OCL 2017, 24(5), A501
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DOI: 10.1051/ocl/2017012

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

RESEARCH ARTICLE OPEN ACCESS

Flavor profiles of monovarietal virgin olive oils produced in the Oriental region of Morocco

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Received 20 October 2016 – Accepted 7 March 2017

Abstract – The purpose of this study is the evaluation of flavor profiles of monovarietal virgin olive oils (VOO) produced in the Oriental region of Morocco via the characterization of volatile compounds, using SPME-GC/MS technique, and the determination of total phenolic content (colorimetric method). The study concerns oils of three European olive cultivars (*Arbosana*, *Arbequina* and *Koroneiki*) which were recently introduced in Morocco under irrigated high-density plantation system. GC/MS aroma profiles of analyzed VOOs showed the presence of 35 volatile compounds. The major compounds in such oils are C6 compounds produced from linoleic and linolenic acids via lipoxygenase pathway such as *trans*-2-hexenal, *cis*-2-hexenal, *cis*-3-hexen-1-ol, *trans*-3-hexen-1-ol, *trans*-3-hexen-1-ol acetate, hexanal and 1-hexanol in different proportions depending on the cultivar ($p < 0.05$). In addition, statistical analyses indicate that the analyzed VOOs have different aroma profiles. *Arbequina* oil has a high proportion of compounds with sensory notes "green" and "sweet" giving it a fruity sensation compared to *Arbosana* and *Koroneiki*. In parallel, *Arbosana* and *Koroneiki* oils are rich in phenolic compounds and provide relatively bitter and pungent tastes to these oils.

Keywords: virgin olive oil / volatile compounds / phenols / solid phase micro extraction

Résumé – Profils organoleptiques d'huiles d'olive vierges monovariétales produites dans la région orientale du Maroc. Le but de cette étude vise l'évaluation des profils organoleptiques des huiles d'olive vierges monovariétales produites dans la région orientale du Maroc par la caractérisation des composés volatils et la détermination de la teneur en phénols totaux. Il s'agit d'huiles d'olive de trois cultivars d'origine européenne (*Arbequina*, *Arbosana* et *Koroneiki*) récemment introduites au Maroc en irriguée super-intensif. Les profils aromatiques obtenus par GC/MS des huiles analysées montrent la présence de 35 composés volatils. Les principaux composés présents dans ces huiles sont les composés C6, tels que le *trans*-2-hexénal, le *cis*-2-hexènal, le *cis*-3-hexén-1-ol, le *trans*-3-hexén-1-ol, l'acétate de *trans*-3-hexén-1-ol, le 1-héxanol et l'hexanal à des proportions différentes selon le cultivar ($p < 0{,}05$). En outre, les analyses statistiques indiquent que les huiles analysées ont des profils aromatiques différents. L'huile d'*Arbéquina* présente une forte proportion des composés offrant des notes sensorielles « vert » et « sucré » lui conférant une sensation de fruité par rapport à l'*Arbosana* et *Koroneiki*. En parallèle, les huiles *Arbosana* et *Koroneiki* sont riches en composés phénoliques et apportent des goûts relativement amer et piquant à ces huiles.

Mots clés : huile d'olive vierge / composes volatils / phenols / micro extraction en phase solide

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1 Introduction

Virgin olive oil (VOO) is the ultimate Mediterranean product. It is increasingly becoming a part of the eating habits of new consumers worldwide nowadays. Its global consumption increased significantly during the past 25 years, from 1 985 000 to 3 075 500 tonnes (IOOC, 2015c). This increase is mainly associated to the unique characteristics of VOOs such as: nutritional properties, aroma, taste and color (Gutiérrez *et al.*, 1999).

In Morocco, olive has been thus far a traditional culture, yet it occupies a privileged place in agriculture contributing 5% of Gross Domestic Product (MAPM, 2014). The olive sector is a future opportunity in the increase in demand coupled with Moroccan agricultural policy through Green Morocco plan. It plans to expand from 760 000 to 1 220 000 ha for the period of 2008–2020 (MAPM, 2014), adopting a super-intensive system. This allows the intensification of production with densities ranging from 1200 to 2500 trees/h (Benito *et al.*, 2013).

Among the suitable olive varieties for intensive cultivation, three have been recently adopted in Morocco; Spanish cultivars *Arbequina* and *Arbosana*, and Greek cultivar *Koroneiki*. They are intended for the production of olive oil (Godini *et al.*, 2011). The choice of these varieties by growers is due to several reasons, mainly their erected habit and low vigor, their precocity and their oil richness in comparison with autochthone varieties as well as their excellent quality (Allalout *et al.*, 2009; Russo *et al.*, 2014; Mansouri *et al.*, 2016).

The high quality of VOO is determined largely by its organoleptic characteristics. These fine properties are particularly related to the presence of phenolic and volatile compounds (Angerosa, 2002; Andrewes *et al.*, 2003; Cerretani *et al.*, 2008). The former compounds are responsible for giving olive oil the following sensory properties: pungency and bitterness (Andrewes *et al.*, 2003; Beltran *et al.*, 2007) while the latter compounds are responsible for sensory notes green and fruity (Angerosa, 2002; Cerretani *et al.*, 2008).

