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Analytical Methods

Virgin olive oil (VOO) production in Tunisia: The commercial potential of the major olive varieties from the arid Tataouine zone

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ABSTRACT

The commercial potential of olive oils from three consecutive crop years, derived from the main autochthonous olive varieties Chemlali Tataouine, Fakhari Douirat, Zarrazi Douirat and Dhokar Douirat grown in the arid region of Tataounine (Tunisia) was examined with regards to stability and nutrition aspects. Several characteristics such as fatty acid composition, the extremely high phenol-type antioxidant content, and the low levels of green pigments suggested that these oils, the only fat source for the local people, are of promising composition. Efforts to develop commercial products from these varieties could improve antioxidant intake of the local population. The introduction of Good Manufacturing Practices is a prerequisite so that the quality observed for oils obtained by an Abencor system to be achieved also in real life. This can be feasible through appropriate oleoculture, which is often the only viable agricultural activity in remote arid regions such as Tataouine.

1. 1.Introduction

Tunisia is the northernmost African country on the Mediterranean coast (Fig. 1) and the smallest (163,600 km^2) among those situated along the Atlas mountain line. The Sahara desert occupies ~40% of Tunisian territory, while the rest largely consists of particularly fertile soil. The Mediterranean coastline (1300 km) is characterized by moderate temperatures, an important factor in agriculture.

Agriculture is one of the mainstays of the Tunisian economy, and cultivation of the olive tree constitutes one of the principal economical and agricultural sectors. Because of the plasticity of the Tunisian olive oil varieties, the olive-growing areas spread from the northern to the southern regions, where a wide range of edapho-climatic conditions prevail, from lower semi-arid to arid conditions. Thus, about 65 million trees are distributed and spread over 1.6 million hectares, representing the one-third of the cultivated area. Olive farming accounts for 57% of the active rural population and ensures 45% of the agricultural exports with an average of 120,000 T of olive oil per year (COI, 2000). Traditional oleoculture uses autochthonous varieties to produce oil exclusively. Tunisia is next to Spain, Italy and Greece in the world olive oil sector regarding the number of trees and olive oil production; Tunisia produced 144,500 T per year between 2000 and 2006 (Codex Alimenarius Commission, 2007), accounting for ~5.2% of world production and is considered to rank second one with regard to surface use.

Although Tunisia possesses substantial genetic diversity among its olive tree varieties, its oleoculture depends on only two: Chétoui in the northern regions and Chemlali in the central and southern parts. These two varieties account for 95% of the total olive tree orchards and contribute more than 90% of the national production of olive oil. Chemlali alone, covering 60% of the olive-growing surface, is spread from the northeast to the extreme south. Almost all studies on Tunisian virgin olive oil have focused on characterization and/or improvement of these two varieties (Baccouri et al., 2007; Ben Temime et al., 2006; Manaï, Haddada, Trigui, Daoud, & Zarrouk, 2007). Other studies have also examined some of the well-known secondary varieties like Chemcheli, Gerboui, Zalmati and Oueslati (Abaza, Msallem, Daoud, & Zarrouk, 2002).

As a consequence, there is a lack of information on the chemical characteristics of several minor varieties that are sustained in restricted areas in different parts of Tunisia. A good example is the case of Tataouine, a region of southern Tunisia (Fig. 1) bordering Algeria and Libya. Because of the severe pedoclimatic conditions (shallow soils, high temperature and low rainfall), the traditional diet in the region is rather simple and is based on some specific plant foods, like cereals or palm fruits, olive oil being the main fat source. For the local people, virgin olive oil is the everyday

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Fig. 1. Position of Tunisia in the world.

source of an array of micronutrients, especially antioxidants in the form of phenolic compounds, vitamin E and carotenes. Although Tataouine is the largest Tunisian governorate (24% of the total area), its olive oil production amounts to only ~150 T per year, corresponding to 0.15% of the total Tunisian olive oil production. Varieties of that region are still commercially unexplored, and their VOO qualities have not been fully characterized.

The present study was motivated by the fact that a rise in temperature, low rainfall and soil erosion are becoming the principal environmental problems associated with olive farming in Mediterranean regions (Tombesi, Michelakis, & Pastor, 1996) and that the genetic heritage of olive tree diversity must be protected from disappearance. Therefore this study assesses the commercial potential of the main olive varieties of the arid Tataouine zone: Chemlali Tataouine, Fakhari Douirat, Zarrazi Douirat and Dhokar Douirat (Trigui & Msallem, 2002). The ultimate objective of this work is to improve local people's diet and income and maintain them in their homelands because oleoculture not only contributes largely to these objectives but is often the only viable agricultural activity in such difficult climates. Studying stability of the above oils with regard to fatty acid composition and to pro-oxidant/antioxidant content of oils from these autochthonous varieties for three consecutive years is the first step to present their commercial potential to producers and draw official attention. A biochemical comparison of these oils with the main Tunisian variety, Chemlali, was also carried out.

2. Materials and methods

2.1. Samples

The analysis was applied to samples of VOOs from the South of Tunisia. The VOO samples chosen for this study were from selected olive varieties: Fakhari Douirat, Dhokar Douirat, Zarrazi Douirat, Chemlali Tataouine, and the main Tunisian variety, Chemlali. The first four cultivars were grown in the locality of Douirat in an arid zone under the same pedoclimatic conditions. Chemlali was grown in the region of Sfax (South of Tunisia). All varieties of olive fruits were harvested by hand from the trees. Only undamaged, healthy drupes were selected. When necessary, sampling was repeated for three consecutive crop seasons (2004–2006) to have representative samples and to see the effect of climatic changes from one year to another on oil characteristics, and two stages of maturity (A and B). Group A included oils obtained from semi-green olives (maturity index (MI) = 2.50–3.55), and group B included oils from mature olives (MI = 4.00–4.63). Maturity index was determined according to the method developed by the Agronomic Station of Jaén as function of fruit colour in both skin and pulp (Uceda & Hermoso, 1998). After harvesting, the olive fruit samples were immediately transported to the laboratory mill, where oil was extracted within 24 h under similar extraction conditions using an Abencor analyser (MC2 Ingenieria y Sistemas, S.L., Sevilla, Spain). A portion of olives (1.5–2 kg) was crushed with a hammer mill, slowly mixed for 30 min at ambient temperature, centrifuged without addition of water or chemicals; then this oil was transferred into dark glass bottles. The bottles were completely filled and stored in the freezer until analysis. Because of the cloudiness of the oil, all the oil samples were filtered in the laboratory using common filter paper before analysis. Sample filtration was performed in the dark.

