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Territorial Investigation Based on the Chemical Composition of Chemlali Virgin Olive Oils

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Abstract: The purpose of this study was to evaluate differences in the chemical composition of virgin olive oils from the Chemlali variety cultivated in different olive growing areas of the Centre of Tunisia. All samples were harvested using the same controlled procedures and were submitted to a controlled processing in the same laboratory mill. Several analytical parameters and indices were determined. Results showed that the oils quality was attributed not only to the olive variety but also to the plantation site, therefore to climatic and pedologic factors. All these parameters showed an important effect on the fatty acid, phenol, α-tocopherol, sterol and volatile contents of the oils.

Key words: Chemlali variety, fatty acids, phenols, sterols, volatiles, virgin olive oils

INTRODUCTION

Virgin olive oil is a unique product extracted from the fruit of olive tree, *Olea europeae*, by only gentle physical procedures, which results in a genuine fruit juice. Over the last few years, the virgin olive oil has been appreciated by consumers, especially for its nutritional value and its organoleptic characteristics. The oil chemical composition is influenced by genetic (cultivar), agronomic and environmental factors (edaphologic characteristics and climatological conditions) (Kiritsakis, 1998). The altitude and temperature at which the olive trees are grown affect the olive oil composition. In addition, the seasonal factors (temperature and rainfall) which belong to the agronomic effects influence the physiology of the plant; so that the olive production area has a large effect on the specific characteristics of olive oil. In consequence, there is an increasing interest in the geographical classification of olive oil as a reliable criterion for olive oil authentication and quality.

Several attempts have been made to define olive oil origin by means of multivariate analysis of chemical parameters. The principal component analysis (PCA) and fatty acid and triacylglycerol profiles have been applied for the geographical classification of Greek oils (Tsimidou and Karakostas, 1993). Using an expert system (so-called SEXIA), Aparicio *et al*. (1994) has studied data from different chemical analysis to classify Spanish oils with respect to their origin and variety. Sacchi *et al*. (1998), the use of the high-field proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and PCA, obtained a classification of Italian olive oils from different regions. Ranalli *et al*. (1999) found that phenols, tocopherols, volatiles and fatty acids were influenced by site of production with different climate and soil parameters. Bortolomeazzi *et al*. (2001) used the hydrocarbon fraction analysis to classify different varieties according to their geographical origin. Salvador *et al*. (2003) assessed the chemical composition and quality of Cornicabra virgin olive oils

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throughout 5 crop years in 5 areas and were able to show differences in composition due to season and environment effects, resulting in a higher quality of oil from South and South-West of Toledo in respect to other regions. In previous investigations (Ben Temine *et al*., 2006, 2008), they demonstrated that the growing area had a significant effect on the percent composition of the volatiles and sterols present in the olive oils produced from Chétoui cultivar with the same harvesting period and processing technology. Analysis of Tunisian Chétoui virgin olive oils from 14 geographic sites by SPME has allowed the identification and the detection of significant differences in volatile compounds from various origins. In fact, (E)-2-hexenal, the principal compound present in the olive oil headspace samples, distinguished the oils from Bouarada, Amdoun, Sers, Chuigui, Borj El Amri, Zaghouan and Testour, whereas, the corresponding alcohols: (E)-2-hexenol and (E)-3-hexenol, characterized the oils from Lakhouet, Gâafour and Slouguia (Ben Temine *et al*., 2006).

Olive tree production, is a major traditional crop, it is cultivated throughout Tunisia with its different bioclimatic stages on a total area of 1, 600, 000 ha. With an annual production of 170.000 tonnes. Tunisia has now more than 67 million olive trees cropped mainly for oil production and are distributed as follows: 11.6% in the North, 34% in the Center and 54.1% in the South. With this olive oil cropping situation, Tunisia becomes the fourth olive oil producer after Spain, Italy and Greece. Despite of its genetic diversity, Tunisian olive grove is traditionally dominated by Chemlali variety cultivated in the Center and the South of Tunisia; it occupied almost 85% of the area reserved to the olive growing and contributes to 80% of oil national production. The Chemlali olive tree is characterized by small fruit size, high productivity and high vigor which tolerate drought, infertile soil and lack of treatment, its olive oil is well known in the international oil market for its excellent taste and flavor.

The aim of this study was to analyze the qualitative and quantitative differences of the fatty acids, minor fractions and oxidative stability of virgin olive oils from the Chemlali variety cultivated in different olive growing regions in Tunisia, namely Sfax, Kasserine and Kairouan located in the Center.

MATERIALS AND METHODS

This study was conducted on olive fruits of the Chemlali variety, from three distinct farms in the Center of Tunisia: Sfax; Kasserine and Kairouan during crop season 2006/2007.