The volatile compounds are low molecular weight molecules which are volatile at room temperature (Angerosa, 2002). They are products of oxidation of unsaturated fatty acids (Kalua *et al.*, 2007). In fact, the products of oxidation by lipoxygenase (endogenous enzymes of olive tree) are responsible for the positive perceptions of VOO flavors, while substances of auto-oxidation and photo-oxidation are generally associated to sensory defects (Kalua *et al.*, 2007).

The majority of molecules produced via lipoxygenase pathway are C5 and C6 compounds produced during oil manufacturing process and during its storage (Kalua *et al.*, 2007). The action of lipoxygenase on linolenic acid and linoleic acid causes the formation of 13-hydroperoxydes, the substrates for the rest of enzymatic reactions leading to the formation of volatile compounds (Angerosa *et al.*, 1999; Luaces *et al.*, 2007).

The level and activity of enzymes involved in different pathways of the lipoxygenase influence the composition of the olive oil volatile fraction (Angerosa, 2002). These enzymes are genetically determined and influenced in particular by the stage of maturity of olives (Campeol *et al.*, 2001; Kandylis *et al.*, 2011), cultivation methods (García-González *et al.*, 2014), extraction methods and storage conditions (Vekiari *et al.*, 2007; Servili, 2014) and geographical origin (Temime *et al.*, 2006; Bajoub *et al.*, 2015).

Studies on the composition and quality of monovarietal VOO from *Arbequina*, *Arbosana* and *Koroneiki* recently introduced in the Oriental region of Morocco have been conducted. They have focused on major and minor compounds (Mansouri *et al.*, 2016). However, little is known about the quality of these oils especially sensory quality. Hence why this study aims to determine the flavor profiles of these three monovarietal VOOs by the characterization of volatile compounds and the assessment of the total phenolic content.

2 Materials and methods

2.1 Samples

The analyzed oil samples are from three olive varieties recently introduced in the Oriental region of Morocco: *Arbequina*, *Arbosana* and *Koroneiki*. These three cultivars are planted since 2007 in a private estate located in the plain of Angad (latitude: 34° 47′N, longitude: 001° 57′W, altitude: 458 m) north-east of Morocco. These are irrigated super-intensive varieties planted with a density of 1666 trees/ha with a distance of 1.5 m/4 m between the trees. The irrigation period is 10 months a year, from January to October.

This study examined three samples of each variety, each sample contains 30 trees. The olives were harvested at the last week of November of the 2013/2014 harvesting season with a ripening index of 3.8–4.0. The olive ripening index was determined according to the method developed by the Agronomic Station of Jaén (Uceda and Hermoso, 1998). This maturity stage is the stage of the change in color from yellow-green olives to purple ones (rotating color).

The harvested olives by straddle harvesters are triturated immediately by a two-phase centrifugation system “Pieralisi” in the “Huiles d’olive de la Méditerranée-Oujda, Maroc” company factory. After deleafing and washing, the olives are crushed by a hammer mill. The obtained olive paste was mixed at 27 °C for 30 min and then centrifuged using a horizontal two-phase centrifuge without addition of water. Thereafter, the oil passes into a vertical centrifuge to remove the maximum of water.

Although physicochemical quality analysis (acidity, peroxide value and UV absorbance) is carried out on site at the factory, all the physicochemical quality tests were retaken in the laboratory using the methods described in the European Commission standard (EEC, 2003). All analyzed oils have values that meet the standards set by the International Olive Oil Council (IOOC, 2015b) for the category of extra virgin olive oils (moisture $\leq$0.1%; acidity $\leq$0.8%, peroxide value $\leq$20 meq O_2 kg^{-1}; $K_{270} \leq 0.22$; $K_{232} \leq 2.5$ and delta $K \leq 0.01$).

2.2 Determination of total phenols

The extraction of phenolic compounds was performed according to the method described by Ollivier *et al.* (2004) using a methanol/water mixture (80/20; v/v). The total phenol content was determined by the Folin–Ciocalteu method (Folin

and Ciocalteu, 1927) described by Ollivier *et al.* (2004) using caffeic acid (Sigma-Aldrich, St. Louis, MO, USA) as standard. The results are expressed in milligrams caffeic acid per kilogram olive oil.

2.3 Analysis of volatile compounds

Analysis of the oil's volatile fraction was performed using the solid phase micro extraction (SPME) technique and gas chromatography (GC-7890A, Agilent Technologies, Palo Alto, CA, USA) equipped with an automatic injector and coupled with quadrupole-type mass spectroscopy (MS-5975C, Agilent Technologies, Palo Alto, CA, USA). For this, 2.5 g of oil were placed in a 20 ml vial and preincubated at 40 °C for 10 min. Volatile compounds in the vials' headspace were adsorbed on a SPME fiber (50/30 μm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA, USA) for 50 min. After the extraction, the volatile compounds were desorbed directly in the injector of the device.

The separation of volatile compounds was done in splitless mode on a DB-Wax ms capillary column (30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, PaloAlto, CA, USA). The used carrier gas is helium (99.999%, Air Liquide, Liège, Belgium) at a flow rate of 1.5 ml/min. The initial oven temperature was 40 °C, kept for 6 min, then the temperature was raised at a rate of 6 °C/min up to 128 °C followed by an increase of 20 °C/min up to 250 °C and then this temperature was maintained for 10 min.

Mass spectra were performed using a method of ionization by electron impact of 70 eV (source temperature: 230 °C and quadrupole temperature: 150 °C) and recorded at a speed of 4.27 scans/s for a range of 35–400 amu (atomic mass unit).