2.2. Solvents and standards

HPLC grade solvents were used without further purification. *n*-Hexane (95%) and 2-propanol (Chromasolv) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany); acetonitrile, acetone and diethyl ether were from Merck KGaA (Darmstadt, Germany). Isooctane and ethanol were from Riedel-de Haën (Seelze, Germany) and ethyl acetate was obtained from Scharlau Chemie S.A. (Barcelona, Spain). The solvents were of appropriate purity. Squalene (98–100%) and chlorophyll *a* (Chl *a*) from Anacystis nidulans algae (free of chlorophyll *b*) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DL-α-Tocopherol (⩾98%) and β-carotene (⩾97.0%) for HPLC were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Fatty acid methyl esters (FAMEs) standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pheophytin α (Pheo α) was obtained by acid treatment of the respective chlorophyll solution (Canjura & Schwartz, 1991).

2.3. Apparatus

Squalene determination was carried out using a solvent delivery system that consisted of a liquid chromatography pump (LC-10AV*vp*, Shimadzu Co., Kyoto, Japan) that was equipped with an injection valve with a 20 μL loop (Model 7125, Rheodyne Cotati, CA, USA). The liquid chromatography system was equipped with a dual-wavelength UV–Vis spectrophotometric detector (SPD-10AV, Shimadzu Co., Kyoto, Japan). A Hewlett Packard 3396 Series II electronic integrator (Avondale, PA, USA) was used to record and quantify the chromatographic peaks.

The solvent delivery system to determine α-tocopherol and pigments consisted of two Marathon IV series HPLC pumps (Rigas Labs, Thessaloniki, Greece), equipped with an injection valve with a 20 μL loop (Model 7125, Rheodyne Cotati, CA, USA). The liquid chromatography system was equipped with a Diode Array Linear UVIS-206 Multiple Wavelength Detector (Linear Instr., Fermont, CA). Column temperature was controlled with an SSI Model 505 column oven. The data were stored and processed using the chromatographic software EZChrom (Scientific Software, Inc., San Ramon, CA, USA).

A Model 1601 UV/VIS spectrophotometer (Shimadzu Co., Kyoto, Japan), together with UV 1601 PC software, was used for spectra recording and analysis. The system was equipped with rectangular quartz cuvettes (optical length 1 cm).

2.4. NP-HPLC of tocopherols

The isocratic elution system was *n*-hexane/2-propanol (99:1, v/v). Separation was achieved on a LiChrospher Si column (particle size 5 μm, 250 × 4.0 mm i.d., Analyzentechnik, Mainz, Germany) maintained at 30 °C, using a flow rate of 1.2 mL/min. The injection volume was 10 μL. Tocopherols were detected at 294 nm by the diode array detector. Peak identification and purity assessment were based on photodiode array spectroscopic data, retention time and peak spiking with authentic standards as previously described (Psomiadou, Tsimidou, & Boskou, 2000). Standards and samples were prepared for analysis by dilution into the elution solvent. Sample solutions were filtered through a 0.45 μm membrane filter (Schleicher & Schuell, Dassel, Germany) just before HPLC analysis. Care was taken to protect samples and standard solutions from sunlight exposure throughout the analytical procedure. α-Tocopherol was quantified with the use of a standard curve (concentration *vs.* peak area, 2.5–25 mg/L) calculated by linear regression analysis ($y = 243.34x + 1652.4$, $R^2 = 0.99$). Repeatability of a standard solution (injection volume) within the same day was satisfactory ($C = 5$ mg/L, CV% = 2.8%, $n = 5$). To determine measurement precision, three olive oil samples were tested three times each, and the calculated coefficients of variation (5.0%, 4.5% and 2.6%) were satisfactory. Each sample solution was then chromatographed in triplicate. The concentration of α-tocopherol standard solutions was calculated from absorbance values at 292 nm divided by a correction factor of 0.0076, as suggested by Pocklington and Dieffenbacher (1988). The calibration curve was tested daily.

2.5. NP-HPLC of pigments

The mixture *n*-hexane/2-propanol (99:1, v/v) (A) and 2-propanol (B) were used as eluents. The gradient used was 0% B for 10 min, increasing to 5% B in 4 min, 5% B for 6 min, decreasing back to 0% B in 4 min and 0% B for 6 min. Separation was achieved on a LiChrospher-100 Si column (particle size 5 μm, 250 × 4.0 mm i.d., Analyzentechnik, Mainz, Germany) maintained at 30 °C, using a flow rate of 1.2 mL/min. The injection volume was 10 μL. Samples were prepared in solvent A and filtered through a 0.45 μm membrane filter (Schleicher & Schuell, Dassel, Germany) just before HPLC analysis. Care was taken to protect samples and standard solutions from sunlight exposure throughout the analytical procedure. Pheo α standards were prepared in diethyl ether. β-Carotene was first dissolved in a small quantity of tetrahydrofuran and then in solvent A. Peak identification and purity was based on photodiode array spectroscopic data (absorption maxima and peak ratios), retention time and peak spiking with authentic standards as previously described (Psomiadou & Tsimidou, 1998). Quantification was carried out at 410 nm (Pheo *a*) and 453 nm (carotenoids). Due to the lack of an available lutein standard, its quantification was based on a β-carotene calibration curve. Standard curves of concentration *vs* peak area (0.25–5.0 mg/L for β-carotene and 0.7–10.0 mg/L for Pheo *a*) were calculated by linear regression analysis ($y = 9430.6x - 760.91$, $R^2 = 1$ for β-carotene and $y = 3457.4x - 3556$, $R^2 = 0.99$ for Pheo *a*). Repeatability of standard solutions (injection volume) within the same day was satisfactory ($C = 2.5$ mg/L, CV% = 1.8, $n = 5$ for β-carotene and $C = 1.0$ mg/L, CV% = 3.9, $n = 5$ for Pheo *a*). Each sample solution was then tested once. Control of standard chlorophyll solution concentrations was carried out through spectrometry using extinction coefficients from the literature (Watanabe et al., 1984). Calibration curves were tested daily.

