4.98±0.14 ^{ba}	4.36±0.07 ^{ab}	***
	2013/2014	11.10±0.46 ^{cb}	6.00±0.82 ^{bb}	4.20±0.24 ^{aa}	***
	Sig. level	***	***	*	
OOO	2012/2013	37.53±0.08 ^{ab}	44.91±0.34 ^{bb}	49.08±0.17 ^{cb}	***
	2013/2014	31.51±0.53 ^{aa}	40.40±1.02 ^{ba}	48.02±0.13 ^{ca}	***
	Sig. level	***	***	***	
POO	2012/2013	25.97±0.39 ^{aa}	27.59±0.03 ^{ca}	26.35±0.12 ^{ba}	***
	2013/2014	26.39±0.43 ^{ab}	29.80±1.30 ^{bb}	26.49±0.38 ^{aa}	***
	Sig. level	**	***	NS	
POP	2012/2013	1.29±0.18 ^{ca}	0.72±0.10 ^{ba}	0.27±0.03 ^{aa}	***
	2013/2014	1.50±0.10 ^{bb}	1.42±0.23 ^{bb}	1.04±0.31 ^{ab}	***
	Sig. level	**	***	***	
SOO	2012/2013	4.30±0.09 ^{bb}	4.11±0.02 ^{bb}	3.52±0.32 ^{ab}	**
	2013/2014	3.81±0.41 ^{ba}	3.74±0.22 ^{ba}	2.68±0.29 ^{aa}	***
	Sig. level	***	***	***	

Values are the means±standard deviations (n=9). For each parameter, significant differences in the same line (comparison among cultivars) are indicated by different superscript letters and significant differences in the same column (comparison between crop seasons) are indicated by different lowercase letters. Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS=not significant. L: Linoleyl; Ln: Linolenyl; O: Oleyl; S: Stearyl; P: Palmityl

composition, mainly the genetic factor and the maturity stage of olives (Giuffrè, 2013), the results of triacylglycerols in the analyzed olive oils can be compared to those found in Tunisian olive oils (OOO (30–46%) and POO (19.7–26.3%)) (Manai-Djebali et al., 2012), Italian olive oils (OOO (21.70–39.37%), POO (18.84–22.01%)) (Giuffrè, 2013) and French olive oils (OOO (32.63–54.59%) and POO (17.22–21.72%)) (Ollivier et al., 2006).

Variations of antioxidant parameters

In EVOO, the main chlorophylls and carotenoids are pheophytin and lutein, respectively. These compounds are also involved in oxidation mechanisms (Minguez-Mosquera et al., 1991). The results of assaying these pigments in the oils of the studied varieties by spectrophotometer are summarized in Table 5. The maximum levels of pigments (calculated from the results in Table 5), in the 2012/2013 and 2013/2014 crop seasons, were observed in *Koroneiki* oil (6.11 and 4.96 mg kg⁻¹, respectively), whereas the lowest values for these compounds were observed in *Arbequina* oil (3.52 and 3.33 mg kg⁻¹, respectively). Statistical analysis shows that the chlorophyll and carotenoid contents are influenced by the varietal factor ($p < 0.001$). Although the levels of carotenoids in oil samples are still lower than those of chlorophylls, the statistical analysis shows that the carotenoid content is visibly influenced by the effect of crop season ($p < 0.001$). Additionally, *Arbequina* and *Koroneiki* oils of the 2013/2014 crop season showed a decrease in carotenoid content compared to 2012/2013 crop season. This variation may be due to environmental factors, especially the temperature. Romero et al. (2003) reported that low temperatures during the ripening stage would lead to alterations or even partial degradation of pigments. In

