

Sterolic composition of Chétoui virgin olive oil: Influence of geographical origin

Sonia Ben Temime^a, Hedia Manai^{a,b}, Kaouther Methenni^a, Bechir Baccouri^a, Leila Abaza^a,
Douja Daoud^a, Jacinto Sánchez Casas^b, Emilio Osorio Bueno^b, Mokhtar Zarrouk^{a,*}

^a *Laboratoire Caractérisation et Qualité de l'Huile d'Olive, Centre de Biotechnologie, Technopole de Borj-Cedria, BP 901, Hammam-Lif 2050, Tunisia*

^b *Instituto Tecnológico Agroalimentario, Dirección General de Infraestructuras y Desarrollo Tecnológico, Junta de Extremadura, Carretera de Cáceres s/n, 06071 Badajoz, Spain*

Received 9 March 2007; received in revised form 6 December 2007; accepted 7 February 2008

Abstract

The sterol profile of Tunisian virgin olive oils produced from Chétoui cultivar, the second main variety cultivated in the north of the country, grown under different environmental conditions, was established by gas chromatography using a flame ionisation detector. More than ten compounds were identified and characterised. As expected for virgin olive oil, the main sterols found in all Chétoui olive oils were β -sitosterol, $\Delta 5$ -avenasterol, campesterol and stigmasterol. Cholesterol, 24-methylenecholesterol, clerosterol, campestanol, sitostanol, $\Delta 7$ -stigmastenol, $\Delta 5,24$ -stigmastadienol, and $\Delta 7$ -avenasterol were also found in all samples, but in lower amounts. Most of these compounds are significantly affected by the geographical origin. The majority of the Chétoui virgin olive oils analysed respected EC Regulation No. 2568, and in all cases total sterol amounts were higher than the minimum limit set by legislation, ranging from 1017 to 1522 mg/kg.

Two triterpenic dialcohols (erythrodiol and uvaol), were also detected besides the sterolic components. Their content was below the upper legal limit of 4% in all analysed samples, with a range from 1.2% to 3.2%. These results suggest that, besides the genetic factor, environmental conditions influence the sterolic fraction.

© 2008 Elsevier Ltd. All rights reserved.

Keywords: Virgin olive oil; Chétoui cultivar; Sterols; Erythrodiol; Uvaol; Production area

1. Introduction

Virgin olive oil has with excellent nutritional, sensorial and functional qualities (Matos et al., 2007), and is a product of major economical importance in the Mediterranean area. Due to its fatty acid composition and content of other functional food components, interest in olive oil as a healthy food source has also increased outside the Mediterranean region (Bandelj, Jakše, & Javornik, 2002). Since the production of olive oil is much lower than demand, there is a need to improve olive cultivation, both to produce more oil and to enhance its quality, particularly with regard to components beneficial to human health, such as natural

antioxidants and sterols (Cercaci, Passalacqua, Poerio, Rodriguez-Estrada, & Lercker, 2007).

Plant sterols, also called phytosterols, make up the greatest proportion of the unsaponifiable fraction of lipids. Research has shown that each oily fruit has a characteristic sterol profile which makes its determination an important tool for detecting adulteration or checking the genuineness of an oil (Gutiérrez, Varona, & Albi, 2000; Mariani, Bellan, Morchio, & Pellegrino, 1999; Salvador, Aranda, & Fragapane, 1998; Vichi, Pizzale, Toffano, Bortolomeazzi, & Conte, 2001). Recently, it has also been proposed that these profiles could be used to classify virgin olive oils according to their fruit variety (Bucci, Magri, Magri, Marini, & Marini, 2002; Cañabate-Díaz et al., 2007; Ranalli et al., 2002). Additionally, it has been suggested that phytosterols have anti-inflammatory,

* Corresponding author. Tel./fax: +216 79 412 948.

E-mail address: mokhtar.zarrouk@cbbc.rnrt.tn (M. Zarrouk).

antibacterial, antifungal, antiulcerative and antitumoral activities (Williamson, 1988). Sterols have also been recognised as cancer-preventive biologically-active substances, together with other secondary plant products. Also, they apparently help to reduce total plasma cholesterol and LDL cholesterol, and as a result these compounds are being considered as ingredients of functional foods (Ostlund, 2002).

Several factors are known to affect the quantitative sterolic profiles of olive fruits. Among these factors, the ripening cycle of the fruit and the nature of the cultivar (Gutiérrez, Jimenez, Ruíz, & Albi, 1999; Hajana, El Antari, & Hafifi, 1998; Koutsaftakis, Kotsifaki, Stefanoudaki, & Cert, 2000), oil extraction and refining procedures and storage conditions (Gracia, 2001; Gutiérrez et al., 2000; Koutsaftakis, Kotsifaki, & Stefanoudaki, 1999; Määttä et al., 1999; Pasqualone & Catalano, 2000; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000). The effects of agronomic and climatic conditions have also been studied (El Antari, Hilal, Boulouha, & El Moudni, 2000; Stefanoudaki, Chartzoulakis, Koutsaftakis, & Kotsifaki, 2001). However, the total effect of these variables on sterol profiles is ambiguous. The diversity of these factors and their interrelationships make it very difficult to completely characterise the sterol profile and erythrodiol+uvaol content of a given product (Sánchez Casas, Osorio Bueno, Montaña García, & Martínez Cano, 2004).