2.6. Quantification of squalene

2.6.1. Sample preparation (Manzi, Panfili, Esti, & Pizzoferrato, 1998)

About 0.1 g of oil was weighed in a 25-mL ground–glass stoppered test tube. Then 3 mL of potassium hydroxide (600 g/L), 2 mL of ethanol and 5 mL of an ethanolic pyrogallol solution (60 g/L) were added to the glass tube. The tube was flushed with nitrogen. After alkaline digestion at 70 °C for 30 min, 15 mL of NaCl (10 g/L) were added, and the suspension was extracted twice with 15 mL of a mixture of *n*-hexane/ethyl acetate (9:1, v/v). The organic phase was evaporated to dryness, and the residue was dissolved in a mixture of acetone/acetonitrile (50:50, v/v) for HPLC analysis (Grigoriadou, Androulaki, Psomiadou, & Tsimidou, 2007). To determine precision, saponification of an olive oil sample was carried out five times. Because the calculated coefficient of variation (6.4%, $n = 5$) was found to be satisfactory, each sample was then saponified once.

2.6.2. RP-HPLC of squalene

Squalene determination was accomplished on a reverse phase Nucleosil C_{18} column (particle size 5 μm, 125 × 4.0 mm i.d., Macherey-Nagel, Düren, Germany). The elution solvent was 100% acetonitrile; the flow rate, 1.2 mL/min; and the injection volume, 10 μL. Detection was achieved at 208 nm, and quantification was carried out using a standard curve [concentration (2.5–75 mg/L) *vs.* peak area] constructed by linear regression analysis ($y = 3 \times 10^7 x + 171{,}719$, $R^2 = 0.99$). Repeatability of injection volume of a standard solution was found to be satisfactory ($C = 25$ mg/L, CV% = 0.3, $n = 5$). Each sample solution was then tested once.

2.7. Spectrometric estimation of chlorophyll content

The content of chlorophyll pigments was estimated from the equation *C* (mg of Pheo *a*/kg of oil) = $345.3[A_{670} - (A_{630} + A_{710})/2]/L$, where A_i is the absorbance of the oil at the respective wavelength and *L* is the cell thickness (mm) (IUPAC, 1987). The repeatability of the determination of total chlorophyll content was checked for five replicates and was 2.8%.

2.8. Determination of fatty acid composition

The fatty acid composition of the oil was determined by gas chromatography (GC) as FAMEs. FAMEs were prepared by vigorous shaking of a solution of each olive oil sample in *n*-hexane (0.2 g in 3 mL) with 0.4 mL 2 N methanolic potassium hydroxide solution. Chromatographic analysis was performed on a Hewlett Packard 4890D gas chromatograph equipped with a flame ionization detector (Hewlett Packard, Palo Alto, CA, USA), using a fused-silica capillary column (30 m × 0.25 μm i.d. × 0.25 μm film thickness, HP Supelco, Inc., Bellefonte, PA, USA). The injector and detector temperatures were maintained at 240 °C and 260 °C, respectively; the oven temperature was set at 210 °C. Nitrogen was employed as the carrier gas with a flow rate of 1 mL min^{-1} according to the method of European Regulation 2568/91 (EEC, 1991) and subsequent amendments. Fatty acids were identified by comparing retention times with those of standard compounds.

2.9. Other quality characteristics of the olive oil samples

Quality of the samples was evaluated based on measurements of acidity, PV and absorbance at 232 and 270 nm (EEC, 1991). The content of total polar phenol and sterols were determined

according to procedures described by Psomiadou and Tsimidou (2002a) and Sanchez Casas, Osorio Bueno, Montano Garcia, and Martinez Cano (2004), respectively.

2.10. Determination of oil oxidative stability

Oxidative stability was evaluated by the Rancimat apparatus (model 743, Metrohm Co., Basel, Switzerland). Stability was expressed as the oxidation induction time (hours) using 3.5 g of oil. The temperature was set at 102 °C, and the air flow rate was 10 L per hour.

2.11. Radical scavenging capacity of the virgin olive oil samples

The olive oil samples were examined for their capacity to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) (Kalantzakis, Blekas, Pegklidou, & Boskou, 2006). Of the oil solution in ethyl acetate (10%, wt/vol), 1 mL 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 shaken vigorously for 10 s in a Vortex apparatus, and the tube was maintained in the dark for 30 min, after which 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 measured daily. 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 % reduction in DPPH· concentration by the constituents of the oils: $\%\ [DPPH^{\cdot}]_{red} = 100 \times (1 - [DPPH^{\cdot}]_{30}/[DPPH^{\cdot}]_0)$, where $[DPPH^{\cdot}]_0$ and $[DPPH^{\cdot}]_{30}$ were the concentrations of DPPH· in the control sample ($t = 0$) and in the test mixture after the 30 min reaction, respectively.

2.12. Statistical analysis

Comparisons of the mean values were performed by one-way analysis of variance, followed by the multiple Duncan test ($p < 0.05$ confidence level) (SPSS 16.0, (SPSS Inc., 2007).

3. Results and discussion

The arid zone of Tataouine (latitude 32°55′ north; longitude 10°09′ east; 80 km south-east from the sea, chalky grounds), especially the locality of Douirat, is surrounded by mountains (see Supplementary material 1).

In this region, the cultivation of the olive tree cannot be considered typical. In fact, because of the lack of rainfall (Fig. 2) and the limited groundwater supply, growing takes place in depressions (see Supplementary material 2) to obtain the maximum available water. It is not rare to observe only a few olive trees per depression. The morphology of the olive tree is unusual, different than what is seen in European orchards. The trees are very high and the branches surround the tree, which looks like a sphere (see Supplementary material 3). In the present study, olives of two maturity stages were harvested from the different tree varieties on the same day because the area surrounding the locality of Douirat is not easily accessible. Olive ripeness was taken into account because the oil composition is less affected by variety at later stages of ripeness (Inglese, 1994). The samples were transferred to the laboratory, and the oil was extracted by the Abencor system within 24 h after sampling. Using the Abencor system meant that the same extraction conditions for all of the samples could be used and that the results could easily be compared with other published data for Tunisian VOOs. It should be noted that the Dhokar Douirat variety is used as a pollinator for the Chemlali Tataouine variety and is grown as a very small number of trees; because of the limited quantity of oil extracted, it was impossible to carry out analyses of all the parameters studied for this variety.

