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Full Length Research Paper

The phenolic compounds and compositional quality of Chétoui virgin olive oil: Effect of altitude

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The differences in the chemical composition of virgin olive oils (VOO) obtained from trees of the Chetoui variety cultivated at different altitudes in Tunisia were evaluated. All samples were harvested using the same controlled procedures and were submitted to a controlled processing in the same laboratory mill. Several analytical parameters such as fatty acid composition, amounts of phenols; o-diphenols and pigments were analyzed. All these parameters showed an important effect on the fatty acids and phenols content of the oils. The total phenol content was positively correlated with the altitude, ranging from 817.33 mg/kg (403 m) to 131.91 mg/kg (10 m). However, the results of the regulated parameters in the potential quality classified all the analyzed oils into the ''extra virgin'' category.

Key words: Orchard elevation, total antioxidant capacity, phenolic compounds, quality index.

INTRODUCTION

Extra virgin olive oil is the principal source of fat in the Mediterranean diet, with important nutraceutic effects due to its abundance of oleic acid, phenolic compounds and an adequate content of linoleic and linolenic acids, two important essential fatty acids. All these chemicals contribute to lower the risk of coronary heart diseases and cancers (Bandelj et al., 2002). Since the production of olive oil is far below demand, there is a need to increase the cultivation of olive trees, both to produce more oil and to enhance its quality, particularly with regard to components beneficial to the human health, such as natural antioxidants and sterols (Cercaci et al., 2007). These benefits have been related both to its well-balanced fatty acid composition, where oleic acid is the main component and to the presence of minor biomolecules, such as phytosterols, carotenoids, tocopherol and phenols (Medeiros, 2001; Perez-Jimenez et al., 2005).

The phenolic compounds are natural antioxidants present in virgin olive oils (VOO) (Baldioli et al., 1996) but not in refined ones (Montedoro and Cantarelli, 1969). They have a significant importance for their nutritional and technological properties. Their antioxidant function and anti-inflammatory effect (Perona et al., 2006) have been related to the preventive action on certain diseases such as atherosclerosis (Huang and Sumpio, 2008) and cancer (Giovannini et al., 2008). Polyphenols also present an important technological value due to their influence on sensory characteristics (Baccouri et al., 2008; Bendini et al., 2007) and the shelf life of virgin olive oil.

Several attempts have been made to define olive oil origin by means of multivariate analysis of chemical parameters; that is, the principal component analysis (PCA), and the profiles of fatty acid and triacylglycerol have been applied for the geographical classification of Greek oils (Tsimidou and Karakostas, 1993). Using an expert system (SEXIA), Aparicio et al. (1994) have studied data from different chemical analysis to classify Spanish oils

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with respect to their origin and variety. Sacchi et al. (1998), used high-field proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and PCA to obtained a classification of Italian olive oils from different regions. Ranalli et al. (1999) found that phenols, tocopherol, volatiles and fatty acids were influenced by the site of production, in particular by the different climate and soil parameters. Salvador et al. (2003) assessed the chemical composition and quality of Cornicabra virgin olive oils throughout five crop years in five areas and were able to show differences in composition due to season and environmental effects, resulting in a higher quality of the oil from South and South-West of Toledo with respect to other regions. In their investigations Temime et al. (2006, 2008), demonstrated that the growing area have a significant effect on the percent composition of the volatiles and sterols in the olive oils produced from Chetoui cultivar harvested and processed in the same period and with the same technology. Analysis by SPME of Tunisian Chetoui virgin olive oils from 14 geographic sites, led to the conclusion that significant differences in volatile compounds from various origins existed.

The aims of the present work were to study the effects of different geographic origin, in particular of the different elevation of the orchards, on the compositional quality and phenolic compounds of the oil.

MATERIALS AND METHODS

Reagents and standards

Analytical grade solvents and reagents were used to perform analyses except high-performance liquid chromatography (HPLC) eluents that were of HPLC grade and purchased from Fluka (Buchs, Switzerland), Sigma-Aldrich Chemical (Sternheim, Germany) and Merck (Darmstadt, Germany). Bidistilled deionised water was used and all the reagents were of suitable analytic purity.

Plant material

The olives were hand-picked from different trees, at the same stage of maturity (MI = 3), at five different altitudes and the same laboratory mill was used to prepare the olive oil samples. Only drupes not damaged, fresh and healthy were selected. After harvesting, the olive fruit samples were immediately transported to the laboratory mill where they were transformed into oil within 24 h. The olives (2.5 to 3 kg) were washed and deleafed, crushed with a hammer crusher and the paste mixed at 25°C for 30 min, then centrifuged without addition of warm water. The oil was transferred into dark glass bottles, kept in the dark at 4°C and analyzed within a week (Youssef et al., 2011).

Quality parameters

Determination of physicochemical quality parameters (free acidity, peroxide value and UV absorption characteristics, K270 (conjugated trienes) and K232 (conjugated dienes), was carried out following the analytical methods described by Regulation EEC/ 2568/91 and EEC/ 1429/92 of European Economic Community Regulation (EEC, Regulation, 1991, 1992).

Fatty acid composition

Fatty acid composition, calculated as the percentage of total fatty acids, was determined by gas chromatography with a Hewlett-Packard (HP 4890 D) chromatograph (Hewlett-Packard Company, Wilmington, DE) equipped with a flame ionization detector (FID), after their conversion to methyl esters, according to regulation EEC 2568/91 (EEC, 1991).