The Chétoui variety used in this study is the second main cultivar cultivated in the north of Tunisia. This variety covers an area of 176,000 Ha and accounts for more than 20% of the olive oil produced in Tunisia. Its growing area is located mainly in the north of the country; in the provinces of Amdoun, Testour, Slouguia, Chuigui, Borj El Amri, and some areas of Siliana (Gâafour, Lakhouet, Bou Arada) and El Kef (Sers, Elles); where the annual rainfall varies between 400 and 800 mm (sub-humid (600–800 mm), semi-arid (400–600 mm)). As well as rainfall, temperature and especially winter temperature is also important. This is not only governed by altitude but by proximity to the sea; inland locations have relatively hotter summers and colder winters than areas which benefit from the buffering effects of the sea. Moreover, Chétoui is a vigorous cultivar, considered late to produce and high in yield. The fruit is medium to large with a characteristically elongated and asymmetric shape, and has dual use as table olives and for oil. The lipid yield is about 20–30% of fresh weight and the oil has a balanced composition, with a medium oxidative stability (Abaza, Taamalli, Ben Temime, Daoud, & Zarrouk, 2005), and its sensorial characteristics are much appreciated by consumers (Manai et al., 2007). However, few works have characterised its sterol composition (Stiti, Msallem, Triki, & Cherif, 2002).

Despite, the economic importance of Tunisian Chétoui olive oil, until recent years the scientific literature contained no complete and reliable data on its chemical composition and properties. Because of the importance of this cultivar for oil production and the need to improve knowledge of

sterols, the aim of our present work was therefore, first to study the sterolic fraction obtained from Chétoui virgin olive oils, and then to determine changes due exclusively to environmental factors. Moreover, the present work aimed to complete the characterisation of this variety, which was started by studying the effect of growing area on the chemical composition and volatile compounds of Chétoui virgin olive oils (Ben Temime et al., 2006; Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006). The work was done in order to enable these oils to become eligible for protection under a designation of origin (DO). This label both guarantees that the product complies with quality requirements and that its unique character is linked to the geographical and climatic characteristics of the area, where the product is traditionally produced.

2. Materials and methods

2.1. Sampling

Olive fruits (*Olea europaea*) of the Chétoui variety were collected, at the mature stage, from ten distinct farms in the north of Tunisia with different pedoclimatic characteristics, during crop seasons 2003/2004 and 2004/2005. The olives were picked by hand from three trees at each location. Only undamaged drupes, which were fresh and healthy, were selected. From all olive trees of each region, a representative 5 kg sample was collected. After harvesting, the olive fruit samples were immediately transported to the laboratory mill where they were transformed into oil within 24 h.

2.2. Oil extraction

An Abencor analyser (Commercial Abengoa SA, Sevilla, Spain) was used to process the olives in a pilot extraction plant. The unit consists of three essential elements: the mill, the thermo-mixer, and the pulp centrifuge. After being processed in the mill, the oil was separated by decanting, transferred into dark glass bottles, and stored in the dark at 4 °C until analysis.

2.3. Analytical method

The qualitative and quantitative sterol contents of the samples were determined according to the European Official Analysis Methods, described in Annexes V and VI of Regulation EEC/2568/91 of the European Union Commission. The determination gave sterols expressed in ppm total sterols only and% individual sterols. The oil sample was saponified with ethanolic potassium hydroxide solution. The unsaponifiable fraction was removed with ethyl ether. The unsaponifiable sterol fraction was separated by chromatography on silica gel plate. Separation and quantification of the silanised sterol fraction was carried out by capillary gas chromatography, on a Hewlett Packard 6890 chromatograph with autosampler and flame ionisation detector (FID) using an HP-5MS capillary column

(30 m × 0.25 mm × 0.25 μm). The working conditions of the chromatograph were: injector 300 °C, isothermal column 260 °C, and detector 325 °C. The injected quantity was 0.2 μl, at a flow rate of 1.1 ml/min, using helium as carrier gas. Quantification was made by addition of an internal standard (α -cholestanol) and apparent β -sitosterol was calculated as the sum of β -sitosterol, $\Delta 5,23$ -stigmastadienol, clerosterol, sitostanol and $\Delta 5,24$ -stigmastadienol. Sterols peak identification was carried out according to the reference method. Fig. 1 shows a chromatogram obtained for one of the samples.

2.4. Statistical analysis

The assays were carried out in triplicate, yielding a total of nine samples from every location. The results are shown as the mean values and standard deviations. Significant differences among sites were determined by an analysis of variance, which applied a Duncan's multiple test. Differences were considered statistically significant when probability was greater than 99% ($p < 0.01$). The statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, 2004).

3. Results and discussion

3.1. Sterol composition

The composition of the sterol fraction of olive oil is a very useful parameter for detecting adulterations or to check authenticity, since it can be considered as a fingerprint (Cert, Moreda, & García-Moreno, 1997; Vichi et al., 2001). Besides, their determination is of major interest due to their health benefits, as discussed before. Table 1 lists the sterol levels obtained for the different virgin olive oils analysed. One observes that in general they lie within the established regulatory limits (CEE Regulation 2568, 1991). The fulfilment of the limits established for the different types of olive oil (CEE Regulation 2568, 1991) were checked, as well as any relationship between the sterols that might contribute to the quality of the oil.