Volatile compounds were identified by comparing their mass spectra with those of PAL600K (Palisade Corporation, USA) and Wiley 275 Spectral databases. The identification of volatile compounds was also carried out by calculating their Kovats retention indices (KI). KI were determined by injecting a hydrocarbon mixture containing a series of alkanes (C7–C30; 1000 μg/ml in hexane, Supelco, Bellefonte, PA, USA) under the same conditions described above.

2.4 Statistical analyses

The results presented in this work are the means of analyses carried out in triplicate with corresponding standard deviations (±SD). The normality of these results was evaluated by the test of the right Henry. The one-way ANOVA statistical analysis and the Duncan Post-hoc test were used to determine the significant differences between the means. The significant difference threshold was set at 5%. The Principal Components Analysis (PCA) was performed on the data sets in order to determine the variables that differentiate the flavor properties of VOOs analyzed depending on the cultivar. The similarities between the analyzed varieties are determined by the hierarchical clustering test.

The applications of these statistical analyses were performed using Windows softwares: IBM Statistical Package for the Social Sciences (SPSS 20.0) and XLSTAT, Version 5.16.03 (Addinsoft, 2014).

3 Results and discussion

The sensory evaluation of VOOs is designed to measure the intensity of olfactory and gustatory sensations of perceived flavors. These sensations are primarily modulated by the presence of volatile and phenolic compounds (Cerretani *et al.*, 2008). The latter compounds are considered the main antioxidants in olive oil and contribute to the oxidative stability of the oil (Botia *et al.*, 2001; Allalout *et al.*, 2009). Moreover, these compounds are responsible for some key sensory properties of olive oil (Andrewes *et al.*, 2003; Beltran *et al.*, 2007). The results of the determination of total phenols, by the colorimetric method, of the analyzed VOOs show levels that fluctuate between 286 and 567 mg of caffeic acid per kg of olive oil. This is well in line with the values suggested by the work of Aguilera *et al.* (2005), who reported that the total phenol concentration in VOO can vary between 50 and 1000 mg kg^{-1}, depending on various factors such as cultivar, climate, location, degree maturation and the production process of olive oil. According to Figure 1, total phenols content is clearly influenced by the cultivar ($p < 0.05$). VOO from *Koroneiki* variety is the richest in total phenols with a content of 566.30 mg kg^{-1}, while the lowest concentration was recorded in *Arbequina* oil whose content of phenols is 286.51 mg kg^{-1}. *Arbosana* oil has an intermediate concentration of phenols (454.80 mg kg^{-1}). Based on these results and according to the sensory classification of VOOs based on phenolic content published by Beltran *et al.* (2007), we can deduce that the *Arbosana* and *Koroneiki* VOOs can be classified respectively in the bitter quite/very bitter and bitter oil categories, while the *Arbequina* oil can be classified in the light bitterness category. The bitterness and pungency perceptions are due to complex phenolic compounds. In fact, an oil containing many complex phenols such as secoiridoid derivatives will be more bitter and pungent (Andrewes *et al.*, 2003; Barbieri *et al.*, 2015). The majority of pungent sensations in VOO was attributed to decarboxymethyl ligustroside aglycone (Andrewes *et al.*, 2003; Barbieri *et al.*, 2015), meanwhile, decarboxymethyl oleuropein aglycone is regarded as the main cause of bitterness (Barbieri *et al.*, 2015). In our previous study (Mansouri *et al.*, 2016), we demonstrated that decarboxymethyl oleuropein aglycone and decarboxymethyl ligustroside aglycone constitute the fraction of secoiridoid derivatives and constitute the majority of the identified phenolic compounds in the analyzed VOOs (over 90%) and their contents vary in the same order as total phenols. Thus, this demonstrates respective tastes of the oils of our analyzed varieties.

Beyond the contribution of phenolic compounds to the sensory determination of VOO, flavor is an important component and criterion to distinguish between VOOs of different olive varieties. This unique and delicate flavor of VOO is attributed to the presence of volatile compounds. Table 1 summarizes the proportions of volatile compounds of analyzed VOOs which are determined by integrating the areas under the peaks of the chromatograms obtained by SPME-GC/MS technique. A total of 35 compounds were identified for VOO of *Arbosana* variety, 34 for *Arbequina* and 33 for *Koroneiki*. The identified volatile compounds are mainly aldehydes (14.28–57.67%), alcohols (23.49–29.30%), esters

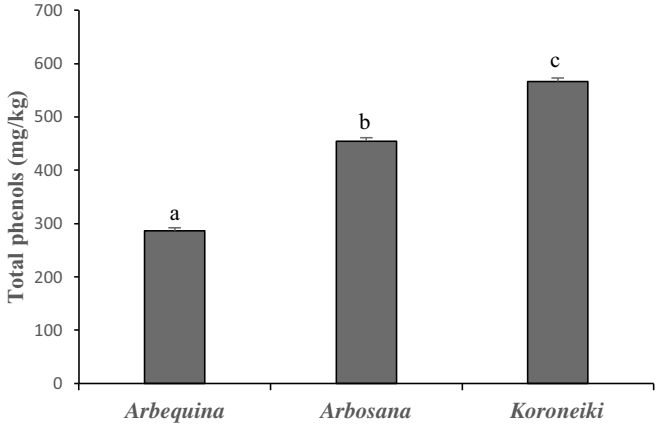

Fig. 1. Total phenols content of the studied virgin olive oils produced in Oriental region of Morocco. Significant differences ($p < 0.05$) are indicated by different letters (a–c). Concentration of total phenols expressed as milligram of caffeic acid per kilogram of olive oil (colorimetric method).