Pigment content

Carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane, using specific extinction values, according to the method of Minguez-Mosquera et al. (1991).

Total phenolic content

Total phenol and *o*-diphenol contents were quantified colorimetrically (Ranalli et al., 1999). Phenolic compounds were isolated by triple extraction of a solution of oil (10 g) in hexane (20 ml) with 30 ml of a methanol-water mixture (60:40, v/v). The Folin-Ciocalteau reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts and the absorption of the solution at 725 nm was measured. Values are given as milligrams of caffeic acid per kilogram of oil (Gutfinger, 1981). *Ortho*-diphenols were also measured colorimetrically at 370 nm after adding 5% (w/v) sodium molybdate in 50% ethanol to the extract (Gutfinger, 1981). Results are given as milligrams of caffeic acid per kilogram of oil.

Oil stability

Oxidative stability was evaluated by the Rancimat method (Gutierrez, 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 743 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g warmed at 100°C with an air flow of 10 l/h.

Antiradical activity

The olive oil samples were examined for their capacity to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Kalantzakis et al., 2006). Oil solution 1 ml in ethyl acetate (10%, w/v) was added to 4 ml of a freshly prepared DPPH solution (10^{-4}M in ethyl acetate) in a screw-capped 10 ml test tube. The reaction mixture was then vigorously shaken for 10 s in a vortex apparatus and the tube was maintained in the dark for 30 min, until a steady state was reached. The absorbance of the mixture was measured at 515 nm against a blank solution. A control sample (no oil) was prepared and daily measured. A refined olive oil (Minerva S.A. edible Oils, Shimatari, Viotia, Greece), devoid of pro-oxidants/antioxidants, was used for comparison. The radical scavenging activity (RSA) toward [DPPH] was expressed as the percent reduction in DPPH concentration by the constituents of the oils:

% [DPPH] red = 100-(1 [DPPH]30/[DPPH]0)

Where, [DPPH]0 and [DPPH]30 were the concentrations of DPPH in the control sample (t = 0) and in the test mixture after the 30 min reaction, respectively.

Phenolic compounds

Analysis of phenolic compounds was performed by reverse phase-

Table 1. Means and standard deviations for the regulated physicochemical quality in the olive oil samples analyzed from the Chetoui olive at different altitudes at the same stage of maturation. The results are expressed as mean ± standard deviation of 3 samples.

Component	Altitude					
	403 m	379 m	354 m	21 m	10 m	EEC regulation
Free acidity (%C18:1)	0.25^{a} ± 0.10	0.35^{a}± 0.01	0.35^{a} ± 0.03	0.40^{ab}± 0.15	0.55^{b} ± 0.02	0.8
Peroxide value (meq O2 kg^{-1})	2.92^{a} ± 0.36	5.16^{ab} ± 1.25	3.66^{a} ± 1.00	7.81^{b} ± 0.40	10.33^{c} ± 1.04	20
UV absorption (K270)	0.18^{a} ± 0 .03	0.13^{a} ± 0.04	0.14^{a} ± 0.02	0.13^{a} ± 0.00	0.13^{a} ± 0.02	0.22
UV absorption (K232)	1.69^{a} ± 0.16	1.41^{a} ± 0.35	1.48^{a} ± 0.18	2.37^{b} ± 0.01	1.94^{a} ± 0.11	2.5
Chlorophylls mg kg^{-1}	4.51^{b}± 0.39	3.63^{a} ± 0.08	4.99^{b} ± 0.00	3.34^{a} ± 0.05	3.10^{a} ± 0.01	-
Carotenoïds mg kg^{-1}	2.15^{b} ± 0.30	1.66^{a} ± 0.09	2.14^{b} ± 0.01	1.36^{a} ± 0.05	1.28^{a} ± 0.05	-
Total phenols mg kg^{-1}	1128.27^{c} ±13.2	828.24^{b} ±58.97	786.66^{b} ± 8.73	158.88^{a} ±13.09	256.35^{a} ± 17.98	-
o-diphenols mg kg^{-1}	282.82^{c} ±40.95	91.90^{b} ± 11.95	243.89^{c} ± 34.4	26.93^{a}± 2 .36	40.51^{a} ± 7.50	-
Stability (h)	73.25^{c} ± 5.81	42.02^{b} ± 0.57	57.63^{bc} ± 0.80	16.78^{a} ± 0.28	34.84^{ab} ± 0.21	-
Radical scavenging activity (%)	94.94^{d} ±1.24	85.17^{c} ±0.99	77.66^{b} ±0.44	60.12^{a}±1.07	54.80^{a} ±0.83	-

$^{a-d}$ Mean ± SD, significant differences within the same row are shown by different letters ($P < 0.05$). EEC, European Economic Community.

high performance liquid chromatography (RP-HPLC) with UV detection (Mateos et al., 2001). A solution of *p*-hydroxyphenyl acetic (0.12 mg/ml) and *o*-coumaric acids (0.01 mg/ml) in methanol was used as standard. A sample of filtered virgin olive oil (2.5±0.001 g) was weighed and 0.5 ml of standard solution was added. The solvent was evaporated in a rotary evaporator at 40°C at reduced pressure and the residue was dissolved in 6 ml of hexane. A diol-bonded phase cartridge was placed in a vacuum elution apparatus and conditioned by the consecutive addition of 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 stationary 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. Finally, the column was eluted with 10 ml of methanol and the collected solvent was evaporated in a rotary evaporator, at room temperature, low speed and reduced pressure until dryness. The residue was extracted with 0.5 ml of a 40°C methanol/water (1:1 v/v) mixture and the obtained solution was left to rest for 4 h.