As shown in Table 1, the amounts of individual sterols varied according to the production area. In the Chétoui variety, the highest phytosterol levels were found for β -sitosterol, followed by $\Delta 5$ -avenasterol, characteristic of the virgin olive oil in the pulp of the olive (Cornforth, 2002). These two major sterols were strongly and negatively correlated, and there was a clear differentiation between sites (Table 1). The other main sterols were stigmasterol, and campesterol. However, small amounts of cholesterol, 24-methylenecholesterol, clerosterol, campestanol, sitostanol, $\Delta 5,24$ -stigmastadienol, $\Delta 7$ -stigmastenol, and $\Delta 7$ -avenasterol were also found in all samples. These results are in good agreement with data published elsewhere (Alves, Cunha, Amaral, Pereira, & Oliveira, 2005; Rivera del Álamo, Fregapane, Aranda, Gómez-Alonso, & Salvador, 2004; Sánchez Casas et al., 2004). Authors have reported that β -sitosterol, $\Delta 5$ -avenasterol and campesterol were the most representative sterols in virgin olive oils from the main Spanish and Portuguese cultivars.

β -sitosterol, the most abundant phytosterol in olive oil, its level represents more than 75% of total sterols. The highest mean per cent value of β -sitosterol was observed in Testour oil (84.4%), whereas Sers oil had the lowest one (76.8%) (Table 1). These values are similar to those reported for other olive oil varieties (Pardo, Cuesta, & Alvarruiz, 2007; Rivera del Álamo et al., 2004). According to Gutiérrez et al. (1999), the content of β -sitosterol generally decreases during ripening, while $\Delta 5$ -avenasterol increases. Other authors reported that β -sitosterol is minimal and $\Delta 5$ -avenasterol is maximal when olives are harvested at their optimum (Koutsaftakis et al., 1999). The health aspects of β -sitosterol have recently been reported in several studies (Awad, Chan, Downie, & Fink, 2000; Awad, Chen, Fink, & Hennessey, 1996). They refer mainly to the reduction of cholesterol levels by opposing its absorption in the intestinal tract and the prevention of many diseases and various types of cancer (colon, prostate, and breast).

Regarding $\Delta 5$ -avenasterol content, Sers virgin olive oil showed the highest value (16.02%), whereas Chétoui virgin olive oil extracted from Testour recorded the lowest one

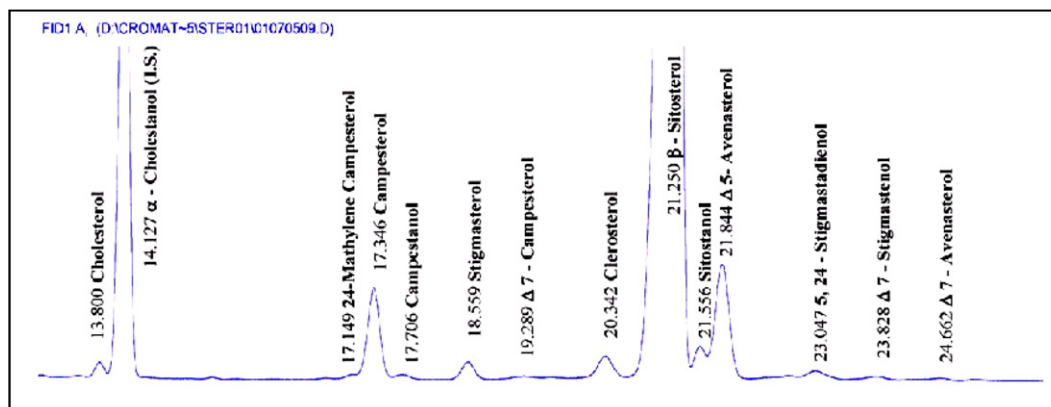


Fig. 1. Chromatogram of the sterol components of one of the analysed samples.

Table 1
Sterol composition (%) of fresh virgin olive oils from Chétoui cultivar grown in ten different geographical areas