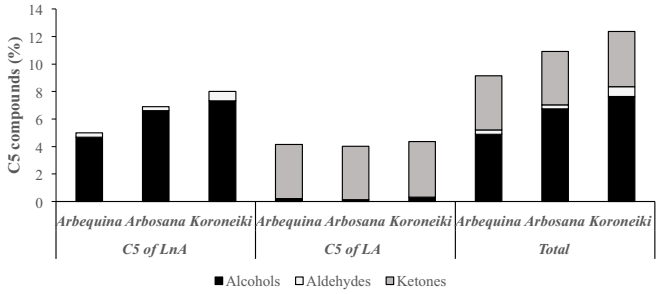

Fig. 2. Percentages of C5 volatile compounds derived from lipoxygenase of linoleic (LA) and linolenic (LnA) acids in monovarietal virgin olive oils of Morocco's Oriental region. C5 of LA: 1-pentanol and 3-pentanone; C5 of LnA: 1-penten-3-ol, *trans*-2-pentenal and *cis*-2-penten-1-ol.

(5.05–31.51%) and carboxylic acids (4.53–15.47%). These four chemical classes represent more than 90% of identified compounds in the analyzed samples. Aldehydes constitute the major fraction in *Arbosana* and *Arbequina* oils with, respectively, 55.87 and 57.67%. While esters and alcohols account for the majority of fractions in *Koroneiki* oil (29.30 and 31.51%, respectively).

The main substances that constitute volatile compounds of studied VOOs are molecules with 6 (C6) and 5 (C5) carbon atoms mainly aldehydes, esters and alcohols. These substances are produced from polyunsaturated fatty acids via lipoxygenase pathway (Cavalli *et al.*, 2004). The level of these compounds depends on the concentration and the activity of each enzyme involved in this pathway, and the fatty acid composition of the oil, which are also dependent on olive variety (Garcia *et al.*, 2012). This may explain the significant differences ($p < 0.05$) observed between these compounds on the analyzed VOOs. The total proportion of these molecules represents 61.18% of the volatile compounds in *Koroneiki* oil, 86.12% in *Arbequina* and 87.27% in *Arbosana* variety. The dominance of these compounds results in a good quality of these oils, because the C5 and C6 molecules represent the largest fraction of volatile compounds in VOOs of high quality in quantitative terms (Reboredo-Rodríguez *et al.*, 2014). In fact, the C6 compounds represent more than 70% of the volatile fraction in *Arbequina* and *Arbosana* oils, while the lowest portion is recorded in *Koroneiki* oil (48.82%). In addition, this fraction is dominated by the compounds from the degradation of linolenic acid by lipoxygenase (more than 85% of C6) such as *trans*-2-hexenal, *cis*-2-hexenal, *trans*, *trans*-2,4-hexadienal, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, *cis*-3-hexen-1-ol acetate and *trans*-2-hexen-1-ol (Fig. 3).

The main esters in the analyzed VOOs are methyl acetate, ethyl acetate, acetate *cis*-3-hexen-1-ol and hexyl acetate. The latter contributes to the sensory rating of "sweet" and "fruity" (Kalua *et al.*, 2007), while acetate *cis*-3-hexen-1-ol is related to pleasant notes "green" and "banana" (Baccouri *et al.*, 2008). Methyl acetate and ethyl acetate also contribute to the "green" and "sweet" notes, respectively (García-González and Aparicio, 2010). However, other authors suggest that ethyl acetate contributes negatively to the flavor of olive oil by adding a winey-vinegary flavor (Morales *et al.*, 2005; IOOC, 2015a). VOO from *Koroneiki* variety has the highest proportion of esters (31.51%) compared to *Arbosana* (8.83%) and *Arbequina* (5.05%). The low levels of hexyl acetate and *cis*-3-hexen-1-ol acetate in *Arbequina* and *Arbosana* oils may be due to low activity of the alcohol acyl transferase which is involved in the generation of C6 esters (Ridolfi *et al.*, 2002).

The alcoholic fraction is composed mainly by C5 and C6, such as 1-penten-3-ol, 1-pentanol, *cis*-2-penten-1-ol, 1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol (Figs. 2 and 3). In all analyzed VOOs, the proportions of C6 are superior to those of C5 compounds. *Koroneiki* oil is characterized by high levels of C6 and C5 alcohols (19.46 and 7.65%, respectively) compared to *Arbequina* (17.37 and 4.89%, respectively) and *Arbosana* (15.03 and 6.74% respectively). *cis*-3-Hexen-1-ol is the major component of this fraction with a proportion ranging from 8.80 (*Arbosana* oil) and 14.53% (*Koroneiki* oil) followed by *trans*-2-hexen-1-ol (2.14–3.74%) and 1-hexanol (2.30–4.46%). The presence of *trans*-3-hexen-1-ol and *trans*-2-hexen-1-ol in VOO also contribute to sensory note "green", while 1-hexanol gives the oil a "fruity" sensation (Kalua *et al.*, 2007). The rest of the alcoholic fraction molecules are present in all samples, but at low levels. Their presence in the oil could have a positive impact on the sensory level generally contributing to the sensory note "fruity" and "green" (Kalua *et al.*, 2007). In addition, high levels of alcohol compounds in *Koroneiki* oil are probably related to an intense enzymatic activity of alcohol dehydrogenase, which results in the reduction of aldehydes to alcohols (Brkić Bubola *et al.*, 2012). On the other hand, Angerosa *et al.* (1999) suggest that the activity of this enzyme is genetically determined for each cultivar.