Then an aliquot of 20 µl of the final solution was injected into a JASCO HPLC system equipped with a double plunger pump and a diode array UV detector. A Lichrospher 100 RP-18 column (250 mm×4 mm id, particle size 5 µm, Merck) was used. The mobile phase consisted of a mixture of water/phosphoric acid (99.5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The gradient elution program used was: 95% (A) / 5% (B) in 0 min, 70% (A) / 30% (B) in 25 min, 62% (A) / 38% (B) in 40 min, 45% (A) / 45% (B) in 45 min, 52.5% (A) / 47.5% (B) in 5 min and 100% (B) in 5 min. Less than 100% (B) was maintained for 5 min and the run was stopped. Quantification of total phenols was carried out at 280 nm using *p*-hydroxyphenyl acetic acid as internal standard and the quantification of flavones and ferulic acid was performed at 335 nm using *o*-coumaric acid as internal standard. Results were expressed in mg kg-1 oil. All the determinations were performed in triplicate.

Statistics

All parameters were determined in triplicate for each sample. Analysis of variance (ANOVA) was processed with the SPSS statistical package (Version 12.0 for Windows, SPSS Inc. Chicago, Illinois, 2003). The significance of differences at a 5% level among means was determined by one way ANOVA, using the Tukey's test. ANOVA was applied to evaluate the influence of growing area conditions on Chetoui olive oil. PCA and hierachical cluster analysis (HCA) were carried out using XLStat-Pro7.5 (2007) for Windows (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

Quality parameters

All the oils (Table 1) showed values well below the maximum admitted for the regulated physicochemical parameters: (acidity ≤ 0.8; peroxide value ≤ 20 meq O2/kg; K270 ≤ 0.22; K232 ≤ 2.5). This value permits all of them to be included in the ''extra virgin'' category, as stated by the European Economic Community Regulation, 2003. Was the decrease significant ($P < 0.05$).

Fatty acid composition

Total fatty acids composition is an important aspect of the quantitative evaluation of olive oils. It is also used to assure their authenticity and to discover frauds, such as mixtures with other vegetable oils (Christopoulou et al., 2004). Among fatty acids, palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acids were identified (Table 2). Palmitic, stearic, oleic and linoleic acids were quantified as the major ones. Palmitoleic, linolenic and arachidic acids were also determined, but in smaller amounts, in all the samples. The study of acidic composition of Chétoui olive oils of various origins allowed distinguishing differences in the levels of the main fatty acids, which result sharply influenced the altitude (Table 2).

Palmitic acid showed inconstant variations among locations, whereas the levels of oleic acid decreased gradually,

Table 2. Changes in fatty acid composition of olive oil obtained from plant cultivated at different altitudes. The results are expressed as mean ± standard deviation of 3 samples.

Fatty acid	Altitude					
	403 m	379 m	354 m	21 m	10 m	ECC regulation
$C_{16:0}$	12.55±0.16	9.02±0.18	11.32±0.18	11.64±0.04	13.04±0.16	7.5-20.0
$C_{16:1}$	0.31±0.00	0.25±0.09	0.33±0.08	0.32±0.07	0.38±0.05	0.3-3.50
$C_{18:0}$	3.28±0.17	2.94±0.04	2.96±0.16	3.14±0.01	3.59±0.08	0.5-5.0
$C_{18:1}$	67.85±0.43	68.73±0.59	63.16±1.28	58.73±0.08	52.92±0.79	55.0-83
$C_{18:2}$	15.01±0.34	18.13±0.02	19.84±0.69	25.78±0.07	28.81±0.60	3.5-21.0
$C_{18:3}$	0.55±0.02	0.67±0.03	0.76±0.09	0.83±0.02	0.76±0.01	≤1.0
$C_{20:0}$	0.40±0.05	0.47±0.01	0.44±0.02	0.28±0.33	0.48±0.01	≤0.6
SFA	16.23±0.12	12.43±0.08	14.72±0.12	15.06±0.13	17.11±0.08	-
MUFA	68.16±0.21	68.98±0.63	63.49±0.68	57.05±0.07	53.30±0.42	-
PUFA	15.56±0.18	18.8±0.02	20.61±0.34	27.06±0.17	29.57±0.30	-
MUFA/PUFA	4.38	3.66	3.08	2.10	1.80	-

C16:0, palmitic; C16:1, palmitoleic; C18:0, stearic; C18:1, oleic; C18:2, linoleic; C18:3, linolenic; C20:0, arachidic; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EEC, European Economic Community.

passing from higher to lower altitudes. On the contrary, linoleic acid levels showed an opposite trend. The ratio unsaturated/saturated fatty acids for the oils obtained at lower altitudes ranged between one and three. This ratio increased at the higher elevations (three to five) (Table 2). This increase was probably related to the fact that lower temperatures favour the oil unsaturation. Low temperatures reduce the rate of chemical reactions and microbial activity that may result in the loss of quality of the olive fruits and consequently, also of quality of the extracted oil. Similar results were reported by Osman et al. (1994) for different cultivars.

Chlorophylls and carotenoids

Chlorophylls and carotenoids are very common pigments that give colour to vegetables and several fruits (Del Giovine and Fabietti, 2005) and play key roles in photosynthesis. Neither chlorophylls nor carotenoids can be synthesized by animals, though they need to obtain these molecules with food that will be chemically modified during assimilation. Several reports have demonstrated the healthy role of many plant pigments (Mayne et al., 1996), that is, the potential health benefit of antioxidants intake of a diet rich in carotenoids for the prevention of cardiovascular and degenerative diseases (Landrum and Bone, 2001).