Sterols	Amdoun	Testour	Bou Arada	Lakhouet	Gâafour	Chuigui	Slouguia	Elles	Sers	Borj El Amri
Cholesterol ^A	0.13 ^a ± 0.02	0.14 ^a ± 0.02	0.10 ^a ± 0.02	0.10 ^b ± 0.00	0.11 ^a ± 0.00	0.17 ^a ± 0.01	0.11 ^a ± 0.02	0.15 ^a ± 0.02	0.15 ^a ± 0.02	0.24 ^b ± 0.01
24-Methylencholesterol ^A	0.22 ^{bc} ± 0.00	0.10 ^a ± 0.02	0.22 ^{bc} ± 0.07	0.18 ^{abc} ± 0.00	0.12 ^{ab} ± 0.01	0.25 ^c ± 0.01	0.20 ^{bc} ± 0.00	0.28 ^c ± 0.00	0.22 ^{bc} ± 0.00	0.14 ^{ab} ± 0.02
Campesterol ^A	2.66 ^{abc} ± 0.01	2.84 ^c ± 0.03	2.45 ^b ± 0.01	2.68 ^{bc} ± 0.11	2.77 ^{bc} ± 0.00	2.72 ^{abc} ± 0.00	2.70 ^{abc} ± 0.01	2.64 ^{abc} ± 0.00	2.47 ^{ab} ± 0.03	2.66 ^{abc} ± 0.23
Campestanol	0.10 ^a ± 0.00	0.09 ^a ± 0.00	0.07 ^a ± 0.00	0.09 ^a ± 0.01	0.08 ^a ± 0.00	0.10 ^a ± 0.00	0.07 ^a ± 0.00	0.08 ^a ± 0.01	0.10 ^a ± 0.00	0.14 ^b ± 0.02
Stigmasterol ^A	1.17 ^{ab} ± 0.00	3.41 ^c ± 0.01	4.93 ^g ± 0.00	3.89 ^f ± 0.02	2.91 ^d ± 0.06	1.10 ^a ± 0.02	4.07 ^f ± 0.05	1.43 ^{bc} ± 0.11	1.61 ^c ± 0.01	3.36 ^c ± 0.21
Clerosterol	1.04 ^a ± 0.01	1.02 ^a ± 0.00	1.09 ^a ± 0.18	0.99 ^a ± 0.03	1.10 ^a ± 0.12	1.10 ^a ± 0.07	1.06 ^a ± 0.03	0.98 ^a ± 0.06	0.92 ^a ± 0.00	0.99 ^a ± 0.04
β-Sitosterol	81.50 ^e ± 0.07	84.40 ^g ± 0.12	79.00 ^b ± 0.06	80.43 ^{cd} ± 0.17	83.22 ^f ± 0.07	79.64 ^{ab} ± 0.03	81.12 ^{de} ± 0.20	77.53 ^a ± 0.57	76.80 ^a ± 0.03	83.15 ^g ± 0.70
Sitostanol	0.67 ^c ± 0.02	0.45 ^{ab} ± 0.02	0.35 ^a ± 0.01	0.40 ^{ab} ± 0.04	0.55 ^{bc} ± 0.02	0.47 ^{ab} ± 0.10	0.38 ^a ± 0.01	0.36 ^a ± 0.01	0.33 ^a ± 0.02	0.41 ^{ab} ± 0.06
Δ5-Avenasterol	11.15 ^c ± 0.02	6.31 ^a ± 0.03	10.62 ^{cd} ± 0.14	9.88 ^{cd} ± 0.21	7.97 ^b ± 0.03	12.95 ^f ± 0.12	9.23 ^c ± 0.20	15.45 ^g ± 0.69	16.02 ^g ± 0.00	7.52 ^b ± 0.17
Δ5,24-Stigmastadienol	0.78 ^{cd} ± 0.09	0.68 ^{abc} ± 0.05	0.70 ^{abc} ± 0.03	0.66 ^{abc} ± 0.01	0.60 ^{ab} ± 0.00	0.86 ^d ± 0.04	0.63 ^{abc} ± 0.03	0.65 ^{abc} ± 0.04	0.75 ^{bcd} ± 0.00	0.56 ^a ± 0.07
Δ7-Stigmastanol ^A	0.16 ^{abcd} ± 0.00	0.24 ^d ± 0.07	0.13 ^{abc} ± 0.00	0.23 ^d ± 0.00	0.21 ^{cd} ± 0.00	0.17 ^{abcd} ± 0.00	0.12 ^{ab} ± 0.02	0.09 ^a ± 0.00	0.13 ^{abc} ± 0.04	0.19 ^{bcd} ± 0.02
Δ7-Avenasterol	0.42 ^c ± 0.00	0.31 ^b ± 0.00	0.34 ^b ± 0.00	0.43 ^c ± 0.01	0.32 ^b ± 0.00	0.44 ^c ± 0.00	0.30 ^b ± 0.00	0.34 ^b ± 0.03	0.49 ^d ± 0.00	0.23 ^a ± 0.02
Apparent β-Sitosterol ^A	95.12 ^h ± 0.05	92.85 ^d ± 0.03	91.77 ^h ± 0.04	92.38 ^b ± 0.06	93.45 ^e ± 0.04	95.05 ^{gh} ± 0.01	92.42 ^b ± 0.03	94.99 ^g ± 0.00	94.81 ^f ± 0.00	92.71 ^e ± 0.00

Values are mean ± SD ($n = 9$).

Values followed by same letters are not significantly different ($p < 0.05$).

^A Limits established by the current European Legislation: cholesterol ≤ 0.5%; campesterol ≤ 4.0%; stigmasterol ≤ 0.5%; Δ7-stigmastanol ≤ 0.5%; apparent β-sitosterol ≥ 93%.

(6.31%) (Table 1). Significant differences were observed in the Δ5-avenasterol content in relation to the geographical area ($p < 0.01$). In the literature this compound has been associated with antioxidant activity (Williamson, 1988).

Stigmasterol is related to various parameters of the quality of virgin olive oil. High levels correlate with high acidity and low organoleptic quality (Gracia, 2001; Gutiérrez et al., 1999). The analysed samples contained low levels of this sterol, which is indicative that the oil came from healthy fruit (Koutsaftakis et al., 1999). The stigmasterol content is significantly affected by the geographical origin.

