DOI 10.1002/ejlt.200700132 *Eur. J. Lipid Sci. Technol.* **109** (2007) 1208–1217

Olfa Baccouri[a]
Lorenzo Cerretani[b]
Alessandra Bendini[b]
Maria Fiorenza Caboni[b]
Mokhtar Zarrouk[a]
Luigi Pirrone[c]
Douja Daoud Ben Miled[a]*

[a] Laboratoire Caracterisation et Qualite de l'Huile d'Olive, Centre de Biotechnologie de Borj-Cédria, Hammam-Lif, Tunisia
[b] Dipartimento di Scienze degli Alimenti, Università di Bologna, Cesena, Italy