Analytical data for the chemical composition and other quality indices of the examined VOOs are presented in Tables 1–3.

3.1. Fatty acid composition

Table 1 presents the results of FAME content for all VOOs for three crop years (2004–2006). The last crop year oils were obtained from two different maturity index olives (A: semi-green, B: black). Differences in the composition of the major fatty acids were discussed using the main Tunisian variety, Chemlali, as the point of reference.

The Chemlali variety yields oils of rather low oleic acid content and high concentrations of linoleic and palmitic acids (Baccouri et al., 2007; Manaï et al., 2007). Fatty acid composition of Dhokar extracted during the two crop seasons (2004 and 2006) resembled that of the main Tunisian variety oil. However, the oils from the three other varieties had high monounsaturated fatty acid (MUFA) content and a moderate level of linoleic acid, in accordance with the general observations for oils from the south of producing countries like Spain, Greece and Italy (Giansante, Di Vincenzo, & Bianchi, 2003; Psomiadou, Karakostas, Blekas, Tsimidou, & Boskou, 2003).

Compared to some secondary Tunisian VOOs (Abaza et al., 2002; Krichen et al., 2007), Chemlali Tataouine, Fakhari Douirat

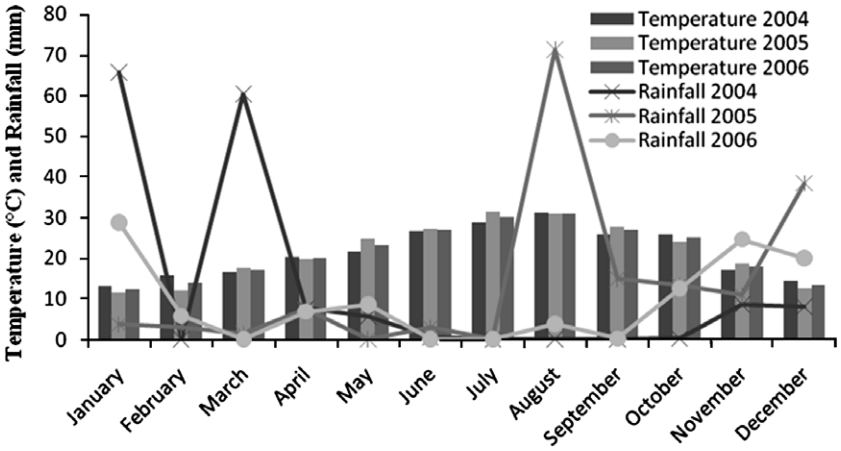

Fig. 2. Registered temperature and rainfall in the region of Tataouine. Monthly values are the mean of three consecutive crops (2004–2006).

Table 1
Fatty acid composition of virgin olive oil samples from the region of Tataouine