Oils obtained from olives cultivated at higher altitudes showed higher chlorophyll content compared with those from lower altitudes. Chlorophyll content varied between 3.1 and 4.5 mg/kg, according to the location. Elevation influenced not only the chlorophyll content but also that of carotenoids that followed the same trend, ranging from 2.15 mg/kg (403 m) to 1.28 mg/kg (10 m) (Table 1).

The chlorophyll content could contribute to the photo-oxidative deterioration of the oil, increasing its peroxide value (Interesse et al 1971; Carlsson et al., 1976). The loss of chlorophylls corresponds to the forma-tion of other pigments, such as anthocyanins, responsible for the purple colour of the olives, or the products of oxidation of phenolic compounds (oleuropein), respon-sible for the blue colour (Fedeli, 1977).

Oxidative stability

Stability to oxidation is an important property of olive oil, which is improved by synergistic interactions between the various antioxidants present in the oil itself, and also depends on the lipid composition. A direct correlation between stability and altitude of cultivation was observed, in particular the olive oil from the higher locations exhibited the highest stability (73.25 h vs. 16.78 h) (Table 1), was significantly different ($P < 0.05$). This charac-teristic could be attributed in part to the lower levels of rainfall in these regions, as reported by Tovar et al. (2002), which hypothesized that oxidative stability is significantly affected by the water regime that in turn determines the phenols content of the olive fruit. On the other hand, genetic factor and the ensuing high levels of mono unsaturated fatty acids (MUFA) could also be causative factors of these findings. Furthermore, the greater stability of the oil from higher elevations could be probably related to the fact that low temperatures favour the oil unsatu-ration. Table 4 shows this relationship for “Chetoui” oils. The correlation obtained for samples over-all showed a good linear relationship between total phenols ($r = 0.74$), *o*-diphenols ($r = 0.90$) and oxidative stability measured by Rancimat.

Antiradical activity

Further experimentation on the radical scavenging

Table 3. Phenols amount (mg/kg) in virgin olive oil from Chetoui variety at different altitudes. The results are expressed as mean ± standard deviation of 3 samples.

Component	Altitude				
	403 m	379 m	354 m	21 m	10 m
Hydroxytyrosol	17.61^{c}±1.03	13.28^{b} ± 0.04	13.18^{b}± 0.06	12.71^{b}±0.05	2.92^{a} ± 0.09
Tyrosol	26.94^{d} ± 0.56	24.25^{c} ± 0.10	23.83^{c} ± 0.05	16.28^{b} ± 0.12	9.32^{a} ± 0.05
Vanillic acid	0.19^{b} ± 0.03	0.06^{a} ± 0.00	0.05^{a} ± 0.002	0.42^{c} ± 0.01	0.62^{c} ± 0.03
Vanillin	0.31^{a} ± 0.01	0.36^{a} ± 0.03	0.35^{a} ± 0.05	0.26^{a} ± 0.01	0.89^{b} ± 0.01
p-coumaric acid	0.53^{c} ± 0.03	0.50^{c} ± 0.01	0.34^{bc} ± 0.04	0.19^{a} ± 0.01	0.25^{b} ± 0.10
Ferulic acid	0.38^{b} ± 0.06	0.33^{b} ± 0.01	0.47^{c} ± 0.01	0.15^{a} ± 0.06	0.25^{a} ± 0.00
Dialdehydic form of oleuropein aglycone	42.60^{c} ± 1.35	29.40^{b} ± 0.34	28.49^{b}± 0.30	27.39^{b} ± 0.47	3.24^{a} ± 0.20
Dialdehydic form of ligstroside aglycone	726.69^{d} ± 5.60	344.72^{c}± 4.87	327.16^{c} ± 1.30	279.24^{b} ± 5.50	114.17^{a}± 6.01
Pinoresinol	0.14^{a} ± 0.01	0.42^{b} ± 0.01	0.36^{b} ± 0.01	0.22^{a} ± 0.00	0.16^{a} ± 0.00
Acetoxypinoresinol	1.94^{c} ± 0.54	2.14^{d} ± 0.12	1.81^{c} ± 0.04	0.98^{b} ± 0.03	0.34^{a} ± 0.23
Total phenols	817.33	415.46	396.04	337.84	131.91

$^{a-d}$Mean ± SD, significant differences within the same row are shown by different letters ($P < 0.05$).

activity (RSA) of these VOO permitted the verification of the characteristics discussed earlier (Table 1). Radical scavenging activity of the Chetoui olive oils varied according to altitude (54.80 to 94.94%). Chetoui oils produced at higher altitudes showed the highest scavenging ability, with an average value of 94.94%. Conversely, at lower altitudes, the average value dropped down to 54.80%. This activity is necessarily linked to the much lower oxidative stability and oleic acid content. Our results show significant variation of the radical scavenging activity of the Chétoui oils due to the effect of cultivar-environment (altitude) interaction. Overall, the correlation showed a positive linear relationship between radical scavenging capacity and oxidative stability measured by Rancimat. (r = 0.67). These results are similar to those reported by several authors for other olive oil varieties (Usenik et al., 2008).