fact, the period of November 2013 was characterized by frequent frosts which explains the difference in the average minimum temperature of this period (0.4 °C) compared to the same phase of the 2012/2013 crop season (5.6 °C). However, the low pigment concentrations in the analyzed oil samples cannot allow to establish a clear link between climatic variations and pigment content. In fact, *Arbosana* showed a different behavior compared to *Arbequina* and *Koroneiki* varieties. Its carotenoid content recorded a significant increase ($p < 0.001$) in the 2013/2014 crop season (1.86 mg kg⁻¹) compared to the 2012/2013 crop season (1.65 mg kg⁻¹). Similarly, the chlorophyll fraction showed a significant difference in its concentration between the two seasons for all the analyzed oils. Regarding the *Koroneiki* oil's chlorophyll, there was a decrease ($p < 0.001$) in its content during the 2013/2014 crop season (3.14 mg kg⁻¹) in comparison with the 2012/2013 crop season (3.94 mg kg⁻¹), while an increase was observed in *Arbosana*'s chlorophyll content (from 1.94 to 2.28 mg kg⁻¹; $p < 0.001$) and *Arbequina*'s chlorophyll content (from 1.86 to 1.94 mg kg⁻¹; $p < 0.05$).

The phenolic compounds of EVOO play an important role in the evaluation of its quality, contributing for a large part to the oil's oxidation resistance (Gutierrez et al., 2001; Mansouri et al., 2017). The results of the total phenols determination for the oils analyzed by colorimetric assay are shown in Table 5. The total phenol content varies significantly according to the cultivar ($p < 0.001$). *Koroneiki* oil is characterized by a richness in phenolic compounds with a 493.66 mg kg⁻¹ content in the 2012/2013 crop season and 566.31 mg kg⁻¹ in 2013/2014 crop season. The lowest levels of total phenols in the two crop seasons

Table 5: Oxidative stability and total phenol, α -tocopherol, chlorophyll and carotenoid contents of monovarietal olive oil samples obtained in two crop seasons

Analytical parameters	Crop season	Olive varieties			Sig. level
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	
Total phenols (mg kg ⁻¹)	2012/2013	241.28±6.70 ^{aa}	411.64±6.70 ^{ba}	493.66±4.89 ^{ca}	***
	2013/2014	286.51±5.63 ^{ab}	454.80±11.87 ^{bb}	566.31±8.79 ^{cb}	***
	Sig. level	***	***	***	
α -Tocopherol (mg kg ⁻¹)	2012/2013	322.36±11.05 ^{aa}	460.07±12.38 ^{cb}	344.58±9.42 ^{ba}	***
	2013/2014	335.29±15.03 ^{ab}	419.00±17.07 ^{ca}	355.66±2.18 ^{ba}	***
	Sig. level	*	***	NS	
Chlorophylls (mg kg ⁻¹)	2012/2013	1.86±0.04 ^{aa}	1.94±0.03 ^{ba}	3.94±0.01 ^{cb}	***
	2013/2014	1.94±0.17 ^{ab}	2.28±0.05 ^{bb}	3.14±0.13 ^{ca}	***
	Sig. level	*	***	***	
Carotenoids (mg kg ⁻¹)	2012/2013	1.66±0.09 ^{ab}	1.65±0.01 ^{aa}	2.17±0.02 ^{bb}	***
	2013/2014	1.39±0.10 ^{aa}	1.86±0.05 ^{bb}	1.82±0.09 ^{ba}	***
	Sig. level	***	***	***	
Oxidative stability (hours)	2012/2013	50.36±0.45 ^{aa}	60.17±0.95 ^{ba}	94.83±0.79 ^{ca}	***
	2013/2014	53.78±1.81 ^{ab}	78.81±0.90 ^{bb}	102.44±0.19 ^{cb}	***
	Sig. level	***	***	***	

Values are the means±standard deviations (n=9). For each parameter, significant differences in the same line (comparison among cultivars) are indicated by different superscript letters and significant differences in the same column (comparison between crop seasons) are indicated by different lowercase letters. Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS=not significant