	Chemlali Tataouine				Fakhari Douirat			
	2004	2005	2006		2004	2005	2006	
	A	A	A	B	A	A	A	B
$C_{16:0}$ (%)	15.24 ± 0.80	13.87 ± 0.47	14.08 ± 0.67	16.06 ± 0.78	12.79 ± 0.54	11.47 ± 0.70	11.50 ± 1.10	10.82 ± 1.65
$C_{16:1}$ (%)	1.85 ± 0.07	1.83 ± 0.04	1.54 ± 0.21	1.75 ± 0.30	0.77 ± 0.03	0.92 ± 0.04	0.63 ± 0.08	0.62 ± 0.06
$C_{18:0}$ (%)	3.6 ± 0.33	3.10 ± 0.14	3.14 ± 0.48	3.44 ± 0.44	4.12 ± 0.27	3.58 ± 0.35	3.64 ± 0.18	3.81 ± 0.21
$C_{18:1}$ (%)	67.12 ± 2.22	72.68 ± 1.33	73.40 ± 2.72	66.88 ± 1.97	69.81 ± 1.65	73.62 ± 1.71	73.74 ± 2.47	72.76 ± 1.61
$C_{18:2}$ (%)	11.05 ± 0.52	7.41 ± 0.69	6.92 ± 0.80	10.84 ± 0.46	11.27 ± 0.62	9.30 ± 1.08	9.06 ± 0.54	10.59 ± 0.67
$C_{18:3}$ (%)	0.61 ± 0.13	0.65 ± 0.04	0.51 ± 0.08	0.52 ± 0.07	0.61 ± 0.05	0.62 ± 0.10	0.65 ± 0.04	0.73 ± 0.08
$C_{20:0}$ (%)	0.53 ± 0.07	0.46 ± 0.03	0.41 ± 0.06	0.51 ± 0.07	0.63 ± 0.18	0.50 ± 0.04	0.78 ± 0.02	0.67 ± 0.08
UFA	80.63 ± 2.93	82.57 ± 0.62	82.37 ± 3.42	79.99 ± 2.03	82.46 ± 0.95	84.64 ± 2.29	84.08 ± 2.60	84.7 ± 2.34
MUFA	68.97 ± 2.29	74.51 ± 0.37	74.94 ± 2.73	68.63 ± 1.98	70.58 ± 1.96	74.72 ± 1.70	74.37 ± 2.41	73.38 ± 1.63
PUFA	11.66 ± 0.64	8.06 ± 0.25	7.43 ± 0.74	11.36 ± 0.52	11.88 ± 0.67	9.92 ± 1.00	9.71 ± 0.56	11.32 ± 0.72
MUFA/PUFA	5.92 ± 3.58	9.24 ± 0.24	10.09 ± 0.71	6.04 ± 0.33	5.94 ± 0.45	7.53 ± 0.75	7.66 ± 0.45	6.48 ± 0.28
SFA	19.37 ± 1.21	17.43 ± 0.62	17.63 ± 0.20	20.01 ± 0.98	17.54 ± 0.88	15.54 ± 1.04	15.92 ± 1.10	15.30 ± 1.61
$C_{18:1}/C_{18:2}$	6.07 ± 4.31	9.81 ± 0.34	10.61 ± 0.90	6.17 ± 0.28	6.19 ± 0.48	7.94 ± 0.94	8.14 ± 0.51	6.87 ± 0.30
IV	83.01 ± 0.42	82.34 ± 0.74	81.47 ± 4.26	82.96 ± 2.67	85.64 ± 0.32	96.39 ± 3.33	85.14 ± 3.29	87.24 ± 1.77
	Zarrazi Douirat				Dhokar Douirat		Chemlali	
	2004	2005	2006		2004	2006	2006	
	A	A	A	B	A	A	A	B
$C_{16:0}$ (%)	9.95 ± 0.31	8.67 ± 0.81	10.56 ± 1.79	10.90 ± 1.97	19.84 ± 0.28	18.89 ± 1.17	16.92 ± 0.85	18.04 ± 1.29
$C_{16:1}$ (%)	0.41 ± 0.01	0.50 ± 0.08	0.22 ± 0.03	0.29 ± 0.08	2.25 ± 0.05	2.73 ± 0.44	2.52 ± 0.23	2.8 ± 0.31
$C_{18:0}$ (%)	3.44 ± 0.22	2.86 ± 0.22	3.53 ± 0.55	3.33 ± 0.29	2.95 ± 0.02	3.16 ± 0.70	2.35 ± 0.51	2.52 ± 0.10
$C_{18:1}$ (%)	71.91 ± 0.32	76.52 ± 2.23	75.25 ± 3.80	70.97 ± 1.91	49.44 ± 0.18	53.67 ± 3.20	61.57 ± 1.51	58.32 ± 2.26
$C_{18:2}$ (%)	12.34 ± 0.12	10.47 ± 0.97	9.46 ± 1.30	13.42 ± 0.77	24.23 ± 0.08	20.23 ± 1.74	15.65 ± 1.45	17.21 ± 1.06
$C_{18:3}$ (%)	0.66 ± 0.04	0.53 ± 0.07	0.53 ± 0.08	0.57 ± 0.07	0.96 ± 0.03	0.78 ± 0.08	0.58 ± 0.05	0.65 ± 0.10
$C_{20:0}$ (%)	1.29 ± 0.63	0.45 ± 0.06	0.45 ± 0.06	0.52 ± 0.06	0.42 ± 0.05	0.54 ± 0.06	0.41 ± 0.03	0.46 ± 0.04
UFA	85.32 ± 0.46	88.02 ± 2.99	85.46 ± 4.58	85.25 ± 1.66	76.88 ± 0.20	77.41 ± 1.86	80.32 ± 1.95	78.98 ± 2.95
MUFA	72.32 ± 0.14	77.02 ± 2.23	75.47 ± 3.82	71.26 ± 1.85	51.69 ± 0.16	56.40 ± 3.53	64.09 ± 1.71	61.12 ± 2.00
PUFA	13.00 ± 0.14	11.00 ± 0.97	9.99 ± 1.25	13.99 ± 0.83	25.19 ± 0.07	21.01 ± 1.67	16.23 ± 1.43	17.86 ± 0.98
MUFA/PUFA	5.56 ± 0.03	7.00 ± 0.49	7.55 ± 0.64	5.09 ± 0.39	2.05 ± 0.01	2.68 ± 0.37	3.95 ± 0.38	3.42 ± 0.08
SFA	14.68 ± 0.40	11.98 ± 1.78	14.54 ± 2.39	14.76 ± 1.74	23.21 ± 0.23	22.59 ± 1.79	19.68 ± 1.31	21.02 ± 1.31
$C_{18:1}/C_{18:2}$	5.32 ± 0.02	7.31 ± 0.53	7.95 ± 0.72	5.29 ± 0.40	2.04 ± 00.00	2.65 ± 0.00	3.93 ± 0.40	3.39 ± 0.08
IV	89.25 ± 0.84	89.73 ± 3.23	86.48 ± 3.20	89.99 ± 1.15	93.28 ± 0.18	89.89 ± 1.41	87.85 ± 3.16	88.34 ± 4.49

All data are means ± sd ($n = 3$).
SFA: saturated fatty acids sum, MUFA: monounsaturated fatty acids sum, PUFA: polyunsaturated fatty acids sum, IV: iodine value.

Table 2
Influence of crop season, maturity index and genetic factor on the quality indices of virgin olive oil samples from the region of Tataouine

	Chemlali Tataouine				Fakhari Douirat			
	2004	2005	2006		2004	2005	2006	
	A	A	A	B	A	A	A	B
Acidity (% oleic acid)	0.43 ± 0.03	0.25 ± 0.03	0.29 ± 0.01	0.38 ± 0.03	0.56 ± 0.03	0.30 ± 0.05	0.25 ± 0.03	0.52 ± 0.02
PV (meq O_2/kg oil)	13.8 ± 0.3	10.0 ± 0.5	7.5 ± 0.4	11.4 ± 0.5	9.7 ± 0.8	7.0 ± 0.4	4.1 ± 0.3	6.2 ± 0.0
K_{270}	0.18 ± 0.12	0.16 ± 0.00	0.18 ± 0.01	0.18 ± 0.12	0.15 ± 0.01	0.14 ± 0.00	0.11 ± 0.00	0.13 ± 0.00
K_{232}	2.34 ± 0.04	2.30 ± 0.03	2.15 ± 0.02	2.07 ± 0.04	2.17 ± 0.04	1.95 ± 0.02	1.73 ± 0.02	1.87 ± 0.01
ΔK	−0.009 ± 0.003	−0.006 ± 0.000	−0.010 ± 0.00	−0.008 ± 0.003	−0.001 ± 0.00	−0.002 ± 0.00	−0.003 ± 0.00	−0.007 ± 0.00
OSI (h)	81.4 ± 7	91.6 ± 1	90.6 ± 8	84.38 ± 3	70.4 ± 2	72.6 ± 4	79.5 ± 5	72.0 ± 2
	Zarrazi Douirat				Dhokar Douirat		Chemlali	
	2004	2005	2006		2004	2006	2006	
	A	A	A	B	A	A	A	B
Acidity (% oleic acid)	0.67 ± 0.12	0.45 ± 0.03	0.37 ± 0.03	0.58 ± 0.02	0.65 ± 0.04	0.25 ± 0.04	0.20 ± 0.02	0.32 ± 0.02
PV (meq O_2/kg oil)	16.1 ± 0.5	15.5 ± 0.6	11.8 ± 0.7	12.8 ± 0.6	17.0 ± 0.5	15.6 ± 2.3	5.7 ± 0.1	8.4 ± 0.1
K_{270}	0.15 ± 0.01	0.14 ± 0.02	0.19 ± 0.04	0.16 ± 0.00	0.12 ± 0.01	0.20 ± 0.02	0.17 ± 0.02	0.16 ± 0.00
K_{232}	2.17 ± 0.04	1.88 ± 0.06	1.78 ± 0.03	1.90 ± 0.01	2.15 ± 0.04	1.95 ± 0.03	1.71 ± 0.01	1.90 ± 0.01
ΔK	−0.001 ± 0.000	0.007 ± 0.001	−0.003 ± 0.000	−0.007 ± 0.00	−0.004 ± 0.000	−0.002 ± 0.000	−0.002 ± 0.000	−0.007 ± 0.00
OSI (h)	75.7 ± 3	100.0 ± 4	97.7 ± 9	77.2 ± 2	20.6 ± 1	32.3 ± 2	32.2 ± 2	26.1 ± 3