Table 1 shows also a significant difference ($p < 0.05$) in the proportions of identified aldehyde compounds. This fraction is constituted exclusively by C5 and C6 compounds, in various proportions depending on the cultivar. *Arbequina* and *Arbosana* oils are characterized by higher proportions of aldehyde compounds (57.67 and 55.87%, respectively) than those observed in *Koroneiki* oil (14.28%). *trans*-2-Hexenal,

Table 1. Percentage peak areas of identified volatile compounds by SPME-GC/MS technique in monovarietal virgin olive oils of Morocco's Oriental region.

Volatile compounds (%)	KI	KI_{ref}	Varieties		
			Arbequina	*Arbosana*	*Koroneiki*
Organic acids					
Acetic acid	1450	1450	3.98 ± 0.28^{a}	4.83 ± 0.34^{b}	14.40 ± 0.30^{c}
Propionic acid	1538	1537	0.13 ± 0.03^{a}	0.26 ± 0.05^{b}	0.42 ± 0.05^{c}
Pivalic acid	1575	1575	0.23 ± 0.04^{a}	0.25 ± 0.04^{a}	0.26 ± 0.12^{a}
Butyric acid	1624	1616	0.06 ± 0.01^{a}	0.07 ± 0.00^{a}	0.13 ± 0.05^{b}
Hexanoic acid	>1800	1829	0.14 ± 0.02^{a}	0.17 ± 0.01^{a}	0.26 ± 0.05^{b}
Σ Organic acids			4.53 ± 0.23^{a}	5.58 ± 0.35^{b}	15.47 ± 0.31^{c}
Alcohols					
Ethanol	928	932	1.23 ± 0.17^{a}	0.95 ± 0.21^{a}	1.34 ± 0.63^{a}
3-Cyclopenten-1-ol	1104	–	0.11 ± 0.00^{b}	0.13 ± 0.02^{c}	ND^{a}
1-Penten-3-ol	1159	1164	2.38 ± 0.24^{a}	3.19 ± 0.41^{b}	2.65 ± 0.13^{a}
3-Methylbutan-1-ol	1204	1211	0.36 ± 0.02^{b}	0.31 ± 0.02^{a}	0.45 ± 0.06^{c}
1-Pentanol	1250	1250	0.21 ± 0.03^{b}	0.13 ± 0.01^{a}	0.33 ± 0.03^{c}
cis-2-Penten-1-ol	1317	1313	2.31 ± 0.13^{a}	3.42 ± 0.08^{b}	4.67 ± 0.29^{c}
1-Hexanol	1351	1354	4.46 ± 0.23^{b}	2.30 ± 0.09^{a}	2.39 ± 0.07^{a}
trans-3-Hexen-1-ol	1360	1366	0.26 ± 0.02^{b}	0.19 ± 0.06^{a}	0.40 ± 0.01^{c}
cis-3-Hexen-1-ol	1379	1385	8.83 ± 0.53^{a}	8.80 ± 0.18^{a}	14.53 ± 0.37^{b}
trans-2-Hexen-1-ol	1401	1408	3.82 ± 0.19^{b}	3.74 ± 0.09^{b}	2.14 ± 0.20^{a}
Propylene glycol	1586	1594	0.19 ± 0.03^{c}	0.15 ± 0.03^{b}	0.09 ± 0.04^{a}
Phenylmethanol	1650	1661	0.03 ± 0.01^{a}	0.05 ± 0.01^{b}	0.07 ± 0.01^{c}
2-Furylmethanol	>1800	–	0.06 ± 0.01^{a}	0.08 ± 0.01^{a}	0.21 ± 0.04^{b}
Phenol	>1800	–	0.04 ± 0.00^{a}	0.05 ± 0.01^{a}	0.04 ± 0.00^{a}
Σ Alcohols			24.29 ± 1.13^{b}	23.49 ± 0.38^{a}	29.30 ± 0.40^{c}
Aldehydes					
Hexanal	1072	1074	6.54 ± 0.34^{c}	3.61 ± 0.26^{b}	3.22 ± 0.23^{a}
trans-2-Pentenal	1125	1117	0.32 ± 0.05^{c}	0.29 ± 0.02^{b}	0.17 ± 0.01^{a}
cis-2-Hexenal	1141	1187	3.37 ± 0.30^{a}	4.96 ± 0.45^{b}	4.72 ± 0.40^{b}
trans-2-Hexenal	1210	1216	47.14 ± 2.95^{b}	46.55 ± 0.88^{b}	5.81 ± 0.70^{a}
trans,trans-2,4-Hexadiènal	1394	1397	0.30 ± 0.02^{a}	0.47 ± 0.04^{c}	0.36 ± 0.03^{b}
Σ Aldehydes			57.67 ± 3.35^{b}	55.87 ± 0.83^{b}	14.28 ± 0.84^{a}
Esters					
Methyl acetate	827	828	1.68 ± 0.31^{a}	1.59 ± 0.07^{a}	8.04 ± 0.57^{b}
Ethyl acetate	889	892	1.07 ± 0.22^{a}	1.49 ± 0.28^{a}	8.21 ± 0.78^{b}
Hexyl acetate	1270	1274	0.27 ± 0.03^{a}	0.21 ± 0.01^{a}	1.15 ± 0.15^{b}
trans-3-Hexen-1-ol acetate	1312	1316	1.98 ± 0.08^{a}	5.54 ± 0.11^{b}	14.11 ± 0.80^{c}
Methyl benzoate	1611	–	0.05 ± 0.01^{b}	ND^{a}	ND^{a}
Σ Esters			5.05 ± 0.29^{a}	8.83 ± 0.32^{b}	31.51 ± 1.23^{c}
Ketones					
3-Pentanone	972	977	3.88 ± 0.48^{a}	3.94 ± 0.43^{a}	4.02 ± 0.54^{a}
Σ Ketones			3.88 ± 0.48^{a}	3.94 ± 0.43^{a}	4.02 ± 0.54^{a}
Other compounds					
3-Ethyl-1,5-octadiene	1006	–	0.21 ± 0.03^{a}	0.20 ± 0.03^{a}	2.00 ± 0.23^{b}
α-Pinene	1011	1020	1.57 ± 0.17^{b}	1.03 ± 0.13^{a}	1.12 ± 0.05^{a}
4,8-Dimethyl-1,3,7-nonatriene	1240	–	0.06 ± 0.02^{a}	0.21 ± 0.02^{c}	0.15 ± 0.03^{b}
5-Ethyl-2(5H)-furanone	1592	–	0.51 ± 0.02^{a}	0.81 ± 0.06^{c}	0.74 ± 0.06^{b}
Dihydro-2(3H)-furanone	1616	1640	0.08 ± 0.03^{a}	0.10 ± 0.04^{a}	0.29 ± 0.07^{b}
Σ Other compounds			2.42 ± 0.24^{a}	2.35 ± 0.16^{a}	4.30 ± 0.21^{b}
Σ C6			76.98 ± 4.21^{b}	76.37 ± 0.96^{b}	48.82 ± 1.03^{a}
Σ C5			9.15 ± 0.43^{a}	10.90 ± 0.60^{b}	12.36 ± 1.68^{c}

