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Characterization of sterolic and alcoholic fractions of some Algerian olive oils according to the variety and ripening stage

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Abstract – The present study reported for the first time the evolution of some minor compounds (sterols and aliphatic alcohols) composition of four Algerian olive oils according to the cultivar and the maturity stage during the season (2013/2014). Olive samples were collected during eight different harvesting dates. The studied cultivars were *Chemlal*, *Aghenfas*, *Buichret* and *Mekki*. Ten sterolic compounds, triterpene dialcohols (erythrodiol + uvaol) and seven fatty alcohols were identified and characterized. The results showed a decreasing level of total sterols, β-sitosterol, triterpenic dialcohols, total aliphatic alcohols, tetracosanol (C24), and an increasing level of Δ^5-avenasterol according to the maturity process. Linear discriminant analysis (LDA) enabled the classification of the studied varieties on the basis of analytical data and a correct classification rate of 96.5% and 88.6% was achieved using the original and cross validation method respectively. The most discriminating variables have been: cholesterol, campestanol, Δ^7-stigmastenol, Δ^7-avenasterol, clerosterol, alcohol C22, alcohol C25, alcohol C27.

Keywords: minor compounds / olive oil / cultivar / maturity / discriminant analysis

Résumé – Caractérisation des fractions stéroliques et alcooliques de quelques huiles d'olive algériennes en fonction de la variété et de l'état de maturité. Cette étude rapporte pour la première fois l'évolution de la composition en stérols et alcools aliphatiques de quatre huiles d'olive algériennes en fonction du cultivar et de la maturité au cours de la récolte 2013/2014. Les échantillons d'olives ont été collectés à huit dates de récolte différentes. Les cultivars étudiés sont : *Chemlal*, *Aghenfas*, *Buichret* et *Mekki*. Dix composés stéroliques, deux dialcools triterpéniques (érythrodiol + uvaol) et sept alcools gras ont été identifiés et caractérisés. Les résultats ont montré une teneur décroissante pour les stérols totaux, le β-sitostérol, les dialcools triterpéniques, les alcools aliphatiques totaux et le tétracosanol (C24) et inversement une teneur croissante en Δ5-avénastérol avec l'augmentation progressive de la maturité. L'analyse linéaire discriminante (*linear discriminant analysis* [LDA]) a permis la classification des variétés étudiées sur la base des données analytiques. Un taux de classification correct de 96,5 % et 88,6 % a été atteint en utilisant respectivement les méthodes de validation originale et croisée. Les variables les plus discriminantes ont été : le cholestérol, le campestanol, le Δ7-stigmasténol, le Δ7-avénastérol, le clérostérol et les alcools gras C22, C25 et C27.

Mots clés : composés mineurs / huile d'olive / cultivar / maturité / analyse discriminante

1 Introduction

Olive oil is one of the most important food products throughout the Mediterranean Basin due to its high nutritional and dietetic value. It is composed essentially of triacylglycerols which represent 98% of the oil and minor components such as tocopherols, phenolic compounds, alcohols and phytosterols (Bianchi, 2003). It has been demonstrated that the unsaponifiable fraction presented a beneficial therapeutic effect for human health (Abuajah *et al.*, 2015; Cilla *et al.*, 2015; Mahaddalkar *et al.*, 2015).

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Qualitative and quantitative sterols analysis is one tool to characterize the quality and the category of olive oil according to the official method provided by regulation EEC No. 2568/1991 (European Union Commission, 2011), to detect possible fraudulent and mixtures with other vegetable oils (Monfreda *et al.*, 2012).

Moreover, sterol esters evaluation has been proposed as useful parameter in studies related to varietal and geographical discrimination (Giacalone *et al.*, 2015). Furthermore, alcohols are identified as highly applicable compounds for chemical authentification (Rivera del Álamo *et al.*, 2004). It is well documented in the literature that the variation in quantitative sterolic profiles in olive oil is related to different factors like geographical origin (Manai-Djebali *et al.*, 2012) harvesting technique, oil extraction systems, storage conditions, refining procedures (Gutiérrez *et al.*, 2000), agronomic and climatic conditions (El Antari *et al.*, 2000). Cultivar and maturity stage are considered among the main factors affecting sterolic composition (Lukić *et al.*, 2013; Lazzez *et al.*, 2008).

In Algeria, olive growing represents an ancient activity, which is very important sector notably for the rural economy, it occupies about 350 000 ha (MADR, 2014) which are located mainly in the Kabilia region situated in the north of Algeria. Thirty-six varieties of olive oil had been identified by Mendil and Sebai (2006). *Chemlal* is considered as the most well-known variety in Algeria, covering approximately 40% of the total Algerian olive orchard. It is distributed mainly in North-Central Algeria, particularly in Kabylia. Recently, it has been pointed that this variety is the predominant cultivar with the highest olive oil production, and the industrial oil yield is about 18 L per 100 kg of olives (Haddadi and Yakoub-Bougdal, 2010). To our knowledge, and after a very advanced literature review, we are confident that the first researches that have been done on the olive varieties in Algeria have been focused much more on the identification and characterization of these varieties based on the characteristics of fruit and the quantity of oil produced by each variety. In fact, there are scarce informations on chemical composition of Algerian olive oils (Douzane *et al.*, 2012), and no published information related to the effect of cultivar or harvesting period on the sterolic and alcoholic compounds of the olive oil. Therefore, the objective of the present work is the characterization of sterol and alcohol fractions of four local Kabylian olive oil varieties: *Chemlal*, *Mekki*, *Aghenfas* and *Buichret*. The determination of the influence of cultivar and harvesting period on the profiles trends of these fractions is also studied. A multivariate statistical analysis ("principal component analysis" [PCA]) as an unsupervised method was applied to study the structure of the data and to visualize possible groups. Afterwards, a "linear discriminant analysis" (LDA) as a supervised method was applied to explain and predict an individual's belonging to a predefined class based on its characteristics measured using predictive variables.

2 Material and methods

2.1 Plant material

The study was carried out during the harvest season 2013/2014 in olive orchards located in Djaafra, department of Bordj Bou Arreridj, North-Eastern part of Algeria, as shown in Figure 1. The latitude and longitude of the area are 36°20′27″N and 4°46′3″E, respectively. Four representative autochthonous kabylian varieties were selected for this study: *Aghenfas*, *Buichret*, *Chemlal* and *Mekki*. Each variety was present in a mono-varietal orchard. The four orchards were situated approximately at an altitude of 400 m and characterized by the same pedo-climatic conditions, subjected to the same cultural practices and conducted without irrigation. Only healthy and fresh olive fruits were hand-picked all around the perimeter of trees. Olive samples were harvested approximately once every two weeks from the end of September until late of January. In each harvesting date, all samples varieties have nearly the same maturity index (MI) which were determined according to the *Estación de Olivicultura de Jaén* (Uceda and Hermoso, 2001). This method is based on the evaluation of the olive skin and pulp colors, and the values range from 0 to 7. For each cultivar, twenty eight olive samples have been extracted separately at sampling period between September and January. The samples were immediately brought to the laboratory for oil extraction.

2.2 Extraction of olive oils

The olive oils were extracted using an Abencor system (MC2 Ingeniería y Sistemas S.L. Sevilla, Spain). First, a representative 3 kg sample of olive fruits was used for olive oil extraction as follows: deleafing, washing, and then crushing in the hammer mill. The obtained paste was well mixed at 25 °C for 20 min, then subjected to centrifugation at 3000 rpm for 3 min without adding warm water. The collected oil was separated by decanting and kept in air-tight, sealed in a dark glass vials, and stored at 4 °C until analysis.

2.3 Physicochemical analysis

2.3.1 Determination of acidity, peroxide value, ultraviolet absorption indices

Free acidity (g oleic acid/100 g olive oil), peroxide value (PV) (meq O_2/kg of olive oil) and the UV absorption for the determination of the extinction coefficients of K232 and K270 were measured following the analytical methods described in regulation EEC/2568/91. All parameters were determined in triplicate for each sample.

2.3.2 Determination of sterols, triterpene dialcohols, and alcohols contents

The content of sterolic and alcoholic fraction extracted from different samples was determined according to the analytical methods described in European regulation EEC 2568/91 and subsequent amendments (European Union Commission, 2011).

2.3.2.1 Sterols and triterpene dialcohols analysis

Five grams of olive oil were added to α-cholestanol used as internal standard and then saponified with 50 ml of potassium hydroxide in an ethanolic solution (2M). After one hour of boiling, the unsaponifiable fraction was extracted with 200 ml of diethyl ether, purified with water and then dried by sodium sulfate and finally the diethyl ether was evaporated. Twenty

Fig. 1. Map of the studied and sampled areas.

milligrams of the unsaponifiable were dissolved in 0.5 ml of chloroform and then deposed on a basic silica gel plate. The sterol fraction was separated by elution with a mixture of hexane and diethyl ether (65/35, v/v). The bands corresponding to the triterpenic dialcohols ($Rf = 0.21$), sterols ($Rf = 0.25$), and aliphatic alcohols ($Rf = 0.42$) were scraped and the sterols recovered from the plate were dissolved in chloroform and filtered through a paper filter. As a final step, the solvent was evaporated under nitrogen and the obtained sterols were transformed into trimethylsilyl ethers by 50 µl of a pyridine/hexamethyldisilazane/trimethylchlorosilane mixture (9/3/1, v/v/v).

Separation and quantification of the trimethylsilyl sterol fraction was carried out using capillary gas chromatography, on a Shimadzu set 17 A chromatograph using an HP-5 capillary column (30 m length x 0.32 mm i.d), coated with a stationary phase formed by 5% of biphenyl and 95% of dimethylpolysiloxane (0.25 µm thickness). The working conditions of the chromatograph were as follow: type of detector: flamme ionisation detector (FID), injector temperature: 280 °C, isothermal column: 260 °C and detector temperature: 290 °C. The injection volume was 5 µl at a flow rate of 1 ml/min using nitrogen as carrier gas.