were observed in *Arbequina* oil samples (241.28 and 286.51 mg kg⁻¹, respectively). Additionally, a significant crop season effect was observed for all the analyzed samples ($p < 0.001$). EVOOs of the 2013/2014 crop season are richer in total phenols than those of 2012/2013 crop season. These differences between the two crop seasons could be attributed to environmental factors, mainly rainfall. In fact, a link was observed between the rainfall of the October-November period, where the trees were deprived of irrigation, and total phenol content. The 2013/2014 crop season, which corresponds to the highest levels, is characterized by lower rainfall in the October-November period (29.5 mm) compared with the same period of 2012/2013 season (155.2 mm). This is in accordance with the results of Romero et al. (2003) who also observed a relationship between the phenolic compounds content in *Arbequina* oil and rainfall accumulation per season. However, the impact of water intake on the phenolic content of olive oil remains controversial. Palese et al. (2010) found a significant decrease in total phenol content of olive oils from irrigated trees. On the other hand, Greven et al. (2009) found that the olive trees deprived from irrigation yielded oils with lower phenol levels compared to irrigated trees (a 33% decrease), while Dag et al. (2008) suggest that moderate water stress would be beneficial to olive oil quality.

Another class of molecules that contribute to the stability of EVOO are tocopherols. α -Tocopherol constitutes the dominant tocopherol fraction in olive oil (more than 95% of total tocopherols) (Aguilera et al., 2005; Mansouri et al., 2016). Their evaluation is important because of their contribution to the final quality of this product. From the results presented in Table 5, α -tocopherol content is strongly influenced by the varietal factor ($p < 0.001$). *Arbosana* oil has the highest concentration of followed by *Koroneiki* oil (344.58 and 355.66 mg kg⁻¹, respectively) and *Arbequina* oil (322.36 and 335.29 mg kg⁻¹, respectively). Additionally, *Arbosana* has a significant difference ($p < 0.001$) in the α -tocopherol content between the two crop seasons. Its content decreased during the 2013/2014 crop season. This can be attributed to the susceptibility of this variety to cold and night/day temperature differences compared to the other two cultivars that seem less sensitive to cold, especially *Koroneiki* which did not show a significant difference ($p > 0.05$). In fact, the minimum temperature of the ripening period in the cultivation site of these varieties experienced frequent frosts before the harvest of olives (November) during the 2013/2014 crop season compared to 2012/2013 crop season (Table 1). Similarly, Romero et al. (2003) revealed that the cold during the olive harvest period causes damage to the fruit by causing a decrease in olive oil's tocopherol content.

Oxidative stability is considered among the main indices of oil quality assessment that could predict its storage aptitude and its qualitative shelf life. It is a tool for estimating the sensitivity (or stability) of an oil to oxidative degradation. The induction times determined during the two crop seasons for the analyzed oils showed that this parameter is influenced by the cultivar factor ($p < 0.001$). *Koroneiki* oil has the highest values (2012/2013: 94.83 h and 2013/2014: 102.44 h) followed by *Arbosana* oil (2012/2013: 60.17 h and 2013/2014: 78.81 h) and *Arbequina* oil (2012/2013: 50.36 h and in 2013/2014: 53.78 h). Our results of oxidative stability could be compared to those determined by Manai-Djebali et al. (2012), at 100 °C and an air flow of 10 L h⁻¹, in virgin olive oils (46.5-113 h) of other varieties but higher than the results reported by Allalout et al., (2009), using an air flow of 10 L h⁻¹ in the same experimental conditions, for virgin olive oils of the same varieties cultivated in Tunisia. Significant differences between the two crop seasons were observed for all the oil samples of the studied varieties ($p < 0.001$). These inequalities can be attributed largely to the difference in phenolic compound contents (Table 5). In fact, several studies on the contribution of the constituents of olive oil to oxidative stability have concluded that the resistance of olive oil to oxidation is related to its monounsaturated fatty acid and polyunsaturated fatty acid content, and to natural antioxidants such as phenolic compounds (Gutierrez et al., 2001; Aguilera et al., 2005). In our case, despite the decrease of the C18:1/C18:2 ratio (Table 3), the analyzed oils recorded a significant increase in induction time for the 2013/2014 olive oil samples compared to those of 2012/2013 crop season. This could be due to the increase of natural antioxidants such as phenolic compounds.