Quantification of phenolic components

The olive oils from different altitudes did not show significant qualitative differences in the HPLC profile of their phenolic fraction. However, significant quantitative differences were observed for many of the phenols (Table 3) was ($P<0.05$). The most representative components were the dialdehydic form of elenolic acid linked to hydroxytyrosol and the dialdehydic form of elenolic acid linked to tyrosol. A higher concentration of these two chemicals was observed in the olive oil obtained from plants cultivated at 403 m, with 17.61 and 26.94 mg kg^{-1}, respectively.

Among secoiridoids, the dialdehydic form of ligstroside aglycone was identified in relatively high amounts, followed by the dialdehydic form of oleuropein aglycone. Both the secoiridoids were produced in greater quantities by plants growing at higher altitude: 114.17 vs. 726.69 mg kg^{-1} for ligstroside aglycone and 3.24 vs. 42.60 mg kg^{-1} for oleuropein aglycone (Table 3). According to ANOVA tests, these differences were significant. Other simple phenols, such as vanillic, vanillin, ferulic acid and *p*-coumaric acid, were detected in very low amounts.

Concerning lignans, the pinoresinol content varied between 0.14 (403 m) and 0.42 mg/kg (379 m), whereas acetoxypinoresinol was detected in larger quantities, ranging between 0.34 (10 m) and 2.14 mg/kg (379 m). Pinoresinol and acetoxypinoresinol were recently described in the olive oil of other cultivars (Brenes, 2000; Owen, 2000). According to Owen et al. (2000), they form the main components of the phenolic fraction of the olive seeds, while they are completely absent in the flesh, leaves and branches. Therefore their presence in the oil is due to the breaking of the pits when the olives are crushed.

The extra virgin olive oil obtained from Chétoui olives collected at different altitudes showed quite high total phenols content, exceeding sometimes 800 mg kg^{-1} (Folin-Ciocalteu method). The total phenol content was positively correlated with the altitude, ranging from 817.33 mg/kg (403 m) to 131.91 mg/kg (10 m) (Table 1). These results are in good agreement with those reported for the Cornicabra variety (Salvador et al., 2001). Similar mean values were also reported for Burlat and Van Cherries by Goncalves et al. (2007) and Gao and Mazza (1995), while lower values for Burlat were reported by Usenik et al. (2008).

As it was hypothesised, it is possible that the lower temperatures of the higher elevation sites trigger the mechanism for producing more phenolics. Similarly, cold stress induced the accumulation of phenolic compounds in apples (Thomai et al., 1998), tomato and watermelon

Table 4. Relationship between content in some phenolic compounds, radical scavenging activity and oxidative stability of the VOO samples obtained from Chetoui variety cultivated at different altitudes. The results are expressed as mean ± standard deviation of 3 samples.

Phenolic compound	Scavenging activity	Oxidative stability
OH. Tyrosol	0.80	0.89
Tyrosol	0.91	0.91
Vanilic	0.81	- 0.73
Vanillin	0.60	- 0.68
p. coumaric	0.71	0.70
Ferulic	0.83	0.67
Dialdehydic form of oleuropein aglycon	0.81	0.90
Dialdehydic form of ligstroside aglycon	0.80	0.91
Pinoresinol	0.17	0.06
Acetoxypinoresinol	0.83	0.83
Total phenol	0.92	0.86
o-diphenols	0.96	0.94
MUFA/PUFA	0.88	0.84

plants (Rivero et al., 2001).

o-Diphenols can be identified as the main chemicals contributing to the overall antioxidant activity of extra virgin olive oils (Servili et al., 2004) and may therefore play a major role in the preservation of the oils and influencing their organoleptic characteristics. Their amount behaved similarly to that of total phenols (Table 1), with values about 63% higher at the highest altitude (282.82 vs 40.51 mg/kg), in agreement with the results reported for other cultivars (Duran, 1990; Osman et al., 1994).

Oxidative stability of the oils was positively correlated to hydroxytyrosol (r = 0.89), tyrosol (r = 0.91), *p*-coumaric acid (r = 0.7), ferulic acid (r = 0.67), vanillin acid (r = 0.83), dialdehydic form of oleuropein aglycon (r = 0.9) and dialdehydic form of ligstroside aglycon (r = 0.91), and negatively correlated to vanillic acid (r = 0.73) and vanillin (r = 0.68) (Table 4). The results of the antioxidant tests of the phenolics suggest that the Chétoui olive oil part of the antioxidant effectiveness of these chemicals on the oil itself can be explained by their radical scavenging activity, as also observed by Morello et al. (2005) with the DPPH test. Radical scavenging activity was positively correlated to tyrosol (r = 0.91), hydroxytyrosol (r = 0.8), vanillic acid (r = 0.81), ferulic acid (r = 0.83), dialdehydic form of ligstroside aglycone (r = 0.81), dialdehydic form of oleuropein aglycone (r = 0.8) and *p*-coumaric acid (r = 0.71). On the contrary, radical scavenging activity was nega-ztively correlated to vanillic acid (r = 0.6) (Table 4).