2.3.2.2 Alcohols analysis

Five grams of olive oil were added to 1-eicosanol used as internal standard and then saponified with 50 ml of potassium hydroxide in an ethanolic solution (2M). The unsaponifiable matter was extracted with ethyl ether. The alcoholic fraction was separated from the unsaponifiable by elution on thin layer

chromatography using a mixture of hexane and diethyl ether (65/35, v/v), then the bands were scraped off and the silica gel containing the alcohols was dissolved in chloroform and filtered through a paper filter. The solvent was evaporated under nitrogen and the alcohols were treated with trimethylsilyl ethers to obtain the trimethylsilyl derivatives by 50 µl of a pyridine/hexamethyldisilazane/trimethylchlorosilane mixture (9/3/1, v/v/v).

The separation and quantification of the trimethyl silyl alcohol fraction was carried out using gas chromatography, on a Shimadzu set 17 A chromatograph using an HP-5 capillary column (30 m length x 0.32 mm i.d), coated with a stationary phase formed by 5% of biphenyl and 95% of dimethylpolysiloxane (0.25 µm thickness). In the following conditions: the column was held at 180 °C for 8 min then programmed to increase at 5 °C/min until 260 °C and stabilized at this temperature for 15 min. The type of detector is FID, injector temperature: 280 °C, detector temperature: 290 °C, gas vector: nitrogen, flow rate: 1 ml/min, volume injected: 5 µl.

The qualitative analysis of the sterolic and alcoholic fractions was performed after the determination of the retention time of their pure compounds, which had been analyzed in the same conditions.

2.4 Statistical analysis

All assays were carried out in triplicates and results were expressed as mean ± SD. Statistical analysis was carried out by using SPSS statistical package software (SPSS for windows,

version 20, SPSS INC, and Chicago, USA). The analysis of variance (ANOVA) was applied on variables studied to detect significant differences between varieties and maturity stages. Tukey's *t*-test was then carried out to detect significant differences between values ($P < 0.05$).

Then a multivariate analysis was performed on the data set by applying PCA and LDA taking into consideration only the variables with $P < 0.01$.

PCA, a commonly used multivariate method, was applied to reduce or group the large number of variables to a smaller number of new, derived variables (called principal components [PCs]). PCs are the linear combinations of the primary variables that are more conducive to making the samples different from each other. They determine which variables contribute the most to such differences.

While LDA is a multivariate probabilistic classification method based on the use of multivariate probability distribution with the hypothesis of normal distribution with the same variance–covariance matrix in all the considered classes. This chemometric technique can be used only with well-defined data matrices, *i.e.* those having a high ratio between number of objects and number of variables inside each category (Field, 2009).

3 Results and discussion

3.1 Chemical quality criteria: acidity, peroxide value, ultraviolet absorption indices

Table 1 shows the chemical quality criteria of olive oils from the four cultivars (*Chemlal*, *Aghenfas*, *Mekki* and *Buichret*). All the analyzed oils showed values falling within the ranges established for "extra virgin olive oil" category (acidity $\leq$0.8%; PV $\leq$20 meq O$_2$/kg; K270 $\leq$0.22; K232 $\leq$2.5), as stated by International Olive Council (IOC) (2016).

At the first harvesting date (the end of September), olive oil ripening index (RI) of all studied varieties ranged between 1.16 and 1.31 in *Buichret* and *Aghenfas* varieties, respectively. On the seven subsequent dates, the ripening index markedly increased at 4.02 to 4.47 in *Aghenfas* and *Chemlal* varieties, respectively. These results indicate that the four varieties were characterized by the same trend of ripening process, while harvesting dates affect significantly ripening index ($P < 0.0001$).

The percentage of free acids is an important used parameter used to measure the olive oil acidity. A significant increase in free acidity along the studied varieties as ripening progressed ($P < 0.0001$) and stage of ripeness ($P < 0.005$) (Tab. 1). During the different harvesting dates, the acidity of olive oils varied from 0.1% to 0.2% in *Chemlal* and *Buichret* olive oils, and from 0.1% to 0.3% in *Aghenfas* and *Mekki* olive oils. A similar slight increase in acidity during the maturation period was found by Bengana *et al.* (2013). In fact, during the olive ripening, there is an increase in lipolytic activity which hydrolyses some triacylglycerols and gives fatty acids, monoacylglycerols and diacylglycerols (Martinez Suarez, 1973).

Measurements of PV and absorbance at 232 nm in the UV region are used to provide information on the primary oxidation of the olive oils. As it is shown in Table 1, the oils obtained from olives at more advanced ripening stages

presented a decrease in the values of PV. This diminution is significant only in *Buichret* and *Mekki* olive oils in which the values varied from 9.24 and 10.07 to 5.34 and 6.50 at the first and last sampling, respectively. This behavior is most likely related to the increasing of enzymatic lipoxygenase activity. Likewise, K232 values decreased as ripening progressed, this result disagree the results found by Desouky *et al.* (2009).

K270 is considered as an indicator of secondary oxidation, as shown in Table 1 this parameter present a slight tendency to decrease during maturity process but not significantly in all studied olive oils, which can been explained by the absence of secondary oxidation during olive maturation.

3.2 Impact of variety and maturity stage on sterolic compound trends

The sterolic composition of olive oils of the studied varieties is given in Table 2. Figure 2 shows a chromatogram of the sterol components obtained for one of *Chemlal* olive oil samples. Ten sterolic compounds were identified, which are cholesterol, campesterol, campestanol, stigmasterol, clerosterol, β-sitosterol, $\Delta^{5,24}$-stigmastadienol, Δ^{7}-stigmastenol, Δ^{7}-avenasterol, and Δ^{5}-avenasterol. From Table 2, it can be seen that all studied olive oils varieties presented higher levels of sterol contents when comparing to the minimum limit (1000 mg/kg) established by the International Olive Oil Council for Virgin Olive Oil (IOC, 2016). In the present study, the mean of total sterol contents ranged between 1187 mg/kg to 2304 mg/kg among the four varieties. The obtained results were similar to other Mediterranean varieties, such as *Chemlali* and *Chetoui* olive oils from Tunisia, with an average contents of 2580 mg/kg and 1522 mg/kg, respectively (Lazzez *et al.*, 2008; Ben Temime *et al.*, 2008), and Italian oils with values ranging from 979 to 1830 mg/kg (Giacalone *et al.*, 2015).

Moreover, the total sterol contents in the studied varieties showed significant variations among olive cultivars and maturity stages ($P < 0.0001$). We noticed that the level of sterols decreased when the ripening stage increased and the contents drop between 9.59% for *Mekki* cultivar to 16.80% for *Chemlal* cultivar, when the first stage was compared with the last.

For *Chemlal*, the level of sterols decreased from 2170 mg/kg to 1744 mg/kg between September and December. Whereas, for *Mekki*, the levels decreased from 1933 to 1747 mg/kg between September and January. *Aghenfas* olive oil presented the highest quantity of total sterols in comparison to the other varieties, which exceeded 2000 mg/kg until the end of November. The highest value was registered in September (2304 mg/kg), whereas the lowest content was recorded in January (1972 mg/kg). The low levels of sterols were observed in *Buichret* olive oil by comparison to other varieties.

With respect to ripening stage, there was a tendency for the sterol levels to decline with increasing ripeness index. This has been noted by other workers (Gutiérrez *et al.*, 1999; Mariani *et al.*, 1991), and is understood as being due to the synthesis of the sterols occurring in the first stages of development of the fruit: with ripening, the sterols become diluted as more oils are produced.

Table 1. Ripening Index (RI) and means ± SDs of chemical quality criteria of *Aghenfas*, *Buichret*, *Chemlal* and *Mekki* olive oils at different olives harvest dates.