Chemicals

Analytical grade solvents and reagents were used to perform analyses except HPLC eluents that were HPLC grade and purchased from Fluka (Buchs, Suitzerland), Sigma-Aldrich Chemical (Sternhelm, Germany) and Merck (Darmstadt, Germany).

Olive Varieties Employed

The study was carried out on virgin olive oils of Chemlali variety from three producing regions in the Center of Tunisia- Sfax (Jbeniana); Kasserine (Sbeitla); and Kairouan (El Alâa) -with different edaphological characteristics and climatological conditions. Virgin olive oil samples were obtained at the end of December 2006. Oils were extracted using an Abencor analyser apparatus (MC2 Ingenierias y Sistemas, Sevilla, Spain) then taken and stored in dark brown glass bottles under cold storage at 4°C, until being used in experimentation. Climatological data were obtained during 2006 from the National Institute of Meteorology of Tunisia (Fig. 1).

Analysis of Fatty Acid Composition

The analytical methods for the determination of fatty acid composition were described in regulation EEC 2568/91. Fatty acids were converted to fatty acids methyl esters before analysis by

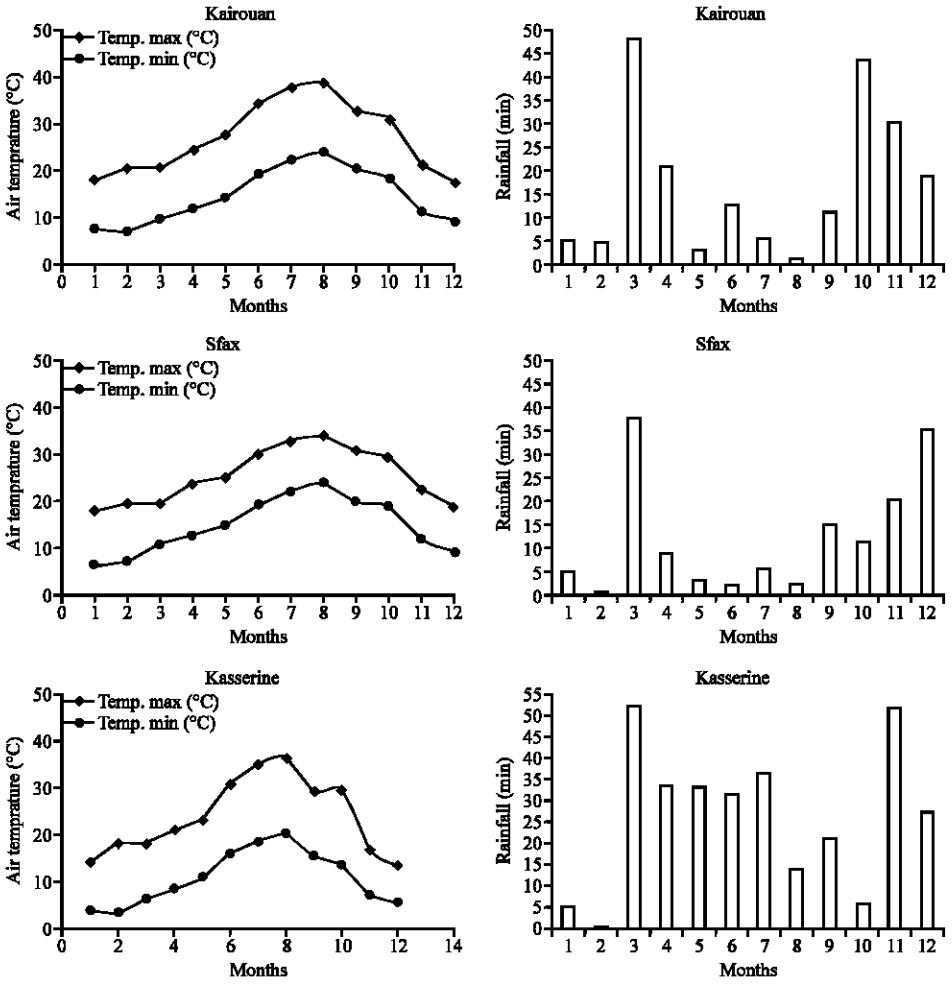

Fig. 1: Annual patterns for year 2006 of air temperature and rainfall in the Tunisian olive growing areas of Kairouan, Sfax and Kasserine

shaking off a solution of 0.2 g oil and 3 mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide and analyzed by a 4890D chromatograph HP, equipped with a capillary column injector system (Suppelcowax: 30 m×0.53 mm; 0.25 μm) and a FID detector. The carrier gas was nitrogen, with a flow of 1 mL min^{-1}. The temperatures of the injector and detector were set at 220 and 250°C, respectively and the oven temperature was set at 210°C. The injection volume was 1 μL.