PV – peroxide values; OSI – oil stability indices.

and Zarrazi Douirat varieties produced oils with excellent fatty acid (FA) composition, i.e., a high level of oleic acid (maximum of 76.52%), low palmitic and linoleic acid content (minimum of 8.67% and 6.92%, respectively) and a stearic acid level lower than the upper limit of 5% established for extra virgin olive oil (EVOO) (EEC, 2003). Some of these FA ratios may prove useful in chemometric studies to discriminate oils on the basis of origin (Aparicio, Roda, Albi, & Gutiérrez, 1999) because they showed great variability depending on the crop seasons, maturity index and the genetic factor. The ratio between oleic and linoleic acids ($C_{18:1}/C_{18:2}$), which has the most marked relationship with stability, showed that all of the studied oils, except for the pollinator Dhokar Douirat,

Table 3
Content of selected minor compounds in virgin olive oil samples from the region of Tataouine

Minor compounds	Chemlali Tataouine				Fakhari Douirat			
	2004	2005	2006		2004	2005	2006	
	A	A	A	B	A	A	A	B
TPP (mg caffeic acid/kg)	590 ± 14	548 ± 11	656 ± 28	738 ± 9	511 ± 8	478 ± 13	524 ± 17	624 ± 7
α-Tocopherol (mg/kg)	590 ± 6	584 ± 10	715 ± 12	544 ± 12	177 ± 8	182 ± 18	269 ± 9	229 ± 2
Squalene	2521	2605	2896	2532	5566	6048	4887	5497
Δ5-Avenasterol (%)	–	19.54	–	–	–	16.14	–	-
Total sterol (mg kg^{-1})	–	1717.02	–	–	–	1040.92	–	-
Total chrolophyll (mg kg^{-1}, as Pheo α)	12.28	12.45	23.98	8.26	10.11	10.02	6.38	3.09
Pheo α (mg kg^{-1})	2.41	5.68	12.93	5.1	4.69	5.80	4.54	2.5
Pyropheo α (mg kg^{-1})	ND	ND	ND	ND	ND	ND	ND	ND
β-Carotenoid (mg kg^{-1})	0.46	1.56	2.23	0.7	0.79	0.80	0.91	0.8
Lutein (mg kg^{-1})	1.24	1.51	2.09	1.2	1.00	0.82	1.22	1.4
Lutein/β-carotenoid	2.71	0.98	0.94	1.64	1.27	1.03	1.34	1.76
Pyropheo α/pheo α	ND	ND	ND	ND	ND	ND	ND	ND
% Pyropheo α/pheo α	ND	ND	ND	ND	ND	ND	ND	ND

	Zarrazi Douirat				Dhokar Douirat	Chemlal	
	2004	2005	2006		2006	2006	
	A	A	A	B	A	A	B
TPP (mg caffeic acid/kg)	496 ± 13	543 ± 9	562 ± 15	907 ± 15	290 ± 9	389 ± 15	241 ± 1
α-Tocopherol (mg/kg)l	226 ± 10	209 ± 14	284 ± 14	309 ± 13	454 ± 12	332 ± 9	391 ± 9
Squalene	5015	3933	4766	2593	2363	2309	2363
Δ5-Avenasterol	–	9.55	–	–	–	–	–
Total sterol (mg kg^{-1})	–	1063.01	–	–	–	–	–
Total chlorophyll (mg kg^{-1} as Pheo α)	6.57	6.95	13.67	2.77	4.11	6.53	9.40
Pheo α (mg kg^{-1})	3.84	4.40	7.35	2.7	2.47	3.51	4.0
Pyropheo α (mg kg^{-1})	2.20	ND	ND	ND	ND	ND	1.2
β-Carotenoid (mg kg^{-1})	0.52	0.69	1.74	0.4	0.97	0.68	1.0
Lutein (mg kg^{-1})	1.33	0.96	1.54	1.1	1.042	1.24	1.3
Lutein/β-carotenoid	2.56	1.38	0.88	2.59	1.08	1.81	1.26
Pyropheo α/pheo α	0.57	ND	ND	ND	ND	ND	0.30
% Pyropheo α/pheo α	57.28	ND	ND	ND	ND	ND	ND

ND – not defined; Pheo – pheophytine; Pyropheo – pyropheophytine; TPP – total polar phenol content.

which had an FA composition outside the norm, had a mean value greater than 7, which is the minimum value proposed by Kiritsakis, Nanos, Polymenoupoulos, Thomai, and Sfakiotakis (1998). Thus, compared to the main Tunisian variety, Chemlali, and with the exception of Dhokar Douirat VOO, the MUFA/PUFA ratio was high for all of the varieties and reached as high as 10.09, which means that the oil theoretically had good stability characteristics (Alvarruiz, Fernández, Montero, Granell, & Pardo, 2003).