	End Sep	Mid-Oct	End Oct	Mid-Nov	End Nov	Mid-Dec	End Dec	Mid-Jan
RI								
Chemlal	1.19 ± 0.06[a]	1.85 ± 0.14[b]	2.07 ± 0.09[b,c]	2.49 ± 0.15[c]	3.38 ± 0.20[d,e]	3.30 ± 0.13[d]	3.72 ± 0.18[e]	4.47 ± 0.19[f]
Aghenfas	1.31 ± 0.19[a]	1.97 ± 0.18[b]	2.30 ± 0.11[b,c]	2.53 ± 0.15[c,d]	3.07 ± 0.22[e]	3.63 ± 0.19[e,f]	3.44 ± 0.44[e,f]	4.02 ± 0.27[f]
Buichret	1.16 ± 0.13[a]	1.70 ± 0.25[b]	2.46 ± 0.09[c,d]	2.39 ± 0.34[c]	3.41 ± 0.40[d]	3.67 ± 0.29[d,e]	3.82 ± 0.19[d,e]	4.35 ± 0.21[e]
Mekki	1.26 ± 0.17[a]	1.74 ± 0.14[b]	2.17 ± 0.26[c,d]	2.10 ± 0.23[c]	3.20 ± 0.19[d]	3.45 ± 0.22[d,e]	3.76 ± 0.23[e,f]	4.14 ± 0.08[g]
Acidty (%)								
Chemlal	0.10 ± 0.02[a]	0.11 ± 0.01[a]	0.12 ± 0.03[a]	0.11 ± 0.04[a]	0.16 ± 0.02[a]	0.19 ± 0.02[a,b]	0.22 ± 0.05[a,b]	0.29 ± 0.06[b]
Aghenfas	0.19 ± 0.04[a]	0.20 ± 0.03[a]	0.22 ± 0.02[a]	0.20 ± 0.03[a]	0.21 ± 0.04[a]	0.25 ± 0.02[a,b]	0.30 ± 0.01[b,c]	0.32 ± 0.01[c]
Buichret	0.13 ± 0.02[a]	0.11 ± 0.02[a]	0.12 ± 0.01[a]	0.22 ± 0.02[b]	0.30 ± 0.06[c]	0.24 ± 0.04[b]	0.28 ± 0.03[b,c]	0.20 ± 0.05[b]
Mekki	0.18 ± 0.03[a]	0.22 ± 0.02[a]	0.21 ± 0.04[a]	0.23 ± 0.05[a]	0.23 ± 0.04[a]	0.29 ± 0.06[a,b]	0.32 ± 0.04[b,c]	0.37 ± 0.06[c]
PV (meq O_2/kg)								
Chemlal	8.46 ± 1.21[a,b]	9.17 ± 2.11[b]	8.45 ± 2.11[a,b]	8.17 ± 1.82[a,b]	7.82 ± 1.84[a,b]	6.18 ± 0.90[a]	5.02 ± 0.80[a]	5.51 ± 0.17[a]
Aghenfas	10.06 ± 0.33[b]	8.13 ± 2.32[a]	9.82 ± 0.73[a,b]	8.55 ± 2.26[a]	8.17 ± 1.09[a]	8.58 ± 3.03[a]	6.89 ± 0.97[a]	6.10 ± 0.60[a]
Buichret	9.24 ± 0.43[c]	8.39 ± 1.69[b,c]	8.21 ± 0.93[a,b]	6.92 ± 0.85[a,b]	6.09 ± 0.28[a]	5.33 ± 0.30[a]	5.42 ± 0.53[a]	5.34 ± 0.22[a]
Mekki	10.07 ± 0.73[b]	11.92 ± 0.72[b]	10.12 ± 0.78[b]	9.89 ± 0.59[b]	10.47 ± 1.42[b]	8.99 ± 1.78[b]	6.70 ± 1.51[a]	6.50 ± 1.58[a]
K_{232}								
Chemlal	1.83 ± 0.12[b]	1.75 ± 0.30[a,b]	1.55 ± 0.22[a,b]	1.31 ± 0.15[a]	1.22 ± 0.08[a]	1.32 ± 0.14[a]	1.12 ± 0.21[a]	1.08 ± 0.12[a]
Aghenfas	1.73 ± 0.18[b]	1.82 ± 0.24[a,b]	1.74 ± 0.25[a,b]	1.57 ± 0.16[a,b]	1.26 ± 0.31[a]	1.13 ± 0.26[a]	1.27 ± 0.11[a]	1.14 ± 0.22[a]
Buichret	2.22 ± 0.33[a,b]	2.23 ± 0.11[b]	2.14 ± 0.18[a,b]	2.10 ± 0.28[a,b]	1.99 ± 0.19[a]	2.03 ± 0.21[a,b]	1.72 ± 0.12[a]	1.65 ± 0.09[a]
Mekki	2.29 ± 0.11[b]	2.22 ± 0.29[a,b]	2.22 ± 0.32[a,b]	2.10 ± 0.25[a]	2.15 ± 0.15[a]	2.03 ± 0.05[a]	1.75 ± 0.12[a]	1.82 ± 0.06[a]
K_{270}								
Chemlal	0.18 ± 0.04[b]	0.16 ± 0.03[a,b]	0.17 ± 0.04[a,b]	0.15 ± 0.03[a]	0.14 ± 0.01[a]	0.15 ± 0.02[a]	0.11 ± 0.02[a]	0.12 ± 0.02[a]
Aghenfas	0.16 ± 0.03[a]	0.15 ± 0.03[a]	0.15 ± 0.01[a]	0.13 ± 0.02[a]	0.11 ± 0.06[a]	0.13 ± 0.01[a]	0.12 ± 0.04[a]	0.13 ± 0.04[a]
Buichret	0.20 ± 0.02[b]	0.19 ± 0.02[a,b]	0.17 ± 0.04[a]	0.18 ± 0.01[a,b]	0.15 ± 0.03[a]	0.13 ± 0.01[a]	0.15 ± 0.02[a]	0.14 ± 0.03[a]
Mekki	0.14 ± 0.01[a]	0.14 ± 0.03[a]	0.15 ± 0.01[a]	0.14 ± 0.03[a]	0.15 ± 0.02[a]	0.13 ± 0.05[a]	0.15 ± 0.04[a]	0.13 ± 0.03[a]

[a,b,c,d] Values with the same letter in the same line are not significantly different at a 95% confidence level ($P \leq 0.05$).

In all studied oils, β-sitosterol was the most abundant phytosterol being found and its level represents more than 75% of total sterols. These results are similar to those reported by Lukić *et al.* (2013). Nevertheless, it is worth noting that all samples contained β-sitosterol with considerable quantities. The values ranged from 966.7 mg/kg to 2123.6 mg/kg (Tab. 2). Statistical analysis showed that the variety and harvesting period had a significant effect on the trend of β-sitosterol amounts in the studied varieties ($P < 0.0001$). The data reported in Table 2 showed a concomitant decrease of both components and total sterols during the process of maturity. In the case of *Chemlal*, β-sitosterol displayed a regular decrease from 1976.1 mg/kg in September to 1468.4 mg/kg at the end of December. As well, the same profile tendency is observed for the other varieties: *Aghenfas* (from 2123.6 mg/kg to 1556.2 mg/kg), *Mekki* (from 1738.0 mg/kg to 1362.1 mg/kg), and *Buichret* (from 1462.2 mg/kg to 996.7 mg/kg). This reduction of β-sitosterol level during ripening stage was also observed by Vekiari *et al.* (2010).

The Δ⁵-avenasterol is the second major sterol found in the sterolic fraction. The Δ⁵-avenasterol and β-sistosterol are the typical components of the pulp of the olive (Christopoulou *et al.*, 1996). The concentration of Δ⁵-avenasterol is important for olive oil quality, as it confers important antioxidant properties at high temperatures (Rossell, 2001).

The obtained results showed a significant differences related to the maturity stage in all evaluated samples ($P < 0.0001$). A significant increase of Δ⁵-avenasterol was recorded during the maturity process with a maximum level in January for *Buichret* (174 mg/kg), in the late of November for *Chemlal* and *Aghenfas* (246 mg/kg and 315 mg/kg, respectively), and at the beginning of December for *Mekki* (344 mg/kg) (Tab. 2).

This increase in Δ⁵-avenasterol is followed by a regular decrease until the end of maturity, except for *Buichret*. The increase of Δ⁵-avenasterol contents observed in the present study is in agreement with the results reported in other previous study (Lazzez *et al.*, 2008).

A negative correlation was noted between β-sitosterol and Δ⁵-avenasterol in all studied oils: $r = -0.78$ for *Chemlal*, $r = -0.87$, for *Aghenfas* and *Buichret*, $r = -0.84$ for *Mekki*. This inverse relationship was previously reported for spanish varieties (Gutiérrez *et al.*, 1999). Fernández-Cuesta *et al.* (2013) have explained this phenomenon to the fact that Δ⁵-avenasterol is the precursor of β-sitosterol.

Aghenfas showed the highest value of β-sitosterol (92.15%) and low level of Δ⁵-avenasterol (2.06%), whereas *Mekki* presented the lowest percentage of β-sitosterol (76.46%) and important level of Δ⁵-avenasterol (18.49%). *Buichret* and *Chemlal* presented high levels of β-sitosterol

Table 2. Sterolic composition (mg cholestanol/kg oil) of *Aghenfas, Buichret, Chemlal* and *Mekki* virgin olive oils during the maturity stage.