Solid Phase Microextraction Analysis of Volatile Composition (Adapted from Vichi *et al.*, 2003)

Solid-phase microextraction (SPME) followed by GC was used to analyse the volatile compounds in the studied virgin olive oil samples. Olive oil (1.5 g) spiked with 4-methyl-2-pentanol (as internal standard) to a concentration of 1.5 μg g^{-1} was placed in a 10 mL vial fitted with a silicone septum. The SPME sampling was performed by exposing the DVB/Carboxen/PDMS fiber (50/30 μm, 2 cm long from Supelco Inc.) for 30 min in the headspace of the sample maintained at 40°C; it was then retracted into the needle and immediately transferred and desorbed for 1 min in the injection port of an Agilent 6890 series gas chromatograph equipped with a flame ionization detector (FID). Compounds were separated on a Supelcowax-10 column (30 m×0.25 mm×0.25 μm, Supelco Inc.) under the following

conditions: injection port temperature, 260°C; helium flow, 0.8 mL min^{-1}; oven temperature ramp, 35°C for 10 min, 3°C min^{-1} to 160°C and then 15°C min^{-1} to 200°C (maintained for 5 min). Volatile compounds were tentatively identified by comparison with standard substances (Sigma Aldrich) added to the refined oils.

Analysis of Sterols, Triterpenic and Aliphatic Alcohols

The determination of sterols, triterpenic and aliphatic alcohols content of olive oil samples were determined according to the European Official Methods Analysis described in Annexes V and VI of Regulation EEC/2568/91 of the European Union Commission (corresponding to AOCS method Ch 6-91, 1989). The olive oil, with added α-cholestanol for sterols and triterpenic alcohols and 1-eicosanol for aliphatic alcohols as an internal standard, was saponified with 2 N ethanolic potassium hydroxide and the unsaponifiable were extracted with ethyl ether. The sterol, triterpenic and aliphatic alcohol fractions were separated from the extract by thin-layer chromatography on a basic gel plate, then recovered from the plate and transformed in to trimethylsilyl ethers and the mixture was analysed by a 6890 chromatograph HP (Agilent Technologies), equipped with a capillary column SGL-5 (25 m×0.25 mm, 0.25 μm). The carrier gas was helium at a flow rate of 1.2 mL min^{-1}. The injector, detector and oven temperatures were 280, 290 and 260°C, respectively. The injection volume was 1 μL.

Phenolic Compounds Analysis

A sample of filtered virgin olive oil was weighed (2.5 g) and 250 μL of a solution of the internal standard (15 ppm of syringic acid in methanol) was added. The solvent was evaporated in a rotary evaporator at 35°C under vacuum and then the oil was dissolved in 6 mL of hexane.

A diol-bonded phase cartridge (Supelco Co., Bellefonte, PA) was placed in a vacuum elution apparatus and conditioned by the consecutive 6 mL of methanol and 6 mL of hexane.

The vacuum was then released to prevent drying of the column. The oil solution was applied to the column and the solvent was pulled through, leaving the sample and the standard on the solid phase. The sample container was washed with 6 mL of hexane, which were run out of the cartridge. The sample container was washed again with 4 mL of hexane/ethyl acetate (85:15, v/v), which were run out of the cartridge and discarded. The column was eluted with 15 mL of methanol and the solvent was evaporated in a rotary evaporator at room temperature and low speed under vacuum until dryness. The residue was dissolved in 250 μL of methanol/water (1:1, v/v) (Mateos *et al*., 2001).

HPLC analysis was performed using an Agilent Technologies series 1100 system equipped with an automatic injector, a column oven and a diode array UV detector. A Spherisorb S3 ODS2 column (250×4.6 i.d. mm, 5 μm particle size) (Waters Co., Milford, MA) was used, maintained at 30°C, with an injection volume of 20 μL and a flow rate of 1.0 mL min^{-1}. Mobile phase was a mixture of water/acetic acid (95:5, v/v) (solvent A), methanol (B) and acetonitrile (C). The elution gradient was from 95% (A)-2.5% (B)-2.5% (C) to 34% (A)-33% (B)-33% (C) in 50 min, followed by 100% (B) for 15 min to clean the column. Chromatograms were taken at 240, 280 and 335 nm. Phenolic compounds were identified and quantified at 280 nm using syringic acid as internal standard.

Statistical Analysis

Statistical analysis was performed using the SPSS of the windows statistical package (version 14.0).

RESULTS AND DISCUSSION

Fatty Acid Composition

Total fatty acids composition is an essential aspect of the qualitative evaluation of olive oil. It is also used as a means allowing to make sure of the authenticity of olive oils and to discover frauds

with other vegetable oils (Christopoulou *et al*., 2004). However, the metabolism and the lipid levels in the olive fruit could be affected by environmental factors, such as light, temperature and water stress (Harwood, 1984).