All the olive oil samples analysed showed low campesterol content, with a global range from 2.45% (Bou Arada) to 2.84% (Testour). Campesterol content was below the threshold established by EU Regulations (4%) in all of the oils studied, indicating a peculiarity of this olive oil variety. Practically no statistically significant differences were observed in the campesterol content in relation to the geographical area. These results are in agreement with others (Salvador, Aranda, Gomez-Alonso, & Fregapane, 2003). The campesterol/stigmasterol ratio has been reported as a quality index of an oil (Ranalli & Angerosa, 1996). As compared to the virgin olive oil of Cornicabra variety, the main cultivar grown in Castilla La-Mancha (Spain), the Chétoui cultivar produced oils with a low level of campesterol of around 3%. The sterol composition of Cornicabra virgin olive oil was characterised by high campesterol content, almost always higher than the limit of 4% established by EEC Regulations. This is a peculiar characteristic of this variety (Rivera del Álamo et al., 2004; Salvador et al., 1998).

Stiti et al. (2002) studied the sterolic composition of seven Tunisian cultivars (Chemchali, Chemlali, Chétoui, Gerboui, Ouelati, Sayali, and Zalmati) and observed that β-sitosterol was the major sterol, with a percentage range from 74.8% to 88.7%, followed by Δ5-avenasterol (4.1–19%) and campesterol (1.9–2.9%). The studied Tunisian cultivars showed very low amounts of campesterol when compared with those from other studied cultivars, namely the Spanish ones studied by Rivera del Álamo et al. (2004) and the Portuguese ones studied by Alves et al. (2005).

The levels of Δ5,24-stigmastadienol were relatively low in all studied virgin olive oils and ranged from 0.56% (Borj El Amri) to 0.86% (Chuigui). The highest sitostanol content was found in Amdoun oil (0.67%), whereas Chétoui virgin olive oil extracted from Sers recorded the lowest (0.33%) (Table 1). Δ7-Stigmastanol content in Elles oil was significantly lower than in the other Chétoui virgin olive oils. The campestanol and clerosterol contents were not useful for discriminating among sites. The highest Δ7-avenasterol content was observed in Sers oil (0.49%), while virgin olive oil produced from Borj El Amri showed the lowest (0.23%) (Table 1).

In the case of apparent β-sitosterol, expressed as the sum of the contents of β-sitosterol and four other sterols formed by the degradation of β-sitosterol (sitostanol,

$\Delta 5,24$ -stigmastadienol, clerosterol and $\Delta 5$ -avenasterol), the majority of the samples analysed contained more than the established limit of 93%. This is the regulatory minimum limit, indicating that the sum of the remaining sterols does

not surpass 7%, thereby confirming the authenticity of the corresponding oil (Sánchez Casas et al., 2004).

All of the olive oil samples studied contained more than 1000 mg/kg of total sterols, the minimum value established by EU Regulations (EEC, 2003), for the category “extra virgin” olive oil. There were, however, major differences between sites, from the lowest level in Chuigui (1017 mg/kg) to the highest in Bou Arada (1522 mg/kg) (Fig. 2). This is undoubtedly a good characteristic of olive oils, due to the great benefits of these compounds for health, as referred before. These findings are in good agreement with those of other authors working on Tunisian olive oil varieties (Stiti et al., 2002). They reported that the content of total sterols in seven monovarietal Tunisian oils (Chemchali, Chemlali, Chétoui, Gerboui, Ouelati, sayali, and Zalmati) varied between 1082 and 2017 mg/kg; these values were low compared with those from Portuguese oils studied by Alves et al. (2005) who found values ranging from 2003 to 2682 mg/kg.

As regards other authenticity indices established by the current legislation (EEC, 2003), cholesterol percentages were below the established limits of 0.5% and the percentages of stigmasterol were lower than those of campesterol in about 50% of the samples analysed (Table 1).

3.2. Erythrodiol and uvaol content

The triterpenic dialcohols (erythrodiol and uvaol), which are also part of the unsaponifiable fraction of the olive oil, are usually analysed together with the sterol fraction. With respect to the levels of erythrodiol+uvaol, there were clear differences between analysed oils (Table 2). The sum of erythrodiol and uvaol in all studied samples was below the established limit of 4.5% for the “extra virgin” olive oil category. These results are consistent with the findings of other authors (Pardo et al., 2007; Sánchez Casas et al., 2004; Stiti et al., 2002). Among the studied samples, Amdoun virgin olive oil had the highest values

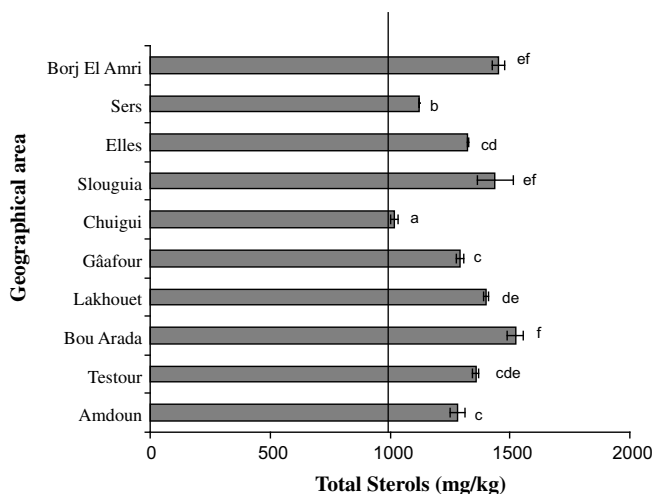


Fig. 2. Total sterol contents of studied olive oil samples.