	End Sep	Mid-Oct	End Oct	Mid-Nov	End Nov	Mid-Dec	End Dec	Mid-Jan
Cholesterol								
Chemlal	4.8 ± 0.3[b]	3.9 ± 0.2[a]	5.3 ± 0.2[b]	3.5 ± 0.5[a]	4.9 ± 0.4[b]	3.8 ± 0.2[a]	3.8 ± 0.3[a]	3.4 ± 0.2[a]
Aghenfas	1.1 ± 0.3[a]	1.2 ± 0.3[a]	1.1 ± 0.1[a]	1.8 ± 0.6[a,b]	1.3 ± 0.3[a]	2.1 ± 0.4[a,b]	2.2 ± 0.2[a,b]	2.5 ± 0,0[b]
Buichret	1.2 ± 0.4[a]	2.0 ± 0.3[a,b]	1.8 ± 0.1[a,b]	2.6 ± 0.3[b]	3.0 ± 0.3[b]	3.4 ± 0.2[b]	3.1 ± 0.2[b]	1.3 ± 0.4[a]
Mekki	3.9 ± 0.5[c]	5.0 ± 0.1[c,d]	5.7 ± 0.1[d]	4.5 ± 0.3[c]	4.3 ± 0.0[c]	2.6 ± 0.1[b]	2.0 ± 0.3[a]	3.2 ± 0.5[b]
Campesterol								
Chemlal	61.7 ± 5.2[c]	65.8 ± 4.1[c]	51.9 ± 3.4[b]	48.2 ± 2.3[b]	54.8 ± 2.6[b]	47.6 ± 3.3[b]	45.8 ± 3.8[b]	39.2 ± 4.0[a]
Aghenfas	80.8 ± 6.6[e]	72.7 ± 2.9[d]	55.2 ± 2.1[b]	65.1 ± 3.2[c]	51.5 ± 3.7[b]	43.4 ± 2.5[a]	51.8 ± 3.5[b]	41.3 ± 2.4[a]
Buichret	32.8 ± 2.4[a]	46.8 ± 2.4[b]	34.7 ± 3.7[a]	36.0 ± 3.7[a,b]	29.0 ± 2.3[a]	33.2 ± 2.2[a]	37.9 ± 2.7[a,b]	41.8 ± 3.1[a,b]
Mekki	56.2 ± 4.1[b]	45.2 ± 4.3[a,b]	52.2 ± 2.0[b]	35.1 ± 4.6[a]	34.54 ± 5.31[a]	43.13 ± 3.09[a,b]	37.80 ± 1.49[a]	34.86 ± 2.71[a]
Campestanol								
Chemlal	0.6 ± 0.2[a]	0.7 ± 0.1[a]	1.4 ± 0.3[b]	2.9 ± 0.3[c]	3.7 ± 0.3[d]	2.9 ± 0.2[c]	3.5 ± 0.2[d]	3.4 ± 0.5[d]
Aghenfas	1.8 ± 0.3[a]	1.6 ± 0.1[a]	1.5 ± 0.2[a]	2.1 ± 0.2[a,b]	1.5 ± 0.3[a]	1.9 ± 0.3[a]	2.8 ± 0.1[b]	3.4 ± 0.4[c]
Buichret	0.5 ± 0.2[a]	0.7 ± 0.3[a]	0.7 ± 0.2[a]	0.5 ± 0.1[a]	1.5 ± 0.3[b]	2.5 ± 0.3[c]	2.5 ± 0.1[c]	2.7 ± 0.4[c]
Mekki	0.8 ± 0.4	0.7 ± 0.2[a]	0.8 ± 0.1[a]	1.8 ± 0.1[b]	1.3 ± 0.0[a,b]	2.6 ± 0.4[b,c]	2.2 ± 0.2[b]	3.0 ± 0.4[c]
Stigmasterol								
Chemlal	18.1 ± 1.3[a,b]	17.8 ± 2.3[a,b]	14.6 ± 1.4[a]	16.5 ± 1.1[a,b]	17.4 ± 2.2[a,b]	11.6 ± 2.1	12.9 ± 1.3[a]	21.2 ± 2.7[b]
Aghenfas	9.2 ± 1.6[a]	10.9 ± 0.9[a]	12.1 ± 1.4[a,b]	9.7 ± 1.0[a]	13.2 ± 1.2[a,b]	16.1 ± 0.9[b]	13.8 ± 1.5[a,b]	10.5 ± 2.0[a]
Buichret	14.3 ± 1.6[b]	14.0 ± 2.5[b]	12.8 ± 3.4[b]	9.1 ± 1.2[a,b]	8.3 ± 1.8[a]	5.5 ± 0.9[a]	7.0 ± 1.3[a]	8.0 ± 1.6[a]
Mekki	7.7 ± 1.2[a]	7.2 ± 2.2[a]	12.9 ± 1.7[b]	6.9 ± 1.1[a]	12.6 ± 3.1[b]	12.5 ± 1.4[b]	17.2 ± 1.7[c]	18.1 ± 3.0[c]
Clerosterol								
Chemlal	16.6 ± 0.8[a,b]	15.7 ± 0.5[a,b]	16.1 ± 0.6[a,b]	17.2 ± 0.6[a,b]	20.0 ± 2.2[c]	18.7 ± 1.2[b,c]	12.7 ± 1.4[a]	14.1 ± 0.1[a]
Aghenfas	16.2 ± 0.9[b]	19.9 ± 0.6[b,c]	18.2 ± 0.5[b]	19.4 ± 1.2[b,c]	20.0 ± 2.7[c]	13.2 ± 1.6[a]	17.2 ± 0.2[b]	16.5 ± 1.4[b]
Buichret	17.9 ± 1.6[b]	18.6 ± 2.2[b]	16.1 ± 1.9[b]	17.8 ± 1.3[b]	11.8 ± 0.8[a,b]	10.5 ± 0.6[a,b]	7.3 ± 1.9[a]	9.2 ± 1.2[a]
Mekki	15.7 ± 0.6[a,b]	18.1 ± 2.1[a,b]	18.5 ± 1.2[a,b]	11.9 ± 1.7[a]	18.7 ± 2.1[b]	12.2 ± 1.0[a]	14.2 ± 2.0[a]	13.1 ± 1.3[a]
β-sitosterol								
Chemlal	1976.1 ± 21.7[d]	1849.8 ± 49.1[c]	1722.7 ± 35.3[b,c]	1667.0 ± 52.6[a,b]	1584.7 ± 40.0[a,b]	1558.4 ± 80.8[a]	1468.4 ± 45.8[a]	1558.0 ± 59.7[a]
Aghenfas	2123.6 ± 10.9[d]	1951.4 ± 50.5[c]	1812.0 ± 42.6[b]	1758.4 ± 38.1[a]	1631.8 ± 55.7[a]	1556.2 ± 56.0[a]	1631.0 ± 26.3[a]	1608.0 ± 44.3[a]
Buichret	1398.9 ± 23.7[e]	1462.6 ± 17.3[f]	1265.4 ± 12.7[d]	1128.2 ± 27.2[d]	1033.2 ± 24.7[b]	1004.8 ± 8.4[b]	966.7 ± 14.1[a]	1051.4 ± 15.1[b,c]
Mekki	1738.0 ± 15.6[d]	1650.6 ± 30.1[c]	1612.2 ± 12.3[c]	1544.0 ± 44.5[b]	1411.9 ± 41.7[a]	1420.9 ± 18.6[a]	1425.5 ± 35.1[a]	1362.1 ± 45.4[a]
Δ5-avenasterol								
Chemlal	73.0 ± 4.6[b]	56.1 ± 5.4[a]	109.2 ± 8.8[c]	184.3 ± 12.1[e]	246.0 ± 14.0[g]	221.5 ± 2.8[f]	178.0 ± 8.9[e]	139.2 ± 10.8[d]
Aghenfas	47.5 ± 6.7[a]	101.5 ± 11.2[b]	189.2 ± 3.5[c]	268.5 ± 7.2[e]	315.0 ± 13.9[f]	226.6 ± 11.0[d]	223.5 ± 3.2[d]	258.6 ± 7.9[e]
Buichret	52.7 ± 2.7[a]	96.8 ± 4.1[b]	113.2 ± 9.8[c]	161.0 ± 3.2[e]	162.8 ± 7.4[e,f]	169.0 ± 8.6[e,f]	146.8 ± 2.7[d]	174.0 ± 9.5[f]
Mekki	88.0 ± 3.4[a]	133.9 ± 4.4[b]	251.17 ± 8.70[c]	318.0 ± 4.4[f]	321.2 ± 12.08[f]	343.6 ± 6.8[g]	297.7 ± 5.3[e]	281.3 ± 9.8[d]

Table 2. (continued).

	End Sep	Mid-Oct	End Oct	Mid-Nov	End Nov	Mid-Dec	End Dec	Mid-Jan
$\Delta^{5,24}$-stigmastadienol								
Chemlal	10.3 ± 1.1^a	$17.8 \pm 0,5^c$	$15.6 \pm 2.2^{b,c}$	9.6 ± 0.8^a	13.4 ± 0.6^b	$15.6 \pm 1.5^{b,c}$	9.1 ± 0.9^a	13.5 ± 1.3^b
Aghenfas	4.5 ± 0.7^a	13.2 ± 1.4^c	16.5 ± 1.1^d	16.8 ± 1.6^d	12.0 ± 1.5^c	9.4 ± 1.5^b	19.4 ± 0.8^e	16.8 ± 0.3^d
Buichret	13.6 ± 1.8^c	$11.3 \pm 1.1^{a,b}$	8.0 ± 0.6^a	$12.8 \pm 0.1^{b,c}$	$9.4 \pm 0.6^{a,b}$	8.7 ± 1.0^a	6.8 ± 1.0^a	$10.0 \pm 1.4^{a,b}$
Mekki	9.7 ± 0.8^a	16.7 ± 0.9^b	14.7 ± 1.2^b	15.1 ± 0.2^b	$11.3 \pm 1.6^{a,b}$	8.5 ± 1.2^a	18.0 ± 2.5^c	17.0 ± 1.1^b
Δ^7-stigmastenol								
Chemlal	2.7 ± 0.3^a	$3.1 \pm 0.5^{a,b}$	2.2 ± 0.4^a	4.1 ± 0.2^b	$3.1 \pm 0.1^{a,b}$	4.6 ± 0.4^b	4.1 ± 0.2^b	5.0 ± 0.2^b
Aghenfas	$4.7 \pm 0.8^{b,c}$	4.4 ± 0.7^b	4.44 ± 0.34^b	2.1 ± 0.4^a	6.0 ± 0.1^c	3.8 ± 0.3^b	$4.7 \pm 0.8^{b,c}$	2.2 ± 0.3^a
Buichret	5.9 ± 0.4^c	3.7 ± 0.3^a	4.3 ± 0.3^a	4.0 ± 0.2^a	$4.8 \pm 0.3^{a,b}$	$5.5 \pm 0.3^{b,c}$	$4.5 \pm 0.5^{a,b}$	3.8 ± 0.8^a
Mekki	5.1 ± 0.9^a	7.1 ± 0.2^b	$7.0 \pm 0.3^{a,b}$	$6.8 \pm 0.5^{a,b}$	5.0 ± 0.4^a	$7.0 \pm 1.0^{a,b}$	$6.1 \pm 0.7^{a,b}$	4.2 ± 0.3^a
Δ^7-avenasterol								
Chemlal	6.2 ± 0.4^a	8.6 ± 0.8^b	5.0 ± 0.7^a	$7.0 \pm 0.3^{a,b}$	$6.7 \pm 1.3^{a,b}$	5.7 ± 0.5^a	$6.43 \pm 0.5^{a,b}$	8.50 ± 1.2^b
Aghenfas	15.2 ± 2.9^b	$11.4 \pm 0.7^{a,b}$	7.2 ± 1.0^a	8.8 ± 0.3^a	$11.6 \pm 0.5^{a,b}$	9.6 ± 0.8^a	8.5 ± 1.5^a	$12.3 \pm 1.4^{a,b}$
Buichret	7.5 ± 0.1^b	$7.0 \pm 0.9^{a,b}$	5.4 ± 1.3^a	4.9 ± 0.1^a	$5.87 \pm 0.4^{a,b}$	4.5 ± 0.3^a	4.5 ± 0.8^a	5.0 ± 0.5^a
Mekki	7.9 ± 1.3^b	$11.9 \pm 0.4^{b,c}$	9.4 ± 0.6^b	8.4 ± 1.0^b	14.2 ± 2.0^c	5.1 ± 0.5^a	7.6 ± 1.4^b	$10.92 \pm 1.0^{b,c}$
Total Sterol								
Chemlal	2170.2 ± 34.6^c	$2039.4 \pm 47.0^{b,c}$	$1944.3 \pm 25.8^{a,b}$	$1960.6 \pm 37.8^{a,b}$	$1954.85 \pm 32.03^{a,b}$	$1890.40 \pm 75.60^{a,b}$	1744.86 ± 61.9^a	1805.8 ± 69.9^a
Aghenfas	2304.6 ± 13.2^d	$2188.2 \pm 35.0^{c,d}$	2117.8 ± 38.7^c	$2152.9 \pm 38.0^{c,d}$	2064.0 ± 35.3^c	1882.3 ± 71.7^a	1974.9 ± 20.2^b	1972.1 ± 46.7^b
Buichret	1545.3 ± 29.3^f	1663.9 ± 14.0^g	1462.7 ± 29.75^e	1377.1 ± 31.4^d	1269.9 ± 29.9^b	1247.7 ± 17.2^b	1187.4 ± 15.6^a	$1307.3 \pm 27.7^{b,c}$
Mekki	$1933.2 \pm 18.5^{b,c}$	$1896.7 \pm 30.3^{b,c}$	1984.7 ± 24.1^c	$1952.6 \pm 41.6^{b,c}$	1835.1 ± 29.0^b	1858.3 ± 29.7^b	1828.4 ± 43.0^a	1747.8 ± 74.7^b
Erythrodiol + Uvaol								
Chemlal	31.0 ± 2.6^b	20.7 ± 1.2^a	21.0 ± 2.3^a	19.8 ± 1.2^a	18.2 ± 2.0^a	20.7 ± 1.7^a	18.8 ± 0.9^a	18.4 ± 1.1^a
Aghenfas	57.9 ± 1.1^d	53.6 ± 3.2^c	52.2 ± 4.2^c	31.9 ± 2.5^b	$34.4 \pm 1.5^{b,c}$	29.4 ± 2.5^b	30.1 ± 1.4^b	18.3 ± 0.7^a
Buichret	29.1 ± 1.8^d	30.9 ± 1.9^c	33.2 ± 2.3^c	32.2 ± 1.7^b	$38.5 \pm 2.6^{b,c}$	31.1 ± 1.4^b	24.7 ± 2.1^b	22.1 ± 1.2^a
Mekki	32.3 ± 2.5^b	32.7 ± 1.5^b	45.9 ± 3.4^d	$42.7 \pm 2.5^{c,d}$	41.5 ± 2.5^c	37.9 ± 1.9^c	33.8 ± 1.8^b	23.0 ± 2.8^a