The study of acidic composition of Chemlali olive oils of various origins allows distinguishing differences in the levels of the main fatty acids which are sharply influenced by pedoclimatic factors (Table 1). The oleic acid, of which its predominance constitutes the main originality of olive oil and it confers the characteristics of mono-unsaturated oil, varies in a significant way between the studied oils. The highest percentages are observed in oils resulting from Sfax (63.57%). While oils resulting from Kairouan possess the lowest percentages (56.24%). Oils from Kasserine present intermediate percentages of 61.98%. Linoleic acid composition in studied Chemlali olive oils is heterogeneous, it evolves practically in an inverse way that of oleic acid. This is confirmed by the extreme values situated between 13.25% (Sfax) and 19.89% (Kairouan). Oils coming from Kasserine always present an intermediate percentage of 16.15 %. Some researchers have observed that oleic acid and the oleic/linoleic acid ratio are connected to the rainfall in the summer period (Angerosa *et al*., 1996). Concerning the palmitic acid, we noticed that there are no significative differences between the three regions, values are around 16.86%. For the other fatty acids (palmitoleic $C_{16:1}$, stearic $C_{18:0}$, linolenic $C_{18:3}$ and arachidic $C_{20:0}$) although their rates change according to the geographic area, are rather minor.

So, we can deduce that the proportions of the various fatty acids of Chemlali oils vary according to the geographic site, but main variations concern two principal fatty acids of olive oil as known the oleic and linoleic acid which rates vary in a significant way from a region to other one.

Another interesting parameter for the varietal characterization consists in considering the proportions of some classes of fatty acids (Table 1). Present results show that the rate of the UFA is variable according to the plantation zone it reaches a maximal value of 84.47% for oils coming from Kairouan and a minimal value of 79.35% for oils resulting from Sfax. Oils from Kasserine have values of 81.67%. Previous studies showed that the rainfall regime could have affected the FA biosynthesis that occurs in plant plastids and that need the concerted activity of two enzymes, acetyl-CoA carboxylase and FA synthase, to regulate a further chain elongation cycle in FA biosynthesis. This step is particularly relevant because it is directly related to the degree of unsaturation of the final oil product (Salas *et al*., 2000). Osman *et al*. (1994) showed that the percentage of the UFA increases when temperature decreases, consequently, the fresh and cold zones produce oil rich in unsaturated fatty acids in comparison with the dry and warm zones. This was confirmed later by Ranalli *et al*. (1997) and Stefanoudaki *et al*. (1999) who noted an inverse correlation between the temperature and percentage in PUFA and MUFA.

Table 1: Fatty acid composition (%) of Chemlali virgin olive oils

Fatty acids (%)	Kairouan	Sfax	Kasserine
C16:0	17.54±0.14^{a}	17.07±0.26^{a}	15.98±0.88^{a}
C16:1	2.44±0.01^{b}	1.77±0.01^{a}	2.14±0.14^{ab}
C18:0	2.46±0.08^{a}	2.68±0.05^{a}	2.21±0.22^{a}
C18:1	56.24±0.04^{b}	63.57±0.73^{a}	61.98±1.77^{a}
C18:2	19.89±0.07^{b}	13.25±0.43^{a}	16.15±1.39^{ab}
C18:3	0.72±0.05^{a}	0.75±0.02^{a}	0.68±0.05^{a}
C20:0	0.46±0.01^{a}	0.60±0.03^{b}	0.52±0.02^{ab}
C18:1/C18:2	2.83±0.01^{a}	4.80±0.21^{b}	3.86±0.41^{ab}
Σ SFAs	20.46±0.08^{a}	20.35±0.28^{a}	18.73±0.91^{a}
Σ MUFAs	58.68±0.04^{a}	65.34±0.72^{b}	64.13±1.75^{b}
Σ PUFAs	20.62±0.12^{b}	14.00±0.45^{a}	16.83±1.43^{ab}
Σ UFAs	84.47±0.81^{a}	79.35±0.30^{a}	81.67±0.34^{a}
UFAs/SFAs	5.69±0.36^{a}	3.90±0.07^{a}	4.49±0.08^{a}
UFAs/PUFAs	5.19±0.51^{a}	5.67±0.20^{b}	8.20±0.05^{ab}

Mean±SD, significant differences in the same raw are showed by different letter(s) ($p<0.001$)

The rate of unsaturated fatty acids/saturated fatty acids (UFA / SFA) varies also according to the geographic site from 3.90 for Sfax's oils followed by Kasserine with 4.49 to 5.69 for Kairouan's oils. According to Koutsaftakis *et al*. (2000), this rate is interesting to study the influence of plantation zone on the maturity of fruits and also for the varietal characterization and it is even observed a positive and a significant correlations between the pluviometry and the contents in UFA or UFA/SFA ratio.

Results obtained in the present study are in agreement with earlier study (Ollivier *et al*., 2000; Ben Temine *et al*., 2004) which consider that the oil fatty acids composition is variable and that this variability can be attributed to numerous factors notably the territorial characteristics.