The values are the means of three different olive oil samples ± standard deviation. Significant differences ($p < 0.05$) in the same line are indicated by different letters (a–c). KI, Kovats Index calculated on DB-Wax column; KI_{ref}, literature Kovats index using DB-Wax stationary phase (Wong and Bernhard, 1988; Buttery *et al.*, 2000; Ruther, 2000; Ferreira *et al.*, 2001; Umano *et al.*, 2002; García-González *et al.*, 2007; García-González and Aparicio, 2010); ND, not identified.

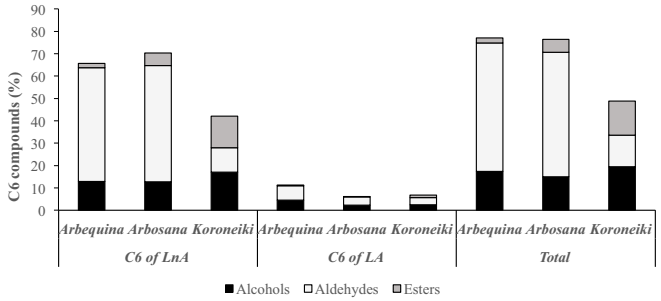

Fig. 3. Percentages of C6 volatile compounds derived from lipoxygenase of linoleic (LA) and linolenic (LnA) acids in monovarietal virgin olive oils of Morocco's Oriental region. C6 of LA: hexyl acetate, 1-hexanol and hexanal; C6 of LnA: *cis*-2-hexenal, *trans*-2-hexenal, *trans*,*trans*-2,4-hexadienal, *cis*-3-hexen-1-ol acetate, *cis*-3-hexen-1-ol, *trans*-3-hexen-1-ol and *trans*-2-hexen-1-ol.

which develops "green" aroma or "fresh cut grass" (Kalua *et al.*, 2007), is among the main volatile compounds identified in *Arbequina* and *Arbosana* oils (47.14% and 46.55%, respectively) while it is present in a very low proportion in *Koroneiki* oil (5.81%). This large difference between analyzed VOOs may be due to the intense activity of isomerization enzymes in the *Arbequina* and *Arbosana* varieties. According to Cavalli *et al.* (2004), *trans*-2-hexenal can be formed from 13-hydroperoxyde via *cis*-3-hexenal, which is either rapidly isomerized into *trans*-2-hexenal by two isomerases or reduced into *cis*-3-hexenol by dehydrogenase. In addition, *trans*-2-hexenal has been used by some authors as a marker of freshness of VOOs (Cavalli *et al.*, 2004).

The remaining aldehyde fraction of the studied oils comprises hexanal, *trans*,*trans*-2,4-hexadienal, *trans*-2-pentanal and *cis*-2-hexenal. Hexanal is considerably present in all tested oils. *Arbequina* oil has the highest rate (6.54%) compared to that of *Arbosana* (3.61%) and *Koroneiki* (3.22%). The high levels of hexanal, are often linked to sensory notes "sweet", "apple" and "green" (Kalua *et al.*, 2007).