Table 2
Triterpenic dialcohols amounts for studied olive oil samples

Sites of plantation	Erythrodiol (%)	Uvaol (%)	Erythrodiol+Uvaol (%)
Amdoun	2.41 ^f ± 0.04	0.79 ^c ± 0.00	3.20 ^e ± 0.04
Testour	1.92 ^e ± 0.01	0.63 ^{bc} ± 0.00	2.55 ^c ± 0.00
Bou Arada	1.65 ^{cd} ± 0.09	0.38 ^a ± 0.02	2.04 ^b ± 0.07
Lakhouet	2.36 ^f ± 0.00	0.53 ^{ab} ± 0.00	2.89 ^d ± 0.00
Gâafour	1.73 ^d ± 0.00	0.63 ^{bc} ± 0.00	2.36 ^c ± 0.00
Chuigui	1.44 ^b ± 0.12	0.63 ^{bc} ± 0.03	2.07 ^b ± 0.09
Slouguia	1.45 ^b ± 0.03	0.43 ^a ± 0.00	1.88 ^b ± 0.04
Elles	0.85 ^a ± 0.02	0.37 ^a ± 0.00	1.23 ^a ± 0.01
Sers	1.59 ^{bcd} ± 0.03	0.43 ^a ± 0.09	2.02 ^b ± 0.14
Borj El Amri	1.53 ^{bc} ± 0.07	0.43 ^a ± 0.08	1.96 ^b ± 0.15

Mean ± SD (n = 9).

Values in the same columns followed by same letters are not significantly different (p < 0.05).

Dendrogram using Average Linkage (Within Group)

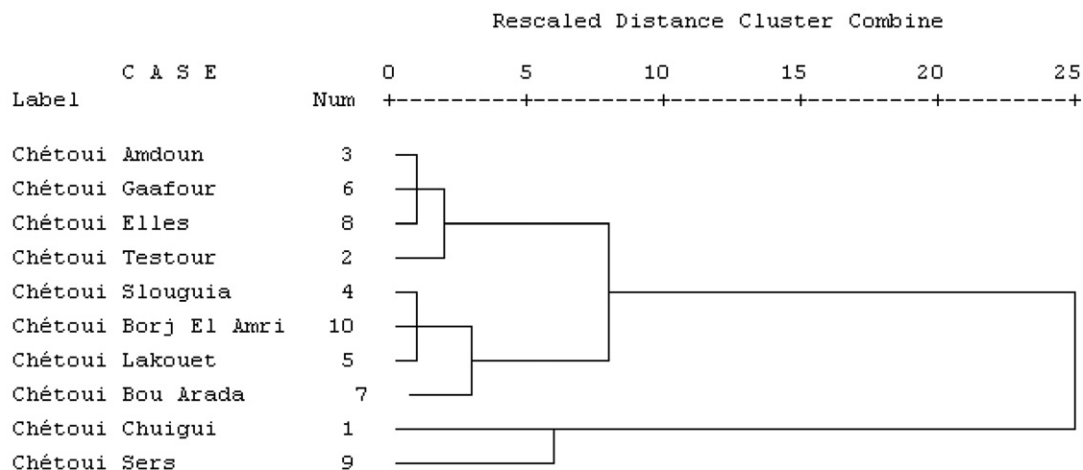


Fig. 3. Cluster analysis of sterolic fraction for studied olive oils.

of erythrodiol and uvaol (2.41% and 0.79%, respectively), whereas virgin olive oil extracted from Elles recorded the lowest (0.85% and 0.37%, respectively); (Table 2). These dialcohols are generally located in the exocarp of the olive (Christopoulou, Lazaraki, Alexiou, Synouri, & Frangis-Cos, 1996). Several workers have described the use of an olive oil's sterol profile to detect possible fraudulent admixtures with lower value fats. The presence of olive pomace in virgin oil can be detected from the levels of erythrodiol+uvaol (Reina, White, & Jahngen, 1997). Total erythrodiol and uvaol have been proposed as applicable to the characterisation of olive oils, since the sterol profile differs from one variety to another.

3.3. Statistical analysis

All collected data were submitted to a classification test using a cluster analysis. The associations obtained based on the similarity in Euclidian distances are shown as a dendrogram in Fig. 3. The dendrogram indicates that, at rescaled distance of twentyfour, the samples are distributed in two major clusters. While, at rescaled distance of seven, there are three groups; the first is constituted by four oil samples which are Amdoun, Gàafour, Testour and Elles. The second group is formed by four oil samples: Slouguia, Borj El Amri, Lakhouet and Bou Arada. Finally, the third group contained the remaining samples, which are Chuigui and Sers.

4. Conclusion

As was demonstrated, the growing area had a significant effect on the percent composition of the sterols present in the olive oils produced from Chétoui cultivar with the same processing technology. Among the studied samples, Testour oil stands out, due to its high β -sitosterol and campesterol contents, and low Δ^5 -avenasterol content; and Sers oil, due to its low β -sitosterol and high Δ^5 -avenasterol contents.

The results show that total sterols, β -sitosterol, Δ^5 -avenasterol, stigmasterol, and the sum of erythrodiol and uvaol are the most important variables for characterising the Chétoui olive oils, according to their geographical origin. As the harvesting period and extraction conditions were similar for all studied samples, the results indicate that besides genetic factors, environmental conditions influence the sterolic fraction. The study of a large number of samples from various years of production would hopefully corroborate the results obtained by this first screening.