Quantification of Phenolic Components

The analysis of phenolic substances using SPE RP-HPLC (Solid Phase Extraction and Reversed Phase-High Performance Liquid Chromatography) as described by Mateos *et al*. (2001), allowed the separation and the identification of 16 phenolic compounds. Results showed no qualitative differences in the HPLC phenolic fraction profile between virgin olive oils from different growing regions. However, significant quantitative differences ($p<0.001$) were observed in a wide number of phenolic compounds (Table 2).

The representative phenolic components were the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and the dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA). A higher concentration of the first component was observed in virgin olive oil from Kairouan with 6.57 mg kg^{-1}, for the second component, virgin olive oil from Sfax showed the highest value (5.92 mg kg^{-1}).

Another phenol which was identified at relatively high concentrations is the oleuropein aglycone derivative of hydroxytyrosol. Its amount ranged from 1.93 mg kg^{-1} in oils from Kasserine to 8.99 mg kg^{-1} in oils from Kairouan. The ligstroside aglycone derivative of tyrosol was represented at low concentrations which vary from 0.41 mg kg^{-1} in oils from Kasserine, to 2.57 mg kg^{-1} in oils coming from Sfax. Duncan's test showed significant differences ($p<0.001$) in their contents (Table 2).

Table 2: Phenolic compounds composition (mg kg^{-1}, as syringic acid) of studied olive oils

[C] (mg kg^{-1})*	Kairouan	Sfax	Kasserine
Hydroxytyrosol	0.29±0.02^{a}	0.19±0.01^{a}	0.23±0.00^{a}
Tyrosol	0.69±0.07^{a}	0.65±0.01^{a}	1.49±0.00^{b}
Vanillic acid	0.12±0.00^{a}	0.22±0.03^{ab}	0.44±0.00^{b}
Vanillin	0.46±0.02^{a}	0.25±0.03^{a}	0.16±0.01^{a}
p-coumaric acid	0.10±0.02^{b}	0.55±0.00^{a}	0.79±0.01^{c}
3,4-DHPEA-AC**	0.02±0.01^{a}	0.03±0.01^{a}	0.05±0.00^{a}
Ferulic acid	0.54±0.01^{b}	0.06±0.01^{a}	0.06±0.00^{a}
3,4-DHPEA-EDA**	6.57±0.43^{b}	2.60±0.33^{ab}	0.28±0.01^{a}
p-HPEA-AC**	0.07±0.02^{a}	0.05±0.01^{a}	0.07±0.00^{a}
p-HPEA-EDA**	4.79±0.36^{ab}	5.92±0.41^{b}	1.28±0.06^{a}
Pinoresinol	4.27±0.15^{a}	7.00±0.14^{b}	3.59±0.01^{a}
Acetoxypinerosol	0.40±0.02^{a}	0.73±0.20^{a}	0.00±0.00^{a}
Oleuropein aglycon	8.99±0.48^{b}	4.80±0.16^{a}	1.93±0.01^{a}
Ligstroside aglycon	2.07±0.08^{b}	2.57±0.09^{b}	0.41±0.01^{a}
Total as syringic acid	234.54±13.31^{b}	184.00±11.15^{ab}	55.67±0.90^{a}
HPLC total phenols***	29.37±1.49^{b}	25.60±1.36^{ab}	10.78±0.08^{a}
Luteolin	1.02±0.08^{b}	1.46±0.05^{b}	0.04±0.00^{a}
Apigenin	0.31±0.31^{a}	0.25±0.02^{a}	0.06±0.00^{a}
Tyrosol secoiridoids	7.54±0.36^{ab}	9.14±0.51^{b}	3.18±0.06^{a}
Hydroxytyrosol secoiridoids	15.85±0.93^{b}	7.58±0.50^{a}	2.44±0.02^{a}

Mean±SD, significant differences in the same raw are showed by different letter(s) ($p<0.001$), *Concentration expressed as mg kg^{-1} of syringic acid, **3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; p-HPEA-AC, 4-(acetoxyethyl)-1-hydroxybenzene; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol, ***Concentration expressed as the absolute concentration of phenols, calculated according to the response factors determined by Mateos *et al.* (2001)

Lignans are present also in considerable amount, particularly pinoresinol which is found in all analysed samples at concentrations ranging from 3.59 mg kg^{-1} in virgin olive oils from Kasserine to 7.00 mg kg^{-1} in virgin olive oils from Sfax, moreover, significant differences ($p<0.001$) were observed in its contents (Table 2). While, the 1-acetoxypinoresinol is found in oils coming from Kairouan and Sfax with a content of 0.40 and 0.73 mg kg^{-1}, respectively.

In relation to simple phenols, hydroxytyrosol was represented at concentrations relatively lower than tyrosol. The higher concentrations of hytroxythyrosol were observed in virgin olive oil coming from Kairouan (0.29 mg kg^{-1}). For tyrosol, virgin olive oils from Kasserine represent higher concentrations with 1.49 mg kg^{-1}.