Discriminant analysis

The application of the principal component analysis algorithm to data and clustering of olive oil samples using all collected data showed three distinctive groups (Figures 1 and 2). By the observation of eigenvalues, it can be concluded that two factors were sufficient to account for 82.44% of the total variance (Factor 1: 63.87%; Factor 2: 18.57%). The first group was composed by Chetoui oils obtained from plants cultivated at the lowest altitudes, the second one by plants grown at average altitudes (about 300 m) and the third one composed by the single sample growing at the highest altitude (>400 m), (Figure 2). These results imply a great difference in terms of fatty acid composition, phenols compounds, *o*-diphenols content and oxidative stability of the oils characterizing these varieties. The PCA algorithm (Figure 1) explains this classification. Indeed, the first group (lower altitude) was correlated with C18:2, poly unsaturated fatty acids (PUFA), C16:1, C18:3, vanillic and vanillin, saturated fatty acid (SFA), PV and K232. The second group (average altitude) was correlated with caffeic, C18:1 and MUFA. The last group (highest altitude) was correlated with C18:1, tyrosol, hydroxytyrosol, carotenoids, total phenols, *o*-diphenols, oxidative stability, MUFA/PUFA and *p*-coumaric acid. These conclusions are in good agreement with the results presented in (Tables 1, 2 and 3).

Conclusion

Summarizing, the elevation of the cultivation site has a marked influence on the antioxidant content, with higher elevation orchards producing olives with larger contents of antioxidant compounds with respect to lower elevation ones. These results indicate that the cultivar and the environmental conditions influence the quali-quantitative production of phenolic compounds. Consequently, phenolic compounds may be used as altitude indicators for Chetoui oils.

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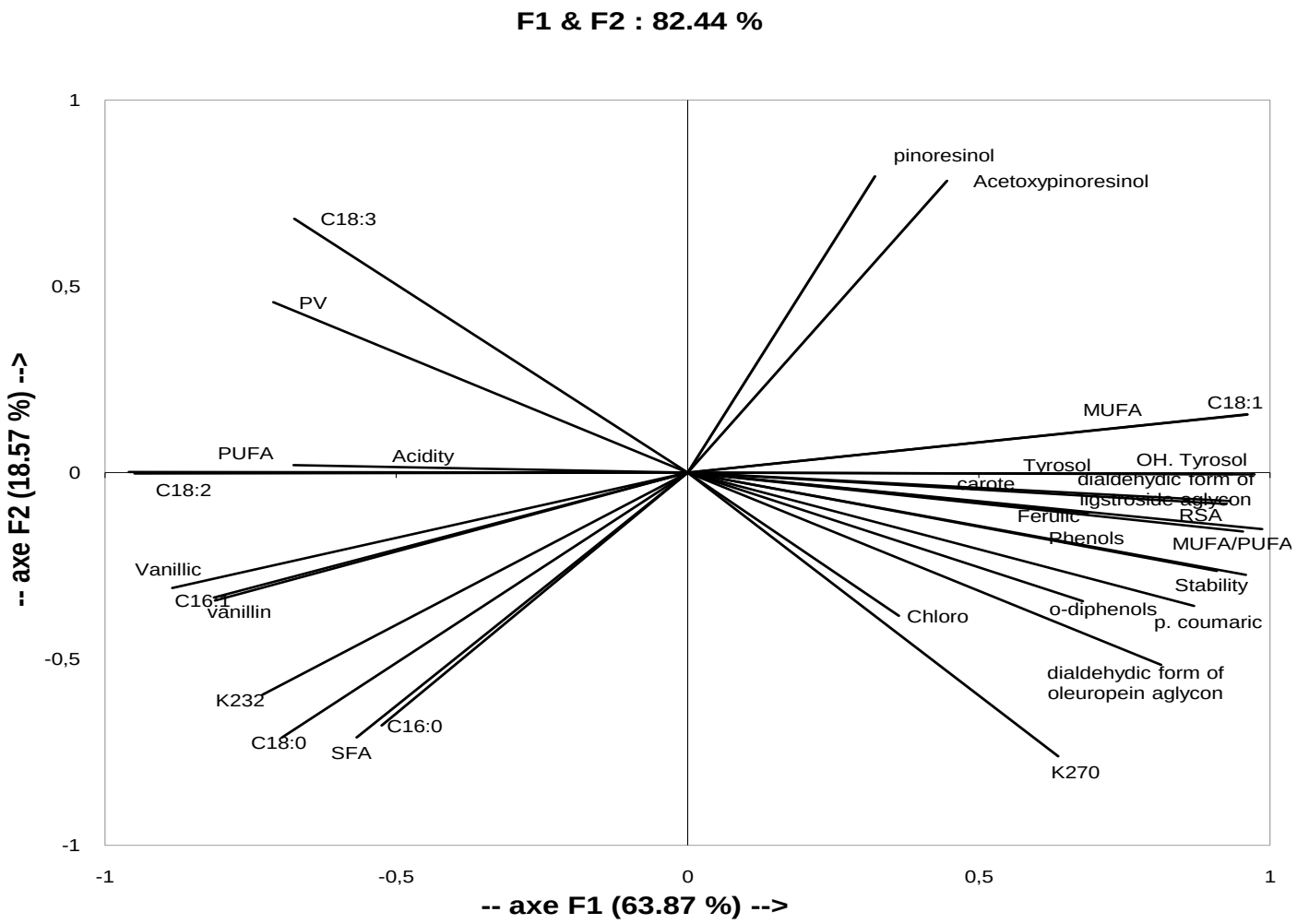

Figure 1. Principal component analysis algorithm. Hydroxytyrosol, tyrosol, *p*-coumaric acid, ferulic acid, dialdehydic form of oleuropein aglycon, dialdehydic form of ligstroside aglycon, Pinoresinol, Acetoxypinoresinol, vanillic acid, vanillin acid, acidity, K232, K270, chlorophylls, oxidative stability, radical scavenging activity, fatty acid, SFA and MUFA/PUFA.