3-pentanone was also detected with significant proportions in all samples. It is the result to the detriment of C6 alcohols and aldehydes formation via homolysis of 13-hydroperoxide (Cavalli *et al.*, 2004). Its presence in oil brings a spicy sensation. *Koroneiki* VOO has the highest rate in 3-pentanone (4.02%), while *Arbequina* and *Arbosana* VOOs have low and similar proportions (3.88 and 3.94% respectively).

Carboxylic acids such as acetic acid, propionic acid, pivalic acid, butyric acid and hexanoic acid were also identified in analyzed oils. Acetic acid is the major component of this fraction with proportions of 3.98% (*Arbequina* oil) and 10.38% (*Koroneiki* oil). The presence of this compound in olive oil may be due to a process of fermentation in the olives which is responsible for the vinegary sensory defect in VOO (Kalua *et al.*, 2007).

Moreover, the results for VOOs from Spanish olive varieties cultivated in Morocco are in agreement with the previous results reported in other studies (Reboredo-Rodríguez *et al.*, 2013a, b; Angerosa *et al.*, 2004), where *trans*-2-hexenal is the main compound of the volatile fraction of the olive oils produced in Spain.

On the other hand, olive oil from the *Koroneiki* variety cultivated in Morocco presents a different profile compared to the results reported in Greece (Kandylis *et al.*, 2011; Kosma *et al.*, 2015). In fact, our results showed that the olive oil from *Koroneiki* variety has a low proportion of *trans*-2-hexenal. This could be explained by the influence of environmental factors (Temime *et al.*, 2006; Kandylis *et al.*, 2011; Bajoub *et al.*, 2015), albeit they are unacknowledged by other studies (Angerosa *et al.*, 2004; Zhu *et al.*, 2013; Zhu *et al.*, 2015). These sources suggest that *trans*-2-hexenal concentration and C6 proportions are not affected by origin and climate; these two parameters would be differentiation markers of monovarietal VOOs.

The identification of changes in volatile compounds of oils of the studied varieties led us to perform a PCA on the identified compounds to study the structure of data and to characterize the oil samples based on their compositions in the headspace of the oils and their total phenols content. The results of the PCA have enabled us to isolate twenty-three factors explaining 100% of the analyzed variances, the first two accounted 79.78% of the total information. The most contributing of factor 1 are methyl, ethyl and hexyl acetates and C6 compounds produced from the lipoxygenase pathway such as *cis*-3-hexen-1-ol, hexyl acetate, *trans*-2-hexenal and *trans*-2-hexen-1-ol (Tab. 2). These compounds are responsible for positive sensory notes such as "green" (Kalua *et al.*, 2007). Organic acids, mainly acetic acid, are part of the variables that present a strong correlation with factor 1. They are often responsible for sensory defects linked to vinegary and pungency (Kalua *et al.*, 2007). Other compounds showed a strong correlation with factor1 such as 3-ethyl-1,5-octadiene. The attribution of a sensory note to this compound in the definition of VOO flavor is not clear. In fact, few studies reported the presence of these compounds in VOO without taking their sensory role into consideration (Vichi *et al.*, 2003; Tanouti *et al.*, 2012). Overall, the volatile compounds with higher levels in *Koroneiki* VOO have mainly a positive correlation with factor 1. On the other hand, 1-hexanol, *trans*, *trans*-2,4-hexadienal, methyl benzoate, hexanal have the most significant correlation with factor 2. Figure 4 shows a projection of different cultivars in the factorial plane defined by the first two main components. The representation of the first factors allows a clear separation for the analyzed samples. *Koroneiki* is completely opposite of *Arbequina* and *Arbosana* on the first factor axis. This means that *Koroneiki* is very different from the Spanish varieties, since the first axis is the one that separates the points best because it explains 61.48% of the information.

The results obtained by PCA were confirmed by the hierarchical cluster test. In fact, the dendrogram obtained from this analysis shows that at a distance of 59.97 cultivars are divided into three groups (Fig. 5). Cluster 1 is formed by the cultivar *Arbequina*, distinguished from other varieties by a high proportion of C6 compounds and low mean value of total phenols. *Koroneiki* variety that forms cluster 3, is characterized by high mean values of *cis*-3-hexen-1-ol, esters, organic acids and total phenols. While cluster 2 includes *Arbosana* which has intermediate values of these parameters. In addition, at a distance of 282.65, oils of the studied varieties are divided into two major groups. The first consists of *Arbequina* and *Arbosana* which are characterized by high levels of 6C

Table 2. Factor loading of parameters classified according to the cultivar on PCA plot.