Low flavonoid levels represented by luteolin and apigenin were observed in all the olive oils analyzed, with concentrations that varied from 0.04 to 1.46 mg kg^{-1} and 0.06 to 0.31 mg kg^{-1}, respectively. In spite of their low concentrations, luteolin showed significant differences ($p<0.001$) between oils from Kasserine and those coming from two other producing regions.

The total phenol content varied between 10.78 and 29.37 mg kg^{-1}, with oils from Kairouan and Sfax having the highest values. This is could be due to the altitude of the growing region as proposed by Moussa and Gerasopoulos (1996), who observed that lower altitudes produced higher phenol contents. In fact, Kasserine, which corresponded to the region with the lowest phenol content, was characterized by an average altitude of 707 m above sea level. In contrast, Kairouan with an average altitude of 60 m above sea level, the oils resulting showed the highest phenol contents. Another parameter has to be considered is the summer rainfall accumulation. Thus, Kasserine was characterized by nearly 82.2 mm accumulated rainfall during the summer period (June-August), while, there was a very low rainfall accumulation in Kairouan with a value of 18.6 mm. It has long known that the level of phenolics in plants tissues can be influenced by environmental factors such as ambient temperature and water availability. With regard to the latter factor, a water deficit tends to generate a stress situation that induces the production of phenolics (Parr and Bolwell, 2000).

O-diphenol concentrations were also studied, significant differences ($p<0.001$) were found in all samples from the different sites and varied in similar way to those observed in the total phenol contents.

Volatile Composition

Given that varietals and processing effects are the most important aspects of volatile composition of olive oil. However, geographic origin, climatic and agronomic conditions of olive growing can affect volatile composition of olive oils obtained by the same cultivar. SPME method has widespread application in analysis of volatiles, but has had limited application to olive oils (Servili *et al*., 2000). Consequently, the identification of volatile compounds contributing to its aroma is considered to be a key for quality and authentication control (Cavalli *et al*., 2004). Components identified in Chemlali oils involved in this study are depicted in Table 3, the chemical compositions of the volatile fraction of Chemlali olive oils were very similar from a qualitative point of view but which varies quantitatively, depending on the region of cultivation. However, significant differences ($p<0.001$) were observed in a wide number of volatile compounds (Table 3).

(E)-2-hexenal was the major volatile in all tested samples. In fact, Chemlali oils from Sfax and Kasserine were characterized by the highest concentrations (41.11 and 39.78 mg kg^{-1}, respectively). Other compounds present in a relatively high concentration as the hexanal particularly in oils resulting from Kasserine with levels of 5.14 mg kg^{-1}.

Concerning alcohol compounds, hexan-1-ol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol were also found to be highly produced by the oils coming from Kasserine (0.77, 0.26 and 3.48 mg kg^{-1}, respectively).

Table 3: Volatile compounds composition (mg kg^{-1}) of Chemlali virgin olive oils

Volatile compounds	Kairouan	Sfax	Kasserine
n-octane	0.39±0.01[a]	0.56±0.43[a]	0.26±0.02[a]
ND (Peak 6.7)	0.82±0.01[a]	0.88±0.04[a]	0.95±0.11[a]
ND (Peak 8.2)	2.56±0.07[a]	3.69±0.55[a]	3.92±0.03[a]
ND (Peak 10.4)	0.00±0.00[a]	0.28±0.03[b]	0.27±0.02[b]
1-penten-3-one	0.74±0.02[a]	0.97±0.16[a]	1.03±0.02[a]
ND (Peak 14.1)	1.51±0.01[a]	4.31±0.95[a]	6.74±0.02[a]
Hexanal	2.07±0.05[a]	4.42±0.27[b]	5.14±0.12[b]
E-2-pentenal	0.09±0.01[a]	0.14±0.05[a]	0.17±0.01[a]
1-penten-3-ol	0.23±0.01[a]	0.44±0.10[a]	0.36±0.00[a]
E-2-hexenal	14.27±0.13[a]	41.11±0.17[b]	39.78±0.12[b]
Hexyl acetate	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Z-3-hexenyl acetate	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
E-2-heptenal	0.36±0.08[ab]	0.00±0.00[a]	0.73±0.01[b]
Hexan-1-ol	0.08±0.00[a]	0.44±0.48[a]	0.77±0.02[a]
Z-3-hexen-1-ol	0.00±0.00[a]	0.27±0.01[b]	0.26±0.01[b]
E-2-hexen-1-ol	0.21±0.00[a]	3.55±0.14[b]	3.48±0.08[b]
Total volatile compounds	26.65±0.08[a]	61.61±1.35[b]	66.52±0.00[b]

Mean±SD, significant differences in the same raw are showed by different letter(s) ($p<0.001$)

The ester fraction represented by two compounds (Z)-3-hexenyl acetate and hexyl acetate are absent in olive oil headspace samples. The absence of this compound in oils allowed pointing out the absence of alcohol acetyl transferases (AATs) catalysing the production of this ester. The SPME method used, allowed the separation and identification of a great number of volatile compounds in VOO samples, some of them not previously described in the literature, these included three compounds designated RT 6.7, RT 8.2 and RT 14.1, which have not yet been identified, although they are quantitatively significant in olive oil headspace particularly in oils from Kasserine (RT 6.7: 0.95 mg kg^{-1}; RT 8.2: 3.92 mg kg^{-1}; RT 14.1: 6.74 mg kg^{-1}, respectively).