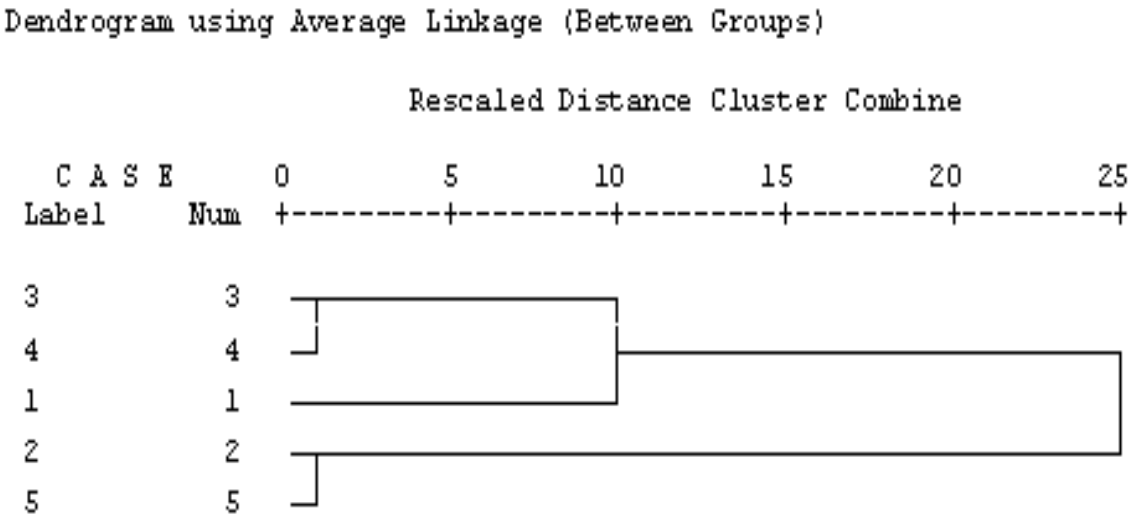

Figure 2. Clustering of olive oil samples using all collected data.1, 403 m; 2, 10 m; 3, 354 m; 4, 379 m; 5, 21 m.

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REFERENCES

Aparicio R, Alonso V, Morales MT (1994). Detailed and exhaustive study of European virgin olive oils by SEXIA expert system. Grasasy Aceites. 45: 241-252.

Baccouri O, Cerretani L, Bendini A, Lercker G, Zarrouk M, Ben Miled DD (2008). Phenol content as correlated to antioxidant activity and gustative characteristics of Tunisian monovarietal virgin olive oils. Ital. J. Fatty Subst. 85: 189-195.

Baldioli M, Servili M, Perretti G, Montedoro G (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. J. Am. Oil Chem. Soc. 73: 1589-1593.

Bandelj D, Jakse J, Javornik B (2002). DNA fingerprinting of olive varieties by microsatellite markers. Food Technol. Biotechnol. 40: 185-190.

Ben Temime S, Campeol E, Cioni PL, Daoud D, Zarrouk M (2006). Volatile compounds from Chetoui olive oil and variations induced by growing area. J. Food Chem. 99: 315-325.

Ben Temime S, Manai H, Methenni K, Baccouri B, Abaza L, Daoud D, Zarrouk M (2008). Sterolic composition of Chetoui virgin olive oil: Influence of geographical origin. J. Food Chem. 110: 368-374.

Bendini A, Cerretani L, Carrasco-Pancorbo A, Gómez-Caravaca AM, Segura-Carretero, A (2007). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules, 12: 1679-1719.

Carlsson DJ, Suprunchuk T, Wiles HM 1976). Photooxidation of unsaturated oils: Effect of single oxygen quenchers. J. Am. Oil Chem. Soc. 53: 656-695.

Cercaci L, Passalacqua G, Poerio A, Rodriguez-Estrada MT, and Lercker 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. J. Food Chem. 102: 66-76.

Christopoulou E, Lazaraki M, Komaitis M, Kaselimis K (2004). Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. Food Chem. 84: 463-474.

Del Giovine L, and Fabietti F (2005). Copper chlorophyll in olive oils: Identification and determination by LIF capillary electrophoresis. Food Control, 16: 267-272.

Duran RM (1990). Relationship between the composition and ripening of the olive and quality of the oil. Acta Hortic. 286: 441-451.

EEC (1991). Characteristics of olive oil and olive pomace and their analytical methods. Regulation EEC/2568/91 and latter modifications. Official J. Euro. Commu. L. 248: 1-82.

EEC (1992). European Union Commission. Regulation EEC/1429/92 amending Regulation EEC2568/91 on the characteristics of olive oil and olive residue oil and on the relevant methods of analysis. Official J. Euro. Commu. L. 150: 17-20.

Fedeli E (1977). Lipids of olives. Program. Chem. Fats Other Lipids, 15: 57-74.

Gao L, Mazza G (1995). Characterization, quantification and distribution of anthocyanins and colorless phenolics in sweet cherries. J. Agric. Food Chem. 43: 343-346

Giovannini C, Scazzocchio B, Matarrese P, Vari R, D'Archivio M(2008). Apoptosis induced by oxidized lipids is associated with up-regulation of p66Shc in intestinal Caco-2 cells: Protective effects of phenolic compounds. J. Nutr. Biochem. 19: 118-128.

Goncalves B, Silva AP, Moutinho-Pereira J, Bacelar E, Rosa E, Meyer AS, (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (Prunus avium L.). J. Food Chem. 103: 976-984.

Gutfinger T (1981). Polyphenols in olive oils. J. Am. Oil Chem. Soc. 58: 966-968.