Phenolic compounds

The phenolic fraction (Table 6) of the EVOO samples of *Arbequina*, *Arbosana* and *Koroneiki* varieties produced during the two crop seasons is characterized by the dominance of secoiridoid derivatives, such as the decarboxymethylated forms of oleuropein aglycone (DOA) and ligstroside aglycone (DLA). These two compounds represent more than 85% (calculated from the results in Table 6) of the phenolic compounds identified in all the analyzed oils, with concentrations that vary respectively between 85-183 mg kg⁻¹ and 108-196 mg kg⁻¹. *Koroneiki* oil shows the highest levels of DOA and DLA during the two crop seasons, followed by *Arbosana* and *Arbequina* oils. Phenolic alcohols, flavonoids, phenolic acids and lignans are also present in the analyzed oils but in small amounts. Their contents do not exceed 15% of the phenolic compounds.

Although significant differences were observed in the total phenol content of the analyzed oils between the two

Table 6: Phenolic compounds composition of extra virgin olive oil samples obtained in two crop seasons

Phenolic compounds (mg kg ⁻¹)	Crop season	Olive varieties			Sig. level
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	
Hydroxytyrosol	2012/2013	1.94±0.03 ^{aa}	9.75±0.19 ^{ba}	14.17±0.16 ^{ca}	***
	2013/2014	2.64±0.07 ^{ab}	11.08±0.07 ^{bb}	16.94±0.19 ^{cb}	***
	Sig. level	***	***	***	
Tyrosol	2012/2013	1.49±0.03 ^{aa}	4.66±0.08 ^{ba}	6.97±0.17 ^{ca}	***
	2013/2014	2.96±0.00 ^{ab}	4.47±0.25 ^{bb}	7.37±0.20 ^{cb}	***
	Sig. level	***	**	***	
Phenolic alcohols	2012/2013	3.43±0.04 ^{aa}	14.41±0.22 ^{ba}	21.14±0.18 ^{ca}	***
	2013/2014	5.60±0.14 ^{ab}	15.54±0.21 ^{bb}	24.31±0.16 ^{cb}	***
	Sig. level	***	**	***	
Decarboxymethyl oleuropein aglycone	2012/2013	85.37±1.43 ^{aa}	128.53±0.20 ^{ba}	146.72±1.79 ^{ca}	***
	2013/2014	104.32±0.92 ^{ab}	153.16±3.54 ^{bb}	183.00±1.17 ^{cb}	***
	Sig. level	***	***	***	
Decarboxymethyl ligstroside aglycone	2012/2013	108.33±1.82 ^{aa}	157.16±0.50 ^{ba}	165.56±1.84 ^{ca}	***
	2013/2014	121.60±4.45 ^{ab}	171.25±6.57 ^{bb}	195.54±5.52 ^{cb}	***
	Sig. level	***	***	***	
Secoiridoid derivatives	2012/2013	193.70±3.06 ^{aa}	285.69±0.69 ^{ba}	312.28±3.04 ^{ca}	***
	2013/2014	225.92±3.74 ^{ab}	324.41±3.88 ^{bb}	378.54±2.84 ^{cb}	***
	Sig. level	***	***	***	
Luteolin	2012/2013	6.89±0.11 ^{bb}	6.71±0.03 ^{aba}	6.54±0.39 ^{ab}	*
	2013/2014	5.04±0.18 ^{aa}	7.21±0.26 ^{cb}	5.45±0.10 ^{ba}	***
	Sig. level	***	***	***	
Apigenin	2012/2013	3.33±0.06 ^{ab}	7.55±0.05 ^{ca}	3.55±0.05 ^{ba}	***
	2013/2014	2.80±0.05 ^{aa}	9.43±0.07 ^{cb}	3.90±0.12 ^{bb}	***
	Sig. level	***	***	***	
Flavonoids	2012/2013	10.22±0.13 ^{ab}	14.26±0.06 ^{ca}	10.09±0.45 ^{bb}	***
	2013/2014	7.83±0.10 ^{aa}	16.64±0.18 ^{cb}	9.35±0.14 ^{ba}	***
	Sig. level	***	***	***	
Pinoresinol	2012/2013	5.24±0.09 ^{bb}	7.08±0.04 ^{ca}	4.79±0.07 ^{ab}	***
	2013/2014	3.87±0.06 ^{aa}	7.43±0.15 ^{bb}	3.87±0.06 ^{aa}	***
	Sig. level	***	***	***	
Lignans	2012/2013	5.24±0.09 ^{bb}	7.08±0.04 ^{ca}	4.79±0.07 ^{ab}	***
	2013/2014	3.87±0.06 ^{aa}	7.43±0.15 ^{bb}	3.87±0.06 ^{aa}	***
	Sig. level	***	***	***	
Vanillic acid	2012/2013	0.41±0.00 ^{ba}	0.25±0.00 ^{aa}	0.43±0.00 ^{ca}	***
	2013/2014	0.52±0.06 ^{bb}	0.36±0.05 ^{ab}	0.62±0.05 ^{cb}	***
	Sig. level	***	***	***	
p-Coumaric acid	2012/2013	0.13±0.00 ^{ca}	0.07±0.00 ^{aa}	0.12±0.01 ^{ba}	***
	2013/2014	0.28±0.01 ^{cb}	0.07±0.01 ^{ab}	0.16±0.02 ^{bb}	***
	Sig. level	***	*	***	
Cinnamic acid	2012/2013	0.12±0.00 ^{aa}	0.17±0.02 ^{ba}	0.55±0.01 ^{ca}	***
	2013/2014	0.19±0.01 ^{ab}	0.24±0.01 ^{bb}	0.68±0.02 ^{cb}	***
	Sig. level	***	***	***	
Vanillin	2012/2013	0.22±0.00 ^{ba}	0.29±0.00 ^{ca}	0.18±0.03 ^{aa}	***
	2013/2014	0.46±0.01 ^{cb}	0.43±0.01 ^{bb}	0.25±0.02 ^{ab}	***
	Sig. level	***	***	***	
Simple phenols	2012/2013	0.87±0.01 ^{ba}	0.78±0.01 ^{aa}	1.28±0.02 ^{ca}	***
	2013/2014	1.45±0.03 ^{bb}	1.11±0.04 ^{ab}	1.71±0.06 ^{cb}	***
	Sig. level	***	***	***	