[a,b,c,d] Values with the same letter in the same line are not significantly different at a 95% confidence level ($P \leq 0.05$).
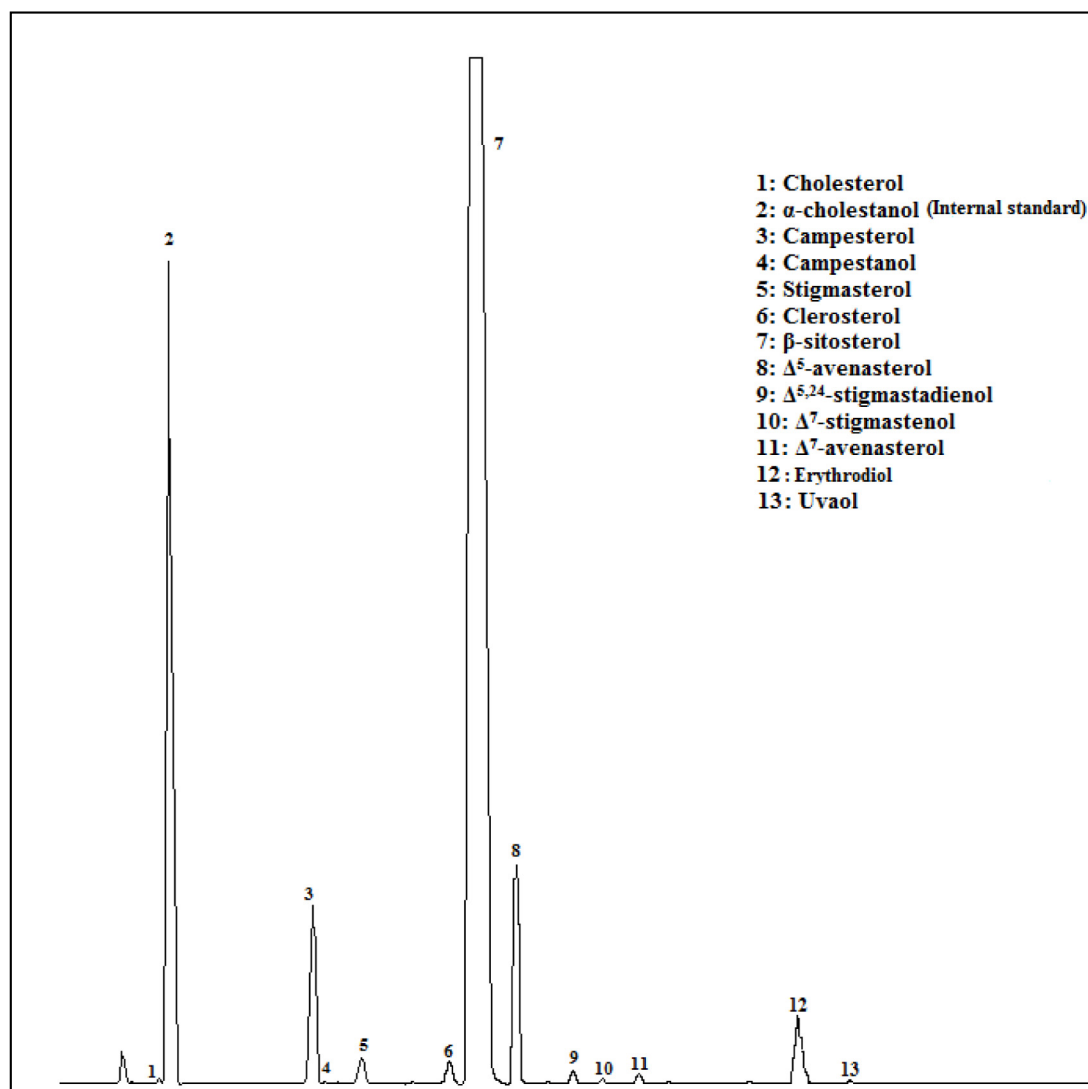
Fig. 2. Chromatogram of the sterol and triterpenic dialcohol components of one of the *Chemlal* analyzed samples.

(90.53–91.06%) and low levels of Δ^5-avenasterol (3.41–3.36%) (data in percentage not shown in tables).

Among identified sterols, campesterol showed a low content compared to the threshold established by the IOC regulations (4%) in all samples (IOC, 2016). As shown in Table 2, the amounts of campesterol varied significantly according to the cultivar. Furthermore, it can be observed that campesterol is significantly influenced by the maturity stage and ranged from 29 to 81 mg/kg.

As well, campesterol showed a clear downward trend during the maturity process except for *Buichret* oil. The amounts of campesterol decreased from 80.8 to 41.3 mg/kg, from 65.8 to 39.2 mg/kg, and from 56.2 to 34.5 mg/kg for *Aghenfas*, *Chemlal* and *Mekki* olive oils, respectively. Lukić *et al.* (2013) have also noted a diminution in campesterol during maturation in Croatian autochthonous olive varieties.

Regarding stigmasterol amounts, all cultivars presented very low contents of stigmasterol, and the values were less than campesterol percentages, as specified by IOC regulations (2016). It is well-known that stigmasterol content evolved unlike the quality of oil, In fact, high level is associated with high acidity and low organoleptic quality of oil (Gutiérrez *et al.*, 2000). A statistically significant difference between the studied varieties was observed ($P < 0.0001$), *Chemlal* variety, presented the highest value (21.2 mg/kg), whereas, *Buichret* presented the lowest concentration (5.5 mg/kg) of stigmasterol. During the maturity stages, the levels of stigmasterol seem to be relatively stable, excepted for the last and sixth samples respectively in *Chemlal* and *Aghenfas* oils, which represents high contents. As well, stigmasterol was constant in *Buichret* but decreased from the beginning of November.

Similarly, a significant difference was observed for the contents of clerosterol, which is related to the variety and the maturity ($P < 0.001$). Moderate amounts of this compounds were found, the values ranged from 7.3 to 20.0 mg/kg. The contents of clerosterol found in Algerian olive oil were higher than those found by Lazzez *et al.* (2008), for *Chemlali* olive oil.

Furthermore, small amounts of cholesterol, campestanol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol and Δ^7-avenasterol were also identified in all analyzed olive oils. Cholesterol and Δ^7-stigmastenol were under the limits established by the IOC regulations (0.5%) (IOC, 2016).

3.3 Impact of variety and olive maturity stage on Triterpene dialcohols compounds trends

The triterpenic dialcohols, which are the erythrodiol and uvaol are concomitantly analysed and evaluated with the sterol fraction because they are also a part of the unsaponifiable fraction. Results of the quantitative analysis of triterpenic dialcohols (erythrodiol and uvaol) of the different olive oil samples are given in Table 2. All values of triterpenic diols were in conformity with the limit established by the International Olive Oil Council's regulation for "edible virgin olive oil", which must not exceed 4.5% of the total sterols (IOC, 2016).

We noticed a significant difference among the studied varieties ($P < 0.0001$) which is in quite agreement with the findings of other studies (Sánchez-Casas *et al.*, 2004; Manai-Djebali *et al.*, 2012). The *Aghenfas* variety presented the highest levels that can reach 58 mg/kg especially in early maturity stage, while the lowest levels were observed in *Chemlal* variety, the other two varieties, namely *Buichret* and *Mekki* showed similar levels in most of maturity stages.

With respect to the levels of erythrodiol and uvaol during maturity process, we generally noted a downward trend when ripeness index increase. Understandably, it can be concluded that the maturity process has a significant influence on the level of the triterpenic dialcohols. These findings agree the results of Yorulmaz *et al.* (2013) and disagree with the outcomes found by Sánchez-Casas *et al.* (2004) in their study on Spanish olive oils.