Gutiérrez F (1989). Determinación de la estabilidad oxidativa de aceites de oliva vírgenes: Comparación entre del método A.O.M. yel método Rancimat. Grasas Aceites. 40: 1-5.

Huang CL, Sumpio BE (2008). Olive oil, the Mediterranean 309 diet and cardiovascular health. J. Am. Coll. Surg. 207: 407-416.

Interesse FS, Ruggier P, Vitagliano M, (1971). Autoxidation of olive oil: Influence of chlorophyll pigment. Ind. Agrar. 9: 318-324.

Kalantzakis G, Blekas G, Pegklidou K, Boskou D (2006). Stability and radical scavenging activity of heated olive oil and other vegetable oils. DOI: 10.1002/ejlt.200500314. Eur. J. Lipid Sci. Technol. 108: 329-335.

Landrum JT, Bone RA (2001). Lutein, zeaxanthin, and the macular pigment. doi:10.1006/abbi.2000.2171. PMID:11361022. Arch. Bioch. Biophys. 385: 28-40.

Mateos R, Espartero JL, Trujillo M, Rios JJ, Leon-Camacho M (2001). Determination of phenols, flavones and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection, 49: 2185-2192

Mayne ST, Janerich DT, Greenwald P, Chorost S, Tucci C (1996). Dietary beta carotene and lung cancer risk in U.S. nonsmokers. doi: 10.1093/jnci/86.1.33. J. Nat. Cancer Inst. 86: 33-38.

Medeiros MD (2001). Olive Oil and Health Benefits. In The Handbook of Nutraceuticals and Functional Foods, Wildman, R.E.C. (Ed.). CRC Press, Boca Raton, FL, Florida, USA. pp. 261-267.

Minguez-Mosquera MI, Rejano L, Gandul B, Higino A, Garrido J, (1991). Color-pigment correlation in virgin olive oil. J. Am. Oil Chem. Soc. 68: 332-336.

Montedoro G, Cantarelli C (1969). Indagini sulle sostanze fenoliche presenti negli oli di oliva. Riv. Ita. Sostanze Grasse. 46: 115-124.

Morello JR, Vuorela S, Romero MP, Motilva MJ, Heinonen M (2005). Antioxidant activity of olive pulp and olive oil phenolic compounds of the Arbequina cultivar. J. Sci. Food Agric. 53: 2002-2008.

Ortega-Garcia F, Peragon J (2009). The response of phenylalanine ammonia-lyase, polyphenol oxidase and phenols to cold stress in the olive tree (Olea europaea> L. cv. Picual). DOI: 10.1002/jsfa.3625, J. Sci. Food Agric. 89: 1565-1573.

Osman M, Metzidakis I, Gerasopoulos D, Kiritsakis A (1994). Qualitative changes in olive oil collected from trees grown at two altitudes. Riu. Ital. Sost. Gras. 71: 187-190.

Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H (2000). Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem. Toxicol. 38: 647-659.

Perez-Jimenez F, De Cienfuegos GA, Badimon L, Barja G, Battino M (2005). International conference on the healthy effect of virgin olive oil. Eur. J. Clin. Invest. 35: 421-424.

Perona J, Cabello-Moruno R, Ruiz-Gutierrez V (2006). The role of virgin olive oil components in the modulation of endothelial function. J. Nutr. Biochem. 17: 429-445

Ranalli A, De Mattia G, Patumi M, Proietti P (1999). Quality of virgin olive oil as influenced by origin area. Grasas Aceites, 50: 249-259.

Rivero RM, Ruiz JM, Garcia PC, Lopez-Lefebre LR, Sanchez E, Romero L (2001). Resistance to cold and heat stress: Accumulation of phenolic compounds in tomato and watermelon plants. Plant Sci. 160: 315-321.

Sacchi R, Mannina L, Fiordiponti P, Barone P, Paolillo L (1998). Characterization of italian extra virgin olive oils using 1H-NMR spectroscopy. J. Sci. Food Agric. 46: 3947-3951.

Salvador MD, Aranda F, Fregapane G (2001). Influence of fruit ripening on Cornicabra virgin olive oil quality: A study of four crop seasons. J. Food Chem. 73: 45-53.

Salvador MD, 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. J. Food Chem. 80: 359-366.

Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro G, Morozzi G (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that

affect their occurence in the oil. J. A. Chromatogr. 1054: 113-127.
Thomai T, Sfakiotakis E, Diamantidis G, Vasilakakis M (1998). Effects of low preharvest temperature on scald susceptibility and biochemical changes in Granny Smith apple peel. doi:10.1016/S0304-4238(98)00133-2. Sci. Hortic. 76: 1-15.
Tovar MJ, Romero MP, Alegre S, Girona J, Motilva MJ (2002). Organoleptic characteristic of oil from Arbequina olive treesolive trees under deficit irrigation. J. Sci. Food Agric. 82: 1755-1763.
Tsimidou M, Karakostas KX (1993). Geographical classification of Greek virgin olive oil by non-parametric multivariate evaluation of fatty acid composition. J. Sci. Food Agric.. 62: 253-257.
Usenik V, Fabcic J, Stampar F (2008). Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry. doi:10.1016/j.foodchem.2007.08.004. J. Food Chem. 107: 185-192.
Youssef O, Guido F, Manel I, Youssef NB, Luigi CP (2011). Volatile compounds and compositional quality of virgin olive oil from Oueslati variety: Influence of geographical origin. Food Chem. 124: 1770-1776.