Previous study of Pannelli *et al*. (1994) and Shiers and Adechy (1998) showed that, in the climatic conditions of central Italy, the rainfall effect is pre-eminent with respect to temperature and that some compounds, such as hexanal and isobutyl acetate, were negatively correlated to rainfall. These results were recently confirmed in Leccino cultivar grown in Tuscany under two different hydric conditions (Gucci *et al*., 2004). But present results seem to disagree with this, as the oils from Kasserine had the highest amounts of hexanal when it possesses the highest value of rainfall. Early studies conducted by Montedoro *et al*. (1995), have shown the ability of volatiles, sampled with the static headspace, in discriminating different oils coming from different Italian regions. The score plot of principal component analysis (PCA) applied to Mediterranean and Australian virgin olive oils, shows a good discrimination of oil according to origin area, when volatile compounds are used as analytical parameters to build the multivariate statistical model (Servili *et al*., 2001).

Analysis of Tunisian Chetoui virgin olive oils from 14 geographic sites by SPME has allowed the identification and the detection of significant differences in volatile compounds from various origins. In fact, (E)-2-hexenal, the principal compound present in the olive oil headspace samples, distinguished the oils from Bouarada, Amdoun, Sers, Chuigui, Borj El Amri, Zaghouan and Testour, whereas, the corresponding alcohols: (E)-2-hexenol and (E)-3-hexenol, characterized the oils from Lakhouet, Gâafour and Slouguia (Ben Temine *et al*., 2006).

Sterol and Aliphatic Alcohol Composition

Sterols are nutritionally important lipids that need to be routinely determined in foods. In olive oil, content and composition of sterols can vary due to the agronomic and climatic conditions, fruit or seed quality, oil extraction and refining procedures and storage conditions (Koutsaftakis *et al*., 1999). The sterol composition of Chemlali olive oils from different regions is shown in Table 4. It is seen that

the main compounds were β-sitosterol and β5-avenasterol. The campesterol was also present at considerably high content and was at the norm and lower than the legal minimum of 4%. Regarding the authenticity markers established by the current legislation, all samples respect the established limits: cholesterol percentages were below the established limits of 0.5%; the percentages of stigmasterol were lower than those of campesterol and the apparent β-sitosterol content was higher than the legal minimum of 93%. The total sterols were remarkably higher than the minimum limit set by legislation (1000 mg kg^{-1}), ranging from 1925 mg kg^{-1} in oils from Sfax to 2250 mg kg^{-1} in oils from Kasserine. This is undoubtedly a good characteristic of olive oils due to the great benefits of these compounds for health. No difference was revealed between oils from the various sites of culture.

The aliphatic alcohols obtained for the different oils are registered in Table 5. In general, dicosanol (C_{22}), tetracosanol (C_{24}), hexacosanol (C_{26}) and octacosanol (C_{28}) were the main components in the aliphatic alcohol fraction. In fact, the most useful parameters for discriminating among the regions of plantation, as regard triterpenic dialcohol and aliphatic alcohol composition, were erythrodiol+uvaol and tetracosanol, respectively, where it was observed significant differences ($p<0.001$) between oils from the various growing area on these parameters. Note that oils resulting from Kairouan has higher tetracosanol (38.78 mg kg^{-1}) and lower erythrodiol+uvaol percentage (0.77%), while, oils from Sfax has lower tetracosanol (16.81 mg kg^{-1}) and higher erythrodiol+uvaol percentage (1.68%).