3.4 Impact of variety and olive maturity stage on alcoholic compounds trends

The aliphatic alcohols compositions of the four varieties, *Chemlal, Aghenfas, Buichret* and *Mekki* are reported in Table 3. Figure 3 shows a chromatogram of the aliphatic alcohol components obtained for one of *Chemlal* olive oil samples. Seven aliphatic alcohols were identified, which are, the docosanol (C22), tricosanol (C23), tetracosanol (C24), pentacosanol (C25), hexacosanol (C26), heptacosanol (C27) and octacosanol (C28). C22, C24, C26 and C28 are the main aliphatic alcohols found in all olive oils, however, C23, C25 and C27 are detected in little quantities. Indeed, these compounds are located inside the olive stone, as consequence their extraction is very practical by solvent extraction methods than mechanical processes (Aparicio *et al.*, 2013). Furthermore, the determination of these minor aliphatic alcohols is very important for the accurate knowledge of alcoholic profiles with other parameters (erythrodiol, uvaol and wax content) during the detection of possible fraudulent addition of olive pomace oil to VOO.

In the present work, the total aliphatic alcohols content varied significantly according to the variety ($P < 0.05$). The mean values of the total aliphatic alcohols ranged from 81.4 mg/kg to 315.1 mg/kg. The maximum and the minimum average values were observed for *Mekki* olive oil (Tab. 3). Our results are similar to those found by Lazzez *et al.* (2008) in *Chemlali* olive oils, who reported values ranging between 70 mg/kg and 317 mg/kg, but very higher than those observed by Rivera del Álamo *et al.* (2004) in Spanish olive oils, in fact the levels observed, ranged from 35.0 mg/kg to 52.6 mg/kg and

from 44.2 mg/kg to 291 mg/kg, respectively. Mariani *et al.* (1991) outlined that high content of total aliphatic alcohols in some olive oils is mainly due to the presence of aliphatic alcohols in the free (non-esterified) form rather than as waxes. This phenomenon is attributed to adverse climatic conditions (prolonged drought).

According to the obtained results, it should be noted that the total aliphatic alcohols contents were significantly affected by the ripening process ($P < 0.0001$). Aliphatic alcohols decreased significantly from early harvest period (September and beginning of October) to advanced stage of maturity (December and January). The highest values were 238.1 mg/kg, 302.6 mg/kg, 315.1 mg/kg and 283.6 mg/kg for *Chemlal, Aghenfas, Mekki* and *Buichret*, respectively. During the maturation of olives, these values decreased progressively to reach 82.3 mg/kg, 86.0 mg/kg, 81.4 mg/kg and 115.7 mg/kg in the same order.

Hexacosanol (C26) is the most important compounds found in the alcoholic fraction in all analyzed samples. The evolution of C26 during maturity stages follow practically the evolution of total alcohols which decreased significantly during maturity. The concentrations of C26 decreased progressively, from 122.4 to 23.7 mg/kg and from 138.0 to 20.2 mg/kg for *Chemlal* and *Mekki*, respectively. Is it the same for the other two varieties, in fact, a downward trend was also recorded, as shown in Table 3. In advanced stages of maturity, the content of C26 seems to be stable especially for the varieties, *Mekki, Chemlal* and *Aghenfas.* According to the variety, we noted that the high levels were recorded in *Mekki* oil especially at the first stages of maturity, whereas, the lowest contents were observed in the oil of *Buichret*. In contrast, Giuffrè (2014) showed that C26 compound is not influenced by cultivar.

Tetracosanol (C24) is considered as the second or the third most abundant alcohol, depending to the variety. The obtained results showed that C24 varied significantly according to the cultivar ($P < 0.0005$) and the harvest period ($P < 0.0001$). Most cultivars showed a decrease in tetracosanol contents over the ripening period. *Aghenfas* contained the highest quantities of C24, which was observed from September until the end of November and the concentration decreased progressively from 94.3 mg/kg in September to 18.8 mg/kg in January. For *Chemlal, Mekki* and *Buichret* olive oils, the concentrations of C24 decreased from 29.3 mg/kg to 23.1 mg/kg, from 44.8 mg/kg to 37.2 mg/kg, and from 65.1 mg/kg to 25.1 mg/kg, respectively (Tab. 3).

With respect to the content of octacosanol (C28), statistical analysis showed a significant differences related to cultivar ($P < 0.0001$) and maturity stage ($P < 0.0001$). The level of C28 decreased clearly during maturity process for *Chemlal* and *Buichret* olive oils which the content range from 56.7 mg/kg to 11.4 mg/kg and from 44 mg/kg to 15.7 mg/kg respectively. Whereas, the concentration showed a different behavior for the other two varieties, the content of C28 increased during the first stages of maturity from 38 mg/kg to 80.5 mg/kg and decreased until the last stage of maturity for the case of *Aghenfas* variety. For the variety *Mekki*, C28 increased until reaching a maximum in October (83.8 mg/kg) and then decrease until the end of maturity. For all cultivars the concentration of octacosanol decreased at the end of the maturity process (Tab. 3), this diminution

Table 3. Alcoholic composition (mg eicosanol/kg oil) of *Aghenfas, Buichret, Chemlal* and *Mekki* virgin olive oils during the maturity stage.

	End Sep	Mid-Oct	End Oct	Mid-Nov	End Nov	Mid-Dec	End Dec	Mid-Jan
C22								
Chemlal	9.5 ± 1.5[a]	15.5 ± 2.5[b]	17.8 ± 2.4[b]	23.7 ± 0.8[c]	17.9 ± 1.5[b]	16.5 ± 1.7[b]	15.0 ± 2.1[b]	15.1 ± 3[b]
Aghenfas	6.3 ± 0.4[e]	5.5 ± 0.8[d]	4.9 ± 0.2[d]	2.8 ± 0.6[c]	1.8 ± 0.4[b]	0.9 ± 0.4[a]	1.9 ± 1.1[b]	1.11 ± 0.3[a]
Buichret	16.3 ± 2.2[a,b]	11.9 ± 2.0[a]	16.0 ± 1.6[a,b]	22.3 ± 1.8[b]	21.1 ± 1.2[b]	10.6 ± 1.5[a]	9.5 ± 1.0[a]	13.5 ± 1.2[a,b]
Mekki	22.7 ± 1.7[c]	28.8 ± 2.1[d]	27.1 ± 1.9[d]	33.0 ± 3.3[e]	15.8 ± 1.2[b]	5.5 ± 0.8[a]	4.7 ± 1.4[a]	3.5 ± 0.9[a]
C23								
Chemlal	5.1 ± 0.1[e]	4.2 ± 0.1[c]	1.9 ± 0.0[a]	3.7 ± 0.0[b]	5.8 ± 0.3[f]	2.1 ± 0.1[a]	2.1 ± 0.0[a]	4.6 ± 0.0[d]
Aghenfas	9.4 ± 0.1[d]	5.5 ± 0.5[c,d]	4.9 ± 0.1[c]	5.7 ± 0.4[c,d]	5.3 ± 0.6[c]	1.9 ± 0.1[b]	1.9 ± 0.1[b]	1.1 ± 0.0[a]
Buichret	5.6 ± 0.2[b]	2.1 ± 0.0[a]	3.62 ± 0.1[a,b]	5.1 ± 0.7[b]	5.3 ± 0.4[b]	3.5 ± 0.3[a,b]	3.1 ± 0.4[a,b]	2.5 ± 0.0[a]
Mekki	8.2 ± 1.1[d]	7.1 ± 0.1[c]	6.3 ± 0.2[c]	7.1 ± 0.7[c]	4.7 ± 0.2[b]	2.3 ± 0.1[a]	1.7 ± 0.1[a]	1.7 ± 0.2[a]
C24								
Chemlal	29.3 ± 2.7[b]	31.5 ± 2.6[b]	38.2 ± 2.9[c]	42.5 ± 1.7[d]	32.8 ± 2.2[b]	25.1 ± 2.1[a]	23.4 ± 1.9[a]	23.1 ± 2.1[a]
Aghenfas	94.3 ± 2.9[f]	76.4 ± 2.4[e]	62.5 ± 3.0[c]	69.5 ± 3.3[d]	54.1 ± 2.9[b]	16.1 ± 0.8[a]	17.0 ± 1.0[a]	18.8 ± 1.9[a]
Buichret	65.1 ± 3.8[d]	45.1 ± 4.4[c]	31.2 ± 3.4[a,b]	30.4 ± 3.0[a,b]	34.1 ± 1.5[a,b]	29.1 ± 2.9[a]	37.0 ± 1.6[b,c]	25.1 ± 1.9[a]
Mekki	44.8 ± 3.8[c]	34.1 ± 2.5[b]	26.1 ± 2.5[a]	44.3 ± 3.8[c]	37.4 ± 1.5[b,c]	34.6 ± 1.8[b]	32.6 ± 1.6[b]	37.2 ± 2.3[b,c]
C25								
Chemlal	9.6 ± 1.5[b]	8.3 ± 1.2[b]	3.7 ± 0.2[a]	5.0 ± 1.1[a,b]	4.7 ± 0.8[a]	3.1 ± 1.0[a]	3.7 ± 0.6[a]	3.6 ± 0.4[a]
Aghenfas	31.5 ± 1.4[c]	33.0 ± 1.6[c]	32.2 ± 1.8[c]	48.6 ± 2.5[e]	37.6 ± 2.1[d]	20.3 ± 1.2[b]	15.2 ± 1.0[a]	15.4 ± 0.8[a]
Buichret	27.2 ± 2.7[c]	14.3 ± 1.7[a,b]	12.5 ± 1.0[a]	14.3 ± 1.3[a,b]	11.1 ± 1.6[a]	13.8 ± 1.4[a]	15.3 ± 1.2[a,b]	18.0 ± 1.4[b,c]
Mekki	29.3 ± 2.7[c]	31.4 ± 2.1[c]	17.5 ± 1.8[b,c]	15.4 ± 1.9[b]	12.4 ± 1.4[b]	6.1 ± 1.2[a]	4.8 ± 1.4[a]	5.5 ± 1.2[a]
C26								
Chemlal	122.4 ± 5.6[e]	94.3 ± 4.3[d]	69.4 ± 2.3[c]	57.8 ± 3.0[b]	31.5 ± 2.8[a,b]	31.3 ± 1.9[a,b]	26.5 ± 2.2[a]	23.7 ± 1.2[a]
Aghenfas	113.4 ± 5.0[f]	89.1 ± 3.9[e]	61.3 ± 2.3[d]	63.7 ± 1.5[d]	43.3 ± 0.8[c]	17.5 ± 1.8[a]	16.9 ± 1.5[a]	26.5 ± 1.3[b]
Buichret	116.5 ± 8.0[d]	56.2 ± 2.0[c]	34.5 ± 1.7[b]	25.1 ± 2.8[a]	34.3 ± 1.7[b]	38.3 ± 2.6[b]	39.8 ± 2.9[b]	40.5 ± 3.0[b]
Mekki	138.0 ± 6.7[f]	121.8 ± 7.6[e]	99.4 ± 5.3[d]	91.7 ± 5.9[d]	66.2 ± 3.8[c]	34.5 ± 3.1[b]	21.4 ± 2.4[a]	20.2 ± 2.3[a]
C27								
Chemlal	5.3 ± 0.2[d]	6.3 ± 0.6[e]	3.1 ± 0.1[c]	3.4 ± 0.2[c]	2.3 ± 0.1[b]	2.6 ± 0.3[b]	0.5 ± 0.0[a]	0.8 ± 0.0[a]
Aghenfas	9.4 ± 0.1[f]	8.3 ± 0.0[e]	7.4 ± 0.1[d]	8.6 ± 0.5[e]	3.1 ± 0.1[b]	1.9 ± 0.1[a]	2.8 ± 0.0[b]	4.4 ± 0.1[c]
Buichret	8.8 ± 0.2[d]	7.5 ± 0.5[c]	5.2 ± 0.1[b]	4.0 ± 0.1[a]	3.7 ± 0.1[a]	5.2 ± 0.6[b]	8.5 ± 0.4[d]	9.3 ± 0.2[e]
Mekki	9.1 ± 0.1[g]	8.0 ± 0.2[f]	6.4 ± 0.1[e]	4.5 ± 0.1[d]	3.3 ± 0.5[c]	2.1 ± 0.1[b]	1.1 ± 0.1[a]	1.4 ± 0.0[a]
C28								
Chemlal	56.7 ± 1.4[f]	52.7 ± 1.7[e]	37.5 ± 2.2[d]	28.6 ± 1.4[c]	15.3 ± 0.7[a]	18.3 ± 1.5[b]	13.8 ± 0.9[a]	11.4 ± 0.9[a]
Aghenfas	38.1 ± 1.7[b]	48.5 ± 2.1[c]	64.1 ± 3.7[e]	80.5 ± 4.2[f]	54.6 ± 2.6[d]	31.1 ± 1.8[a]	30.2 ± 2.0[a]	29.8 ± 1.7[a]
Buichret	44.1 ± 3.7[c]	24.5 ± 2.3[b]	11.5 ± 1.0[a]	11.6 ± 1.9[a]	23.2 ± 2.0[b]	15.0 ± 1.8[a]	13.7 ± 1.5[a]	15.6 ± 1.0[a,b]
Mekki	47.9 ± 3.6[b,c]	83.8 ± 3.6[d]	64.7 ± 4.1[c]	43.7 ± 3.3[b]	42.2 ± 3.0[b]	18.2 ± 1.4[a]	15.1 ± 1.5[a]	15.7 ± 1.5[a]
Total alcohol								
Chemlal	238.1 ± 13.2[d]	212.8 ± 12.8[c]	171.7 ± 10.1[b]	164.8 ± 8.2[b]	110.4 ± 8.5[a,b]	99.1 ± 5.5[a]	85.1 ± 7.8[a]	82.3 ± 9.8[a]
Aghenfas	302.6 ± 11.7[e]	266.4 ± 11.3[d]	237.4 ± 11.0[c]	279.4 ± 8.1[d]	199.9 ± 7.7[b]	89.7 ± 6.3[a]	86.0 ± 3.6[a]	97.1 ± 6.1[a]
Buichret	283.6 ± 4.9[c]	161.6 ± 12.8[b]	114.6 ± 8.9[a]	112.9 ± 8.4[a]	132.9 ± 8.5[a,b]	115.7 ± 10.5[a]	127.0 ± 9.1[a]	124.6 ± 8.7[a]
Mekki	300.2 ± 11.9[b,d]	315.1 ± 18.2[d]	247.6 ± 15.5[c]	239.9 ± 15.2[c]	182.1 ± 11.2[b]	103.3 ± 8.3[a]	81.4 ± 8.5[a]	85.2 ± 8.5[a]