Values are the means±standard deviations (n=9). For each parameter, significant differences in the same line (comparison among cultivars) are indicated by different lowercase letters and significant differences in the same column (comparison between crop seasons) are indicated by different superscript letters. Significance level: *** $p<0.001$; ** $p<0.01$; * $p<0.05$; NS=not significant

crop seasons (Table 5), quantitative analysis of phenolic compounds by HPLC also showed an influence of climatic variations on the contents of these compounds in oil samples of the studied varieties (Table 6). With the

exception of flavonoids and lignans, all the identified phenolic fractions showed an increase in their levels in olive oils from the 2013/2014 crop season. The main variations were recorded in the fraction of secoiridoids. DLA and

DOA levels showed a significant difference between the two crop seasons ($p < 0.001$). As stated in the total phenols part, this variation could be due to environmental factors, mainly precipitation. The strong increase in DLA and DOA concentration was recorded in *Koroneiki* oil, from 165.56 mg kg⁻¹ in the 2012/2013 crop season to 195.54 mg kg⁻¹ in 2013/2014 crop season and from 146.72 to 183.00 mg kg⁻¹, respectively. Another variation was observed in *Arbosana* oil on flavonoid and lignan fractions. Their contents increased significantly ($p < 0.001$) between the two crop seasons (from 14.26 and 7.08 mg kg⁻¹ in 2012/2013 to 16.64 and 7.43 mg kg⁻¹ in 2013/2014). *Arbequina* and *Koroneiki* differ from *Arbosana* variety. Their contents showed a significant decrease ($p < 0.001$), passing, respectively from 10.22 and 10.09 mg kg⁻¹ in the 2012/2013

crop season to 7.83 and 9.35 mg kg⁻¹ in 2013/2014 crop season for flavonoids and from 5.24 and 4.79 mg kg⁻¹ in the 2012/2013 crop season to 3.87 mg kg⁻¹ in 2013/2014 crop season for lignans.