The stage of ripeness of the olive fruit affected the fatty acid composition (Table 1). There was an increase in linoleic and palmitic acid content and a decrease in oleic acid content with ripening. As was explained by Gutiérrez, Jimenez, Ruiz, and Albi (1999), triacylglycerol biosynthesis is active during ripening and yields mainly oleic acid, which is subsequently transformed into linoleic acid by the enzyme oleate desaturase.

3.2. Oxidative stability and other quality parameters

The above found fatty acid composition of the examined variety VOOs suggested a high oxidative stability, which was verified using the Rancimat apparatus (Table 2). In Table 2, the values for free acidity (%), a well-established quality index, reflected our effort to collect only undamaged, healthy drupes. Moreover, initial values for the oxidative state of the oils obtained by the Abencor system were acceptable according to Codex Alimentarius recommendations and EU standards.

In detail, the oil stability indices (OSI) during the three crop years varied over the ranges 20.6–81.4 h (2004), 72.6–100.0 h (2005) and 32.3–97.7 h (2006). OSI values of VOOs depended strongly on olive variety. Except Dhokar Douirat, all the varieties showed remarkably high stability values (>70 h). Zarrazi Douirat VOO stability was similar to that found for oils of the Spanish varieties Picual and Conicabra (Salvador, Aranda, Gómez-Alonso, & Fregapane, 2001). Moreover, comparison with published data on other secondary Tunisian varieties and on the main Chemlali variety (Abaza et al., 2005; Ben Temime et al., 2006; Manaï et al., 2007) indicate that Zarrazi Douirat, Chemlali Tataouine and Fakhari Douirat yield VOOs of unusually high stability two to threefold higher than other Tunisian secondary varieties. This characteristic may be partially attributed to the low level of rainfall in the Tataouine region as demonstrated by Tovar, Romero, Alegre, Girona, & Motilva, 2002, who showed that oxidative stability is affected significantly by water regime that determines the phenol content of the olive fruit. On the other hand, genetic factor and the ensuing high level of MUFAs could also be causative factors of our findings. Within each variety, differences in oil stability were observed for different stages of maturity. Stability decreased with maturity in agreement with the literature (Gutiérrez et al., 1999). The oxidative stability findings led us to study other compounds that affect VOO stability in the dark or under light exposure (Psomiadou & Tsimidou, 2002a, 2002b) and are desirable for their nutritional value. Therefore, the content of total polar phenols, tocopherols, squalene, sterols and pigments was examined using well-established procedures.

3.3. Phenolic type antioxidants

The concentration of total polar phenols, expressed as caffeic acid, had a great variability depending on the variety, crop season and maturity index, ranging from a minimum of 290 to a maximum of 907 mg/kg. The ranges of total phenols for the samples from each year of harvest (2004–2005, 2005–2006, 2006–2007)

were also wide (496–590, 478–548, and 290–907 mg/kg, respectively). The oils from the last crop year had a higher mean content of polar phenols in comparison to the oils from the first and second crop years. The latter is possibly related to low rainfall accumulation of that year (Fig. 2), as it is known that water shortage tends to generate a stress situation in the olive tree that induces phenol production in the olive fruit (Tovar et al., 2002).

As with oxidative stability, the total polar phenol contents of Chemlali Tataouine, Fakhari Douirat and Zarrazi Douirat VOOs were among the highest of all Tunisian varieties, comparable to that reported for the second main Tunisian "Chétoui" variety (Abaza et al., 2005; Ben Temime et al., 2006) and for some VOOs derived from Spanish varieties (Aparicio & Luna, 2002). Similar remarkable levels were observed in Dhokar Douirat and Chemlali VOOs.

The correlation between polar phenol compounds and VOO stability is well-established (e.g. Psomiadou et al., 2003). This was also verified in our study in all cases. Dhokar Douirat VOO, which had the lowest total polar phenol content, showed the lowest oxidative stability. It is worth noting the very high level of total phenol in the Zarrazi Douirat variety: ~900 mg CA kg^{-1}. The high level of total phenol in almost all varieties (>500 mg CA kg^{-1}) is of great importance for both the stability of the oil as a product as well as its nutritional value.

Based on the above findings, VOO produced in the Tataouine region may contain high amounts of phenolic compounds if Good Manufacturing Practices are introduced in the production line. The phenol intake from these oils can complement those of other sources in an everyday diet. For example, local people drink approximately three cups per day of very concentrated black or green tea containing up to 36% of polyphenols on a dry weight basis, whereas consumption of fresh fruits is rather limited.

We also compared levels of total phenol between olives harvested at different stages of maturity. No clear trend between levels of total phenol and maturity index was found. This finding could be related to the fact that the olives of two maturity stages were harvested on the same day. Probably the reduction of the olive phenol content observed in other studies (e.g. Yousfi, Cert, & García, 2006) is attributed to the harvest period than to the skin colour. The enzymatic and oxidative degradation of the phenols in the olive fruit increases with the harvest period probably as consequence of the senescence process of the fruit tissue. If the sampling was done in two different harvest periods, probably differences could have been observed for the oils under examination, too.

Regarding α-tocopherol content, Table 3 shows that the ranges for samples were really wide (177–590, 182–584 and 198–716 mg/kg for 2004, 2005 and 2006, respectively). All of the varieties yielded oils with α-tocopherol levels (>200 mg/kg) higher than those found in Algerian VOO (Douzan & Bellal, 2005) but among the highest reported for either Tunisian (Ben Temime et al., 2006; Krichen et al., 2007) or European origin oils (Fedeli & Cortesi, 1993; Manzi et al., 1998; Psomiadou et al., 2000; Salvador, Aranda, & Fregapane, 1998). The high level of α-tocopherol, together with the phenolic compounds, contributes to oil stability (Psomiadou & Tsimidou, 1998). It is interesting to underline the very high levels of α-tocopherol in the samples of Chemlali Tataouine. These amounts were almost two-fold higher than those found for Chemlali or previously reported for other VOOs (Boskou, Blekas, & Tsimidou, 2006; Psomiadou et al., 2000). This unusually high level of α-tocopherol is promising in view of nutritional needs. Because the per capita consumption of olive oil in Tunisia is ~6 kg (COI, 2000), the intake of α-tocopherol from olive oil can cover a significant part of the daily requirement. This finding is of practical importance because the major dietary source of vitamin E for local people is VOO extracted from these varieties. Once more, the results point toward the critical importance of educating local producers about how they should treat olives after harvest and how they should extract the oil in order to preserve the above-mentioned benefits.