[a,b,c,d] Values with the same letter in the same line are not significantly different at a 95% confidence level ($P \leq 0.05$).

was also reported in previous studies (Lazzez *et al.*, 2008; Giuffrè, 2014).

Concerning the docosanol C22, all samples showed a content inferior to 33 mg/kg. The concentration of docosanol (C22), ranged between 0.9 mg/kg and 33.0 mg/kg. Rivera del Álamo *et al.* (2004), in his study reported values ranging between 8.2 mg/kg and 72.5 mg/kg for the C22. The obtained results, showed a significant differences according to maturity ($P < 0.0001$) and cultivar ($P < 0.0001$). During the maturation process the content of docosanol C22 increased and then decreased until the end of maturity, especially for the *Chemlal*,

Mekki and *Buicheret*, whereas in the oil of *Aghenfas*, which presented the lowest levels, we noted a decrease from 6.3 mg/kg at the first stage of maturity to a low content about 1mg/kg at the end of maturity. In contrary, Giuffrè (2014) has found that in general samples presented a constant docosanol level through the maturity process.

Regarding the C25 aliphatic alcohol, the results presented in Table 3 showed that *Chemlal* olive oil contained the low quantities compared to the other varieties, which don't exceed 10 mg/kg in all maturity stages. However, the high quantities were found in *Aghenfas* variety which reached 49 mg/kg
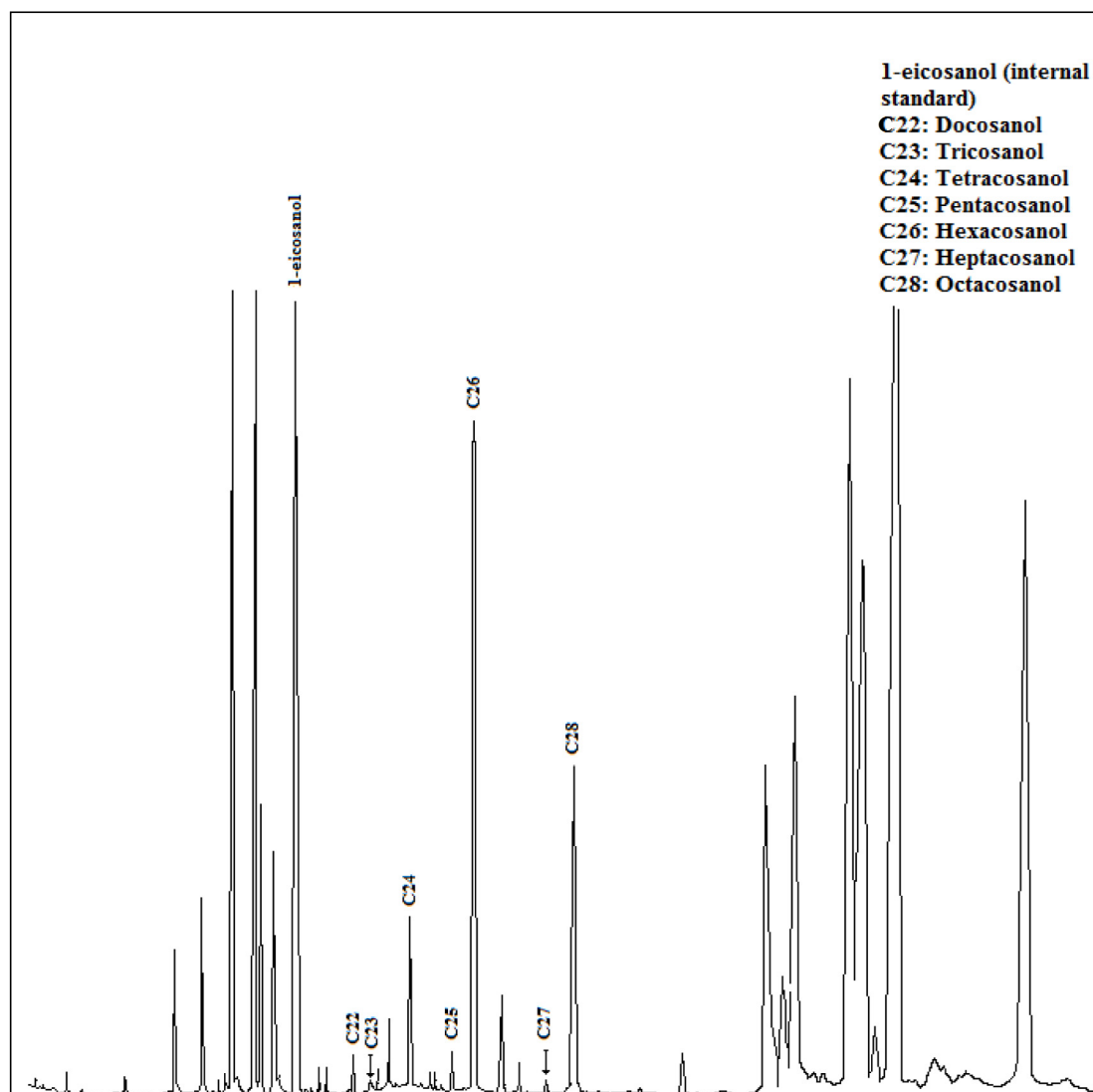
Fig. 3. Chromatogram of the aliphatic alcohol components of one of the *Chemlal* analyzed samples.

in November. During maturity process, the level of C25 decreased in the case of the following cultivars, *Chemlal, Mekki* and *Aghenfas.* We noted a richness of Algerian varieties in C25 content when comparing to the varieties of southern Italy, in fact, Giuffrè (2014), in his study showed a maximum content of pentacosanol with a mean of 12.7 mg/kg.