Phytosterol composition

With the exception of $\Delta^{5,24}$ -stigmastadienol, stigmasterol and erythrodiol, sterol profiles of the analyzed EVOOs (Table 7) did not reveal any significant differences in phytosterol levels and proportions between the two crop seasons ($p > 0.05$). Giuffrè and Louadj (2013) reported that some compounds, such as stigmasterol, β -sitosterol, sitostanol, Δ^7 -avenasterol, were influenced by the effect of crop season. While other authors have observed that the majority of sterol compounds in EVOO is influenced

Table 7: Composition of phytosterol fraction of monovarietal extra virgin olive oils obtained in two crop seasons

Phytosterols (% of total phytosterols)	Crop season	Olive varieties			Sig. level
		<i>Arbequina</i>	<i>Arbosana</i>	<i>Koroneiki</i>	
Cholesterol	2012/2013	0.17±0.00 ^{ca}	0.12±0.00 ^{ba}	0.16±0.01 ^{ba}	***
	2013/2014	0.18±0.02 ^{ca}	0.12±0.00 ^{ba}	0.16±0.01 ^{ba}	***
	Sig. level	NS	NS	NS	
24-Methylene cholesterol	2012/2013	0.39±0.03 ^{ba}	0.19±0.01 ^{aa}	0.42±0.02 ^{ca}	***
	2013/2014	0.38±0.02 ^{ba}	0.18±0.01 ^{aa}	0.43±0.00 ^{ca}	***
	Sig. level	NS	NS	NS	
Campesterol	2012/2013	2.86±0.04 ^{ba}	3.39±0.02 ^{ca}	2.57±0.11 ^{aa}	***
	2013/2014	2.89±0.10 ^{ba}	3.42±0.13 ^{ca}	2.52±0.11 ^{aa}	***
	Sig. level	NS	NS	NS	
Stigmasterol	2012/2013	0.73±0.01 ^{ba}	0.78±0.01 ^{ca}	0.40±0.01 ^{aa}	***
	2013/2014	0.75±0.02 ^{bb}	0.77±0.02 ^{ca}	0.41±0.01 ^{aa}	***
	Sig. level	*	NS	NS	
Clerosterol	2012/2013	1.17±0.00 ^{ca}	1.05±0.02 ^{ba}	0.96±0.13 ^{aa}	**
	2013/2014	1.17±0.11 ^{ca}	1.03±0.03 ^{aa}	0.97±0.20 ^{aa}	*
	Sig. level	NS	NS	NS	
β -Sitosterol	2012/2013	79.26±0.74 ^{ca}	77.48±0.12 ^{ba}	75.79±0.39 ^{aa}	***
	2013/2014	78.81±0.78 ^{ca}	77.36±0.49 ^{ba}	75.65±0.85 ^{aa}	***
	Sig. level	NS	NS	NS	
Δ^5 -Avenasterol	2012/2013	12.10±0.70 ^{aa}	13.53±0.10 ^{ba}	15.83±0.35 ^{ca}	***
	2013/2014	12.54±0.86 ^{aa}	13.51±0.35 ^{ba}	15.97±0.52 ^{ca}	***
	Sig. level	NS	NS	NS	
$\Delta^5,24$ -Stigmastadienol	2012/2013	0.67±0.02 ^{bb}	1.04±0.05 ^{ca}	0.56±0.01 ^{aa}	***
	2013/2014	0.65±0.02 ^{ba}	1.11±0.02 ^{cb}	0.57±0.01 ^{ab}	***
	Sig. level	*	**	*	
Δ^7 -Stigmastenol	2012/2013	0.41±0.02 ^{ba}	0.27±0.00 ^{aa}	0.40±0.02 ^{ba}	***
	2013/2014	0.39±0.01 ^{ba}	0.27±0.01 ^{aa}	0.42±0.02 ^{ca}	***
	Sig. level	NS	NS	NS	
Δ^7 -Avenasterol	2012/2013	0.97±0.05 ^{ca}	0.67±0.01 ^{aa}	0.73±0.04 ^{ba}	***
	2013/2014	0.95±0.06 ^{ba}	0.69±0.02 ^{aa}	0.72±0.06 ^{aa}	***
	Sig. level	NS	NS	NS	
Erythrodiol	2012/2013	1.30±0.03 ^{aa}	1.49±0.03 ^{ba}	2.19±0.12 ^{ca}	***
	2013/2014	1.28±0.03 ^{aa}	1.54±0.04 ^{bb}	2.18±0.33 ^{ca}	***
	Sig. level	NS	*	NS	
Total phytosterols (mg kg ⁻¹)	2012/2013	1978±16 ^{ba}	1950±5 ^{ba}	1604±57 ^{aa}	***
	2013/2014	1971±7 ^{ca}	1941±29 ^{ba}	1596±39 ^{aa}	***
	Sig. level	NS	NS	NS	