Table 4: Sterol composition (%) of Chemlali virgin olive oil samples

Sterol	Kairouan	Sfax	Kasserine
Cholesterol*	0.19±0.00^a	0.40±0.16^a	0.16±0.03^a
Brassicasterol*	0.08±0.02^a	0.00±0.00^a	0.00±0.00^a
24-methylencholesterol	0.39±0.03^a	0.20±0.02^a	0.29±0.01^a
Campesterol*	3.39±0.09^a	3.15±0.07^a	3.23±0.04^a
Campestanol	0.28±0.02^a	0.29±0.01^a	0.25±0.01^a
Sigmasterol*	0.38±0.01^a	0.69±0.32^a	0.46±0.04^a
Δ7-campesterol	0.14±0.10^a	0.12±0.01^a	0.00±0.00^a
Δ5.23-stigmastadienol	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
Clerosterol	0.94±0.01^a	0.94±0.05^a	0.84±0.01^a
β-sitosterol	80.49±0.03^a	82.66±0.77^a	80.93±0.12^a
Sitostanol	0.59±0.02^a	0.45±0.09^a	0.00±0.00^a
Δ5-avenasterol	10.91±0.05^a	9.34±0.44^a	11.44±0.24^a
Δ5.24-stigmastadienol	0.81±0.22^a	0.64±0.29^a	0.81±0.06^a
Δ7-stigmastenol*	0.53±0.04^a	0.28±0.04^a	0.79±0.10^a
Δ7-avenasterol	0.75±0.02^a	0.68±0.05^a	0.82±0.06^a
Apparent β-sitosterol*	93.87±0.06^a	94.18±0.51^a	93.98±0.16^a
Total sterol (mg kg^{-1})*	2119.60±19.26^a	1925.59±42.63^a	2250.83±20.66^a

*Limits established by the EU regulation: cholesterol, ≤0.5; brassicasterol,≤0.1; campesterol, ≤4.0; stigmasterol, <campesterol; Δ7-stigmastenol, ≤0.5; apparent β-sitosterol, ≥93; total sterols, ≥1000 mg kg^{-1}, Mean±SD, Significant Differences in the same raw are showed by different letter(s) ($p<0.001$)

Table 5: Alcohol composition (mg kg^{-1}) of Chemlali virgin olive oil samples

Alcohol	Kairouan	Sfax	Kasserine
Dicosanol (C22)	25.27±18.77^a	14.31±2.11^a	40.37±10.14^a
Tricosanol	2.20±0.07^a	1.27±0.73^a	2.61±0.84^a
Tetracosanol (C24)	38.78±0.06^b	16.81±0.50^a	26.66±1.07^c
Pentacosanol	3.02±0.10^a	4.76±0.08^a	4.87±0.60^a
Hexacosanol (C26)	32.89±0.02^a	39.72±0.83^a	33.74±1.56^a
Heptacosanol	4.99±0.10^a	4.85±0.04^a	4.59±0.19^a
Octacosanol (C28)	21.15±6.91^a	31.16±16.30^a	22.32±1.05^a
Total aliphatic alcohols	118.09±11.89^a	101.99±13.85^a	123.09±11.73^a
Erythrodiol	14.52±0.04^a	30.26±2.21^a	27.29±0.38^a
Uvaol	1.95±0.45^a	2.53±0.95^a	0.89±0.12^a
Erythrodiol+Uvaol (%)*	0.77±0.01^a	1.68±0.10^b	1.24±0.02^{ab}

*Limits established by the EU regulation:≤4.5%, Mean±SD, Significant Differences in the same raw are showed by different letter(s) ($p<0.001$)

The relationship between the Andalusian oil chemical composition and the altitude, as an indirect parameter related to climate of their olive grows zones, has been pointed by Ferreiro and Aparicio (1992). It was found that sterols and some triterpenic alcohols showed changes in relation to altitude. In fact, sterols (β-sitosterol, stigmasterol, campesterol and total sterols) decrease with the altitude, while triterpenic alcohols (cycloartenol and 24-methylencycloartanol) increase with altitude. Thus, oils were classified into two groups of altitude, valley (altitude inferior than 400 m) and mountains (altitude superior than 700 m).

The authentication of extra virgin olive oils from different regions of Spain, Italy and Portugal, by mean of their alcohols, sterols and methyl sterols content and the application of multivariate statistical methods and Evidence's Theory has been investigated. Results showed a great ability using the stigmasterol, docosanol, tetracosanol and erythrodiol for discriminating European olive oils from different sites (Aparicio *et al*., 1994).

CONCLUSION

This study allowed evaluating the chemical composition of virgin olive oils from the Chemlali cultivar growed mainly in the Centre of Tunisia. It can be said that Chemlali olive oils show good characteristics in what the analyzed parameters are concerned:

- The proportion of the various fatty acids vary according to the geographic zone, main variations concern essentially two principal fatty acids (oleic and linoleic acids), percentage of UFA and the UFA/SFA ratio
- For the main phenolic compounds, the 3,4-DHPEA-EDA was higher in virgin olive oil from Kairouan. While, the *p*-HPEA-EDA was accounted to virgin olive oil from Sfax.
- (E)-2-hexenal, hexan-1-ol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol permitted to distinguish the oils from different sites. They were found to be highly produced by the oils coming from Kasserine
- With regard to sterols, No differences were revealed between oils from the various sites of culture

Finally, it was possible to conclude that the results obtained for such parameters allow the differentiation of Chemlali virgin olive oils and also permitted, in some cases, to appreciate the influence of the territory.

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