Acknowledgements

This work has been done as a part of a national research project. We thank the Ministry of High Education Scientific Research and Technology for supporting financially this programme.

References

- Abaza, L., Taamalli, W., Ben Temime, S., Daoud, D., & Zarrouk, M. (2005). Natural antioxidant composition as correlated to stability of some Tunisian virgin olive oils. *La Rivista Italiana Delle Sostanze Grasse*, *LXXXII*, 12–17.
- Alves, M. R., Cunha, S. C., Amaral, J. S., Pereira, J. A., & Oliveira, M. B. (2005). Classification of PDO olive oils on the basis of their sterol composition by multivariate analysis. *Analytica Chimica Acta*, *549*, 166–178.
- Awad, A. B., Chan, K. C., Downie, A. C., & Fink, C. S. (2000). Peanuts as a source of β -sitosterol, a sterol with anticancer properties. *Nutrition and Cancer*, *36*, 238–241.
- Awad, A. B., Chen, Y. C., Fink, C. S., & Hennessey, T. (1996). β -sitosterol inhibits HT-29 human cancer colon cell growth and alters membrane lipids. *Anticancer Research*, *16*, 2797–2804.
- Bandelj, D., Jakše, J., & Javornik, B. (2002). DNA Fingerprinting of olive varieties by microsatellite markers. *Food Technology Biotechnology*, *40*(3), 185–190.
- Ben Temime, S., Baccouri, B., Taamalli, W., Abaza, L., Daoud, D., & Zarrouk, M. (2006). Location effects on Chétoui virgin olive oil stability. *Journal of Food Biochemistry*(30), 659–670.
- Ben Temime, S., Campeol, E., Cioni, P. L., Daoud, D., & Zarrouk, M. (2006). Volatile compounds from Chétoui virgin olive oil and variations induced by growing area. *Food Chemistry*, *99*, 315–325.
- Bucci, R., Magri, A. D., Magri, A. L., Marini, D., & Marini, F. (2002). Chemical authentication of extra virgin olive oil varieties by supervised chemometric procedures. *Journal of Agricultural and Food Chemistry*, *50*, 413–418.
- Cañabate-Díaz, B., Segura-Carretero, A., Fernández-Gutiérrez, A., Belmonte Vega, A., Garrido Frenich, A., Martínez-Vidal, J. L., et al. (2007). Separation and determination of sterols in olive oil by HPLC-MS. *Food Chemistry*, *102*, 593–598.
- CEE Regulation 2568. (1991). On the olive oils characteristics and their analytical methods. CEE Official Report L248, 1–48.
- Cercaci, L., Passalacqua, G., Poerio, A., Rodriguez-Estrada, M. T., & Lercer, G. (2007). Composition of total sterols (4-desmethyl-sterols) in extravirgin olive oils obtained with different extraction technologies and their influence on the oil oxidative stability. *Food Chemistry*, *102*, 66–76.
- Cert, A., Moreda, W., & García-Moreno, J. (1997). Determinación de esteroides y alcoholes triterpénicos en aceite de oliva mediante separación de la fracción por cromatografía líquida de alta eficacia y análisis por cromatografía de gases. Estandarización del método analítico. *Grasas y Aceites*, *48*(4), 207–218.
- Christopoulou, E., Lazaraki, M., Alexiou, F., Synouri, S., & Frangis-Cos, E. (1996). Influence of certain factors on the composition of olive-pomace oils. Part II sterol, triterpenic dialcohols and aliphatic alcohols. *La Rivista Italiana Delle Sostanze Grasse*, *LXXXIII*(Maggio), 201–211.
- Cornforth, J. W. (2002). Sterol biosynthesis: The early days. *Biochemical and Biophysical Research Communication*, *292*, 1129–1138.
- EEC (2003). Characteristics of olive and olive pomace oils and their analytical methods. EEC Regulation 1989/2003. *Official Journal of the European Communities*, *295*, 57–66.
- El Antari, A., Hilal, A., Boulouha, B., & El Moudni, A. (2000). Estudio de la influencia de la variedad, los factores ambientales y la técnica de cultivo en las características de los frutos y la composición química del aceite de oliva virgen extra de Marruecos. *Olivae*, *80*, 29–36.
- Gracia, M. S. (2001). Composición química de distintas calidades de aceites de oliva virgen de la variedad «Empetre» en el bajo Aragón. *Grasas y Aceites*, *52*(1), 52–58.
- Gutiérrez, F., Jimenez, B., Ruíz, A., & Albi, A. M. (1999). Effect of Olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and the different compounds involved. *Journal of Agricultural and Food Chemistry*, *47*, 121–127.
- Gutiérrez, F., Varona, I., & Albi, M. A. (2000). Relation of acidity and sensory quality with sterol content of olive oil from stored fruit. *Journal of Agricultural and Food Chemistry*, *48*, 1106–1110.