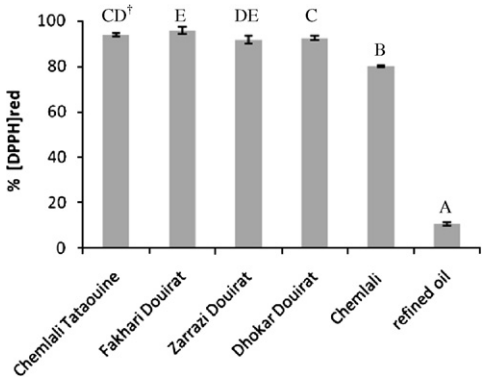

Fig. 3. Radical scavenging activity of the VOOs from varieties grown in the Tataouine region compared to that of both the main Tunisian variety and a refined olive oil; † different characters indicate results significantly different ($p < 0.05$).

Regarding α-tocopherol content, the observed trend for oils from different maturity stages was in agreement with other investigators, who were also unable to show a clear relationship between the content of tocopherols and the maturation degree of olives (Psomiadou et al., 2000).

Further experimentation on the RSA of the studied VOOs verified the exceptional characteristics discussed above (Fig. 3). It can be seen from Fig. 3 that the RSA of VOOs from the Tataouine region, extracted from fruits of 2006 crop season (group A), was much higher than that of refined olive oil. This activity is necessarily due to the much higher tocopherol and phenol content of the examined oils (Tables 1 and 3), in line with previous work (Kalantzakis et al., 2006). Duncan's test showed significant ($p < 0.05$) differences in RSA in some cases for the oils obtained from the autochthonous varieties. All of them were much higher than that of the oil from the standard Tunisian variety oil.

3.4. Squalene content and related alcohol composition

Table 3 presents the squalene amount during three consecutive crop seasons. Ranges for samples from each year of harvest were quite similar with regards to values reported for commercial oils [2521–5566 (2004), 2605–6048 (2005) and 2363–5497 mg/kg (2006)]. All of the analyzed samples contained levels expected for good quality VOOs (800–12,000 mg/kg) (Grigoriadou et al., 2007; Lanzón, Albi, Cert, & Gracián, 1994). The presence of squalene in VOO has a protective effect on the oxidative stability under heating. These VOOs are the most important source of dietary squalene for the local people because they do not consume seafood.

Squalene is the precursor of sterols, the total composition of which in VOOs is of utmost importance in VOO authentication. Analysis of the oils from the 2005 crop season focused on the content of total sterols and, in particular, that of Δ5-avenasterol (Table 3), which is related to VOO stability during heat treatment. All of the olive oil samples contained more than 1000 mg/kg, the minimum value established by EU Regulations for the category 'extra virgin' olive oil. Moreover, a very high Δ5-avenasterol content of the samples was observed, which reached 19.53% of total sterol content in the case of Chemlali Tataouine oil. This amount was similar to that found for oils from the "Koroneiki" variety grown in Crete (Greece) (Koutsaftakis, Kotsifaki, & Stefanoudaki, 2000) but was almost twice as high as that found for both Spanish VOOs from the variety "Cornicabra" (Salvador, Aranda, & Fregapane, 2001) and some other Tunisian varieties.

3.5. Pigment content

The presence of pigments not only determines the colour of the product but also plays an important role in the oxidative activity of processed foodstuff, due to their antioxidant nature in the dark and pro-oxidant activity in the light.

The pigment contents in the analyzed Tunisian oils (Table 3) varied depending on the crop season, variety and ripening index. Pheophytin *a* was the major component (2.41–12.93 mg kg^{-1}; 59–75% total pigment), followed by the major yellow pigment lutein (0.82–1.24 mg kg^{-1}; 11–32% total pigment); β-carotene was also well-represented (0.46–2.22 mg kg^{-1}; 9–22%). Although almost all of the major pigments were present in the oils at levels similar to those found in the Chemlali variety and to those reported in some Algerian varieties (Douzan & Bellal, 2005), it is noteworthy that they were low compared to the corresponding values for olive oils from Morocco (Rahmani & Csallany, 1991), Spain (Gandul-Rojas & Mínguez-Mosquera, 1996), Greece (Psomiadou & Tsimidou, 2001) and Italy (Giuffrida, Salvo, Salvo, La Pera, & Dugo, 2007). This may be attributed to varietal differences with characteristic biosynthetic or catabolic pathways and/or to geographical differences (Roca & Minguez-Mosquera, 2001).

The lutein/β-carotene ratio, which could be useful for differentiating oils from a single cultivar, was less than 3 in all varieties analysed and was similar to oils from Greek cultivars (Psomiadou & Tsimidou, 2001) but different from Spanish and Italian varieties, where the ratio was reported to range between 1.3–5.1 and 0.17–0.4, respectively (Gandul-Rojas & Mínguez-Mosquera, 1996; Giuffrida et al., 2007).

It is generally known that pigment concentration in olives decreases during ripening (Gandul-Rojas & Mínguez-Mosquera, 1996), and the varieties tested in the study, except for the main Tunisian variety, agree with this (Table 3). In fact, as ripening progresses, photosynthetic activity decreases, and the level of both chlorophylls and carotenoids decreases progressively (Criado, Motilva, GoñI, & Romero, 2007). It can be argued that the chlorophyll content in oils from the Tataouine region is not a negative factor for their stability because commercial oils are expected to have much lower levels.

4. Conclusion

This work is the first evaluation of the potential of the main autochthonous olive varieties from the arid region of Tataouine to produce good quality VOO. The examined oils showed some unusually high levels of antioxidants together with a high level of oleic acid. These findings should encourage the local people who are responsible for the sector to take care of this heritage and introduce Good Manufacturing Practices (GMP) into production. The creation of a GMP-based oleoculture must be achieved for the health and well-being of the people in this arid zone of Tunisia.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.06.041.

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