With regard to C23, the analyzed samples showed values raging between 1.1 mg/kg and 9.4 mg/kg. The autochthonous cultivars presented higher contents at the beginning of maturity and then the content of C23 decreased until a little value about 2 and 1mg/kg, except for *Chemlal,* which the content increased in January. These contents are higher than those found by Krichène *et al.* (2010) on Tunisian olive oils (1.3 mg/kg to 5.3 mg/kg).

Heptacosanol (C27) also showed a significant difference in relation to maturity and variety ($P < 0.0001$). The contents ranged between 0.5 mg/kg to 9.4 mg/kg. In almost cultivars, heptacosanol tend to decrease during maturity process except, the *Buichret* variety, in which after a slight decrease in contents levels occur until the end of November, we observed an increase in the three last sampling.

3.5 Discriminant analysis

In order to understand which variables have a discriminating power among the four varieties, a multivariable statistical approach was applied. First we used an ANOVA test on all data, related to sterol compounds and aliphatic alcohols. The analysis of variance (ANOVA) highlighted statistically significant differences ($P < 0.01$) of all the variables, except, the stigmasterol, the β-sitosterol and the Δ7-stigmastenol. However, despite the recorded differences, after applying a post hoc test (honestly significantly different [HSD] for unequal N tukey) on the obtained results, it was found that none of the used variables could perfectly classify the olive oils samples as regards to variety.

For that and in order to get a better overview of the ability of the whole set of variables to differentiate between varieties a PCA was applied. It was found that 76% of total variance was explained by four components (PCs), taking into consideration the eigenevalues and internal consistency by means of varimax rotation (Tab. 4). Selected PCs are usually discussed in terms of component scores and loadings. The four first components,

Table 4. Loadings, eigenvalues, and percent of cumulative variance for the first four principle components.

	Component			
	1	2	3	4
Alcohol C27	0.896	0.011	−0.093	0.101
Alcohol C25	0.712	0.321	−0.364	0.156
Campestanol	−0.694	−0.027	−0.280	−0.078
Alcohol C24	0.675	0.396	−0.334	0.165
Alcohol C23	0.674	0.367	0.322	0.335
Clerosterol	0.135	0.773	0.115	−0.115
Δ^7-avenasterol	0.004	0.708	−0.190	0.444
Campesterol	0.344	0.707	−0.149	−0.274
Alcohol C22	0.217	−0.165	0.883	0.152
Cholesterol	−0.269	0.074	0.871	−0.042
Δ^7-stigmastenol	0.067	−0.194	0.164	0.885
Erythrodiol and uvaol	0.559	0.258	−187	0.568
Eigenvalues	4.33	2.17	1.42	1.18
% Cumulative variance	36.09	54.19	66.02	75.93

Table 5. Standardized canonical discriminant function coefficients.

	Function		
	1	2	3
Cholesterol	−0.254	0.817	0.016
Campestanol	0.207	0.301	−0.530
Δ^7-stigmastenol	−0.101	0.308	0.834
Δ^7-avenasterol	0.412	0.505	0.157
Clerosterol	0.011	0.324	−0.486
Alcohol C22	−0.594	−0.316	−0.108
Alcohol C25	1.185	0.184	0.011
Alcohol C27	−0.495	−0.398	0.167

called PC1 to PC4 explain respectively, PC1 (27.31%), PC2 (17.75%), PC3 (17.56%) and PC4 (13.31%) of the total variance.

The rotated component matrix showed that the PC1 is correlated to Campestanol, alcohols C23, C24, C25 and C27; PC2 is correlated to clerosterol, Δ^7-avenasterol and campesterol. PC3 is correlated to alcohol C22 and cholesterol, PC4 is correlated to erythrodiol and uvaol and Δ^7-stigmastenol.

The applied PCA on the variables was not sufficient to visualize possible groups between the studied olive oil varieties when the scores of olive samples displayed with respect to the three first components

As a consequence, a LDA was applied on the data as a suitable method to discriminate the olive of samples on the basis of the variety. To apply LDA, it was necessary to reduce the number of variables for the model, therefore we took into consideration only the variables with $P < 0.01$ which showed significant differences between varieties.

The results showed that three statistically discriminant functions are formed (Wilk's lambda = 0.034, χ^2 = 300.535, df = 24 for the first, Wilk's lambda = 0.195, χ^2 = 145.51, df = 14 for the second and Wilk's lambda = 0.488, χ^2 = 63.93, df = 6 for the third with P = 0.000 in all cases). The first discriminant function accounts for 69.8% of the total variance, the second accounts for 20.7% of the total variance while the third accounts for 9.5% of the total variance. All three accounts for 100% of the total variance. The variables selected by the SPSS algorithm, and the corresponding model standardized coefficients, showing the predictors with large discriminant capabilities are given in Table 5. The most discriminating variables have been: cholesterol, campestanol, Δ^7-stigmastenol, Δ^7-avenasterol, clerosterol, alcohol C22, alcohol C25, alcool C27.

Figure 4 showed a projection of the score corresponding to olive oil samples in the two-dimensional space defined by the first and second discriminant functions. As it is demonstrated, the VOOs from the four varieties are adequately separated. The

olive oil of *Chemlal*, *Aghenfas* and *Buichret* are clearly separated while that of *Mekki* variety is adequately separated. The overall correct classification rate is 96.5% using the original and 88.6% using the cross validation method. Correct classification (100%) was obtained for *Chemlal*, *Aghenfas* and *Buichret* olive oil varieties. However, *Mekki* is not perfectly separated from *Aghenfas* and *Buichret* which explains the 88.2% of correct classifications for *Mekki* cultivar.

These results are in agreement with data found in the literature; the content of sterols and alcohols in olive oils have been used for the classification of olive oils on the basis of cultivars (Lukić *et al.*, 2013; Grati-Kammoun and Zarrouk, 2012).

The obtained results showed that the cloud point of *Chemlal* variety, have a negative score on the first function and positive score for the second function, means that the oils from *Chemlal* variety were caracterised by high value of cholesterol, clerosterol, Δ^7-stigmastenol, alcohol C22, and alcohol C27. In the contrary, the cloud point of *Aghenfas* variety, have a positive score on the first function and positive score for the second function, means that the oils of *Aghenfas* were caracterised by high cholesterol, campestanol, Δ^7-stigmastenol, Δ^7-avenasterol, clerosterol and alcohol C25.

Buichret have a negative score on the first and second functions, so the oils of *Buichret* variety were characterized by high C22 and C27 values.

4 Conclusion

In general, the values of quality parameters (free acidity, peroxide value and spectrophotometric absorption K232 and K270) and those of sterolic and alcoholic compounds of four monovarietal Algerian olive oils (*Aghenfas, Buichret, Chemlal* and *Mekki*) remained within estimated limits of the International Olive Oil Council's regulation for "edible virgin olive oil" along the maturation process, which should be taken into consideration in commercial dealings and regulatory studies.

Furthermore, it has been proven that the cultivar and the ripening stage had a significant effect on the trend of several sterolic and alcoholic contents during ripening process and the results showed a decreasing level of total sterols, β-sitosterol, triterpenic dialcohols, total aliphatic alcohols, tetracosanol (C24), and an increasing level of Δ^5-avenasterol according to the maturity process.

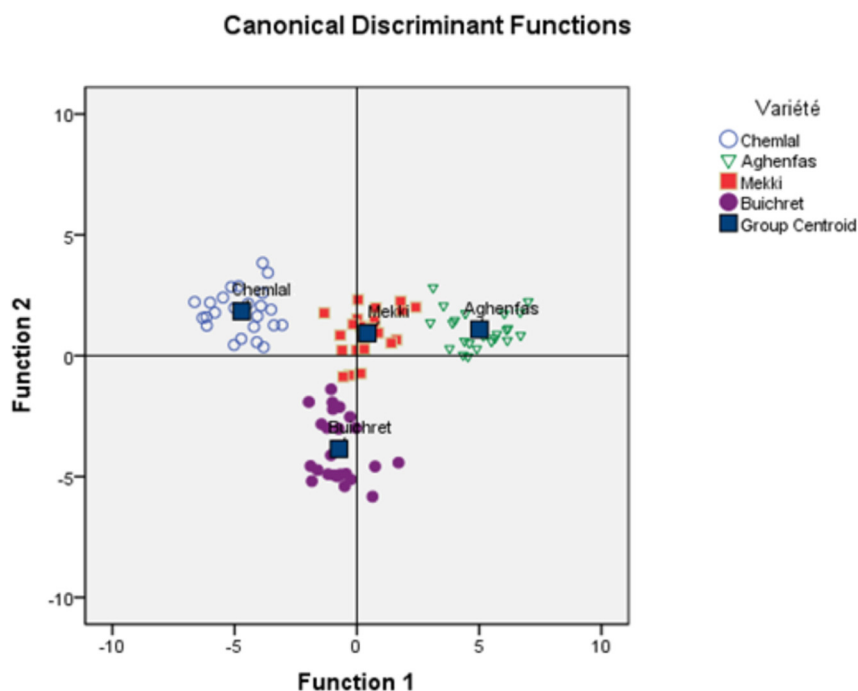## Canonical Discriminant Functions

Fig. 4. A graphical representation of a discriminant function analysis on sterolic and alcoholic compounds of the four varieties: *Aghenfas*, *Buichret*, *Chemlal* and *Mekki*.

Principal component analysis (PCA) on the compositional data was not effective in visualizing possible groups of olive oil varieties. While, LDA showed that the cultivars can be adequately distinguished on the basis of sterolic and alcoholic compositions. The results of this study provide important data, which can be employed in olive oil authenticity studies in Algeria. Finally, additional analysis should be performed for a better characterization of Algerian olive heritage.

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