- Hajana, H., El Antari, A., & Hafifi, A. (1998). Fatty acids, sterol evolution during the ripening of olives from the Moroccan Picholine cultivar. *Grasas y Aceites*, 49(5–6), 405–410.
- Koutsaftakis, A., Kotsifaki, F., & Stefanoudaki, E. (1999). Effect of extraction system, stage of ripeness, and kneading temperature on the sterol composition of virgin olive oils. *JAOCs*, 76(12), 1477–1481.
- Koutsaftakis, A., Kotsifaki, F., Stefanoudaki, E., & Cert, A. (2000). Estudios sobre las variaciones de determinados parámetros químicos y de los componentes menores de los aceites de oliva virgen obtenidos de aceitunas recolectadas en distintas fases de maduración. *Oliva*, 80, 22–27.
- Määttä, K., Lampi, A. M., Petterson, J., Fogelfors, B. M., Pironen, V., & Kamal-Eldin, A. (1999). Phytosterol content in seven oat cultivars grown at three locations in Sweden. *Journal of the Science of Food and Agriculture*, 79, 1021–1027.
- Manai, H., Gonzalez, L. G., Haddada, F., Sánchez-Casas, J., Bueno, E. O., & Zarrouk, M. (2007). Características sensoriales de aceites de oliva virgen procedentes de cultivares tunecinos monovarietales e híbridos con variedades europeas. *Grasas Y Aceites*, 58(2), 163–169.
- Mariani, C., Bellan, G., Morchio, G., & Pellegrino, A. (1999). Free and esterified minor components of olive and hazelnut oils: Their potential utilisation in checking oil blend. *Rivista Italiana delle Sostanze Grasse*, 76, 297–305.
- Matos, L. C., Cunha, S. C., Amaral, J. S., Pereira, J. A., Andrade, P. B., Seabra, R. M., et al. (2007). Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. *Food Chemistry*, 102, 406–414.
- Ostlund, R. E. (2002). Phytosterols in human nutrition. *Annual Review of Nutrition*, 22, 533–549.
- Pardo, J. E., Cuesta, M. A., & Alvarruiz, A. (2007). Evaluation of potential and real quality of virgin olive oil from the designation of origin 'Aceite Campo de Montiel' (Ciudad Real, Spain). *Food Chemistry*, 100, 977–984.
- Pasqualone, A., & Catalano, M. (2000). Free and total sterols in olive oils. Effects of neutralization. *Grasas y Aceites*, 80, 939–966.
- Piironen, V., Lindsay, D., Miettinen, T., Toivo, J., & Lampi, A. M. (2000). Plant sterols: Biosynthesis, biological function and their importance to human nutrition. *Journal of the Science of Food and Agriculture*, 79, 1021–1027.
- Ranalli, A., & Angerosa, F. (1996). Integral centrifuges for olive oil extraction—The qualitative characteristics of product. *Journal of the American Oil Chemists' Society*, 73, 417–422.
- Ranalli, A., Pollastri, L., Contento, S., Di Loreto, G., Iannucci, E., Lucera, L., et al. (2002). Sterol and alcohol components of seeds, pulp, and whole olive fruit oils. Their use to characterise olive fruit variety by multivariate. *Journal of the Science of Food and Agriculture*, 82, 854–859.
- Reina, R. J., White, K. D., & Jahngen, E. G. E. (1997). Validated method for quantitation and identification of 4, 4-desmethylsterols and triterpene diols in plant oils by thin-layer-chromatography-high resolution gas chromatography-mass spectrometry. *Journal of AOAC International*, 80(6), 1272–1280.
- Rivera del Álamo, R. M., Fregapane, G., Aranda, F., Gómez-Alonso, S., & Salvador, M. D. (2004). Sterol and alcohol composition of Cornicabra virgin olive oil: The campesterol content exceeds the upper limit of 4% established by EU regulations. *Food Chemistry*, 84, 533–537.
- Salvador, M. D., Aranda, F., & Fragapane, G. (1998). Chemical composition of commercial Cornicabra virgin olive oil from 1995/96 and 1996/97 crops. *JAOCs*, 75, 1305–1311.
- Salvador, M. D., Aranda, F., Gomez-Alonso, S., & Fregapane, G. (2003). Influence of extraction system, production year and area on Cornicabra virgin olive oil: A study of five crop seasons. *Food Chemistry*, 80, 359–366.
- Sánchez Casas, J., Osorio Bueno, E., Montaña García, A. M., & Martínez Cano, M. (2004). Sterol and erythrodiol+uvaol content of virgin olive oils from cultivars of Extremadura (Spain). *Food Chemistry*, 87, 225–230.
- Stefanoudaki, E., Chartzoulakis, K., Koutsaftakis, A., & Kotsifaki, F. (2001). Effect of drought on qualitative characteristics of olive oil of cv Koroneiki. *Grasas y Aceites*, 52(3-4), 202–206.
- Stiti, N., Msallem, M., Triki, S., & Cherif, A. (2002). Etude de la fraction insaponifiable de l'huile d'olive de différentes variétés Tunisiennes. *La Rivista Italiana delle Sostanze Grasse*, 79(10), 357–363.
- Vichi, S., Pizzale, L., Toffano, E., Bortolomeazzi, R., & Conte, L. (2001). Detection of hazelnut oil in virgin olive oil by assessment of free sterols and triacylglycerols. *Journal of AOAC International*, 84(5), 1534–1541.
- Williamson, E. (1988). The antioxidant activity of Δ^5 -avenasterol. PhD Thesis, University of Reading, UK.