Parameters	Factors		Parameters	Factors	
	1	2		1	2
cis-3-Hexen-1-ol	0.98	0.13	Propanoic acid	0.79	0.53
Methyl acetate	0.98	0.12	2-Furylmethanol	0.76	0.53
3-Ethyl-1,5-octadiene	0.97	0.13	Propylene glycol	−0.71	−0.39
trans-2-Hexenal	−0.97	−0.15	Butyric acid	0.67	0.24
3-Cyclopenten-1-ol	−0.97	−0.01	Hexanal	−0.47	−0.87
Acetic acid	0.97	0.21	Methyl benzoate	−0.37	−0.90
Hexyl acetate	0.97	0.08	1-Hexanol	−0.34	−0.93
Ethyl acetate	0.95	0.19	*trans*,*trans*-2,4-Hexadienal	−0.25	0.90
trans-2-Hexen-1-ol	−0.95	−0.19	1-Penten-3-ol	−0.25	0.75
1-Pentanol	0.93	−0.25	*cis*-2-Hexenal	0.23	0.85
trans-3-Hexen-1-ol	0.93	−0.15	Ethanol	0.22	−0.19
cis-3-Hexen-1-ol acetate	0.90	0.41	α-Pinene	−0.21	−0.86
Dihydro-2(3H)-furanone	0.90	0.22	5-Ethyl-2(5H)-furanone	0.17	0.94
Phenylethyl alcohol	0.88	0.24	Pivalic acid	0.08	0.15
trans-2-Pentanal	−0.86	−0.31	3-Pentanone	0.03	0.01
3-Methylbutan-1-ol	0.86	−0.20	Total phenols	−0.04	0.40
Hexanoic acid	0.80	0.32	*trans*-4,8-Dimethyl-1,3,7-nonatriene	0.01	0.94
cis-2-Penten-1-ol	0.80	0.58			

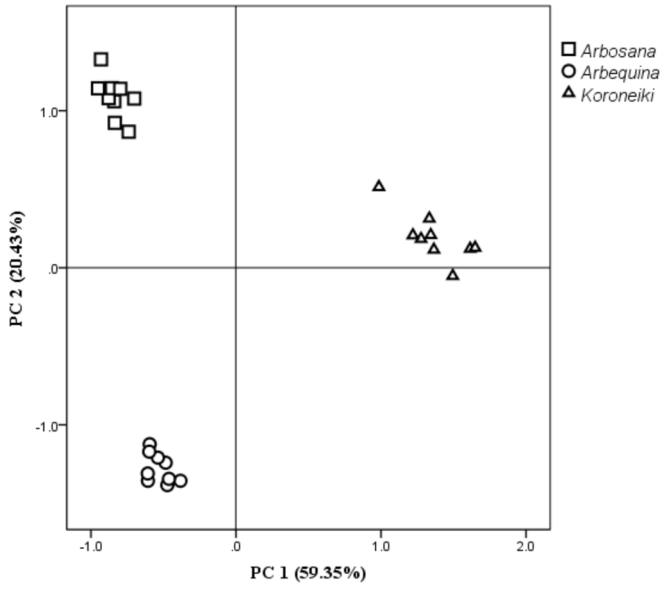

Fig. 4. PCA plot with factors 1 and 2 based on 36 variables (volatile compounds and total phenols) of the studied virgin olive oils.

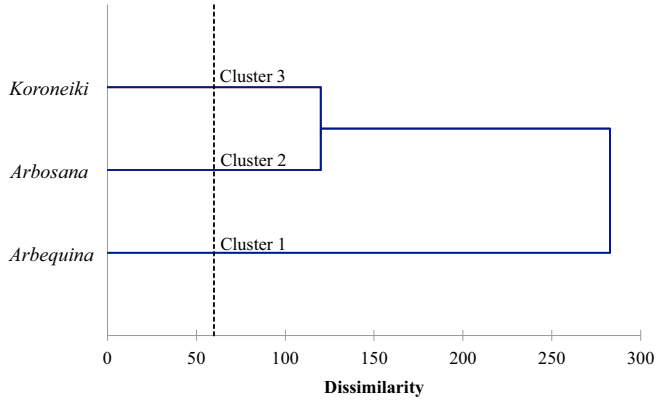

Fig. 5. Euclidean distance dendrogram of volatile compounds and total phenols of monovarietal virgin olive oils of Morocco's Oriental region.

compounds. Both varieties are also characterized by small percentages of esters and organic acids compared with those of *Koroneiki* oil which forms the second group.

4 Conclusion

Analysis of the volatile fraction of oil samples of European varieties produced in the Oriental region of Morocco by SPME-GC/MS has enabled us to identify the presence of 35 compounds from different chemical classes (mainly aldehydes, alcohols, esters and carboxylic acids). The proportions of the majority of the identified compounds clearly vary depending on the cultivar ($p < 0.05$). The major volatile compounds in these oils are C6 compounds, such as *trans*-2-hexenal, hexanal acetate, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol and 1-hexanol. In addition, statistical analyses of hierarchical cluster and principal components showed that the three analyzed VOOs have different flavor profiles. Compared to *Arbosana* and *Koroneiki*, *Arbequina* variety oil showed a high proportion of compounds which are responsible for sensory notes "green" and "sweet" giving it a fruity sensation. As for *Arbosana* and *Koroneiki* oils, their richness in phenolic compounds makes them taste bitter.

The low phenolic content of the *Arbequina* oil is the cause of its instability to oxidation and therefore its low storability. Preservation of the best sensory quality of *Arbequina* oil could be achieved by a meticulous assembling with oils of the other two varieties. This would at the same time help create new products, diversifying the consumer choice.

Acknowledgements. We are grateful to the "Moroccan-Belgian cooperation program" for the financial support of this research through a "WBI-Projet 2/9, 2012-2014". Our thanks are also due to the Company "Huiles d'olive de la Méditerranée" for its collaboration and Mr. Abdelouadoud Omari for the time spent in reviewing this paper.

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Cite this article as: Mansouri F, Ben Moumen A, Richard G, Fauconnier M-L, Sindic M, Serghini Caid H, Elamrani A. 2017. Flavor profiles of monovarietal virgin olive oils produced in the Oriental region of Morocco. *OCL* 24(5): A501.