Values are the means±standard deviations (n=9). For each parameter, significant differences in the same line (comparison among cultivars) are indicated by different lowercase letters and significant differences in the same column (comparison between crop seasons) are indicated by different superscript letters. Significance level: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS=not significant

by the crop season (Salvador et al., 2003). Moreover, several studies have documented that cultivar is the main determinant of olive oil's sterol profile (Giuffrè and Louadj, 2013; Mansouri et al., 2015). This factor was very prominent in our study. In fact, variance analysis shows that the sterol profile of the analyzed oils is highly dependent on the olive variety ($p < 0.05$). The lowest total phytosterol concentration was observed in *Koroneiki* oil (1596 mg kg⁻¹), while the highest level was observed in *Arbequina* oil (1978 mg kg⁻¹). The total phytosterol values and their composition are within the ranges indicated by the International Olive Council for high-quality oils (IOC, 2013). From the results presented in Table 7, we note for all the analyzed oils the dominance of β -sitosterol, whose proportions fluctuate between 75 and 80% during the two crop season. The highest value is recorded in *Arbequina* oil (79.26%) followed by *Arbosana* oil (77.48%) and *Koroneiki* oil (75.79%). As for Δ^5 -avenasterol, its content varies inversely with β -sitosterol. *Koroneiki* oil is the richest in this substance (15.83%) followed by *Arbosana* and *Arbequina* oils (13.53 and 12.54%, respectively). Despite intervarietal variations, for the rest of the identified compounds, cholesterol, 24-methylene cholesterol, campesterol, stigmasterol, clerosterol, $\Delta^{5,24}$ -stigmastadienol, Δ^7 -stigmastenol, Δ^7 -avenasterol and erythrodiol, their percentages remain low compared to those of β -sitosterol and Δ^5 -avenasterol. These compounds make up less than 10% of the sterol fraction in all the samples of the analyzed oils. The 10% is dominated by campesterol, which accounts for more than 30% of these compounds (calculated from the results in Table 7), followed by erythrodiol (more than 15%). Campesterol is found the most in *Arbosana* oil with an average of 3.42% of total phytosterols, while the highest value in erythrodiol is observed in *Koroneiki* oil (2.19%).

CONCLUSION

This work is the first evaluation of the crop season's influence on the quality and chemical composition of the olive oils of *Arbequina*, *Arbosana* and *Koroneiki* cultivars grown under irrigated super-high-density planting system in the Oriental region of Morocco. The results obtained from this study indicate that the composition, with the exception of phytosterols, and the quality indices of extra virgin olive oils from these cultivars are strongly influenced by the variation of the climatic conditions. The main variations observed in the analyzed olive oils between the two crop seasons concerned antioxidant parameters, such as phenolic compounds, pigments and oxidation stability. Fatty acids and triacylglycerols are also influenced by climatic variations but the behavior of the studied varieties

in relation to climatic conditions is different. Despite these observed variations, the studied cultivars produce oils of excellent quality with a chemical composition that meets the requirements of the International Olive Council for high-quality oil.

ACKNOWLEDGEMENTS

This study was supported by the Moroccan–Belgian cooperation program “the Wallonia Brussels International project 2.9, 2012-2014”. We wish to thank Mr. Abdelouadoud Omari for his assistance and the company “Huiles d'olive de la Méditerranée” for its collaboration.

Authors' Contributions

Conception and design of the study: Mansouri and Elamrani; Acquisition of data: Mansouri, Gaetan and Fauconnier; Analysis and interpretation of Data: Mansouri, Ben moumen and Belhaj; Drafting the manuscript: Mansouri and Belhaj; Critical revision: Serghini Caid and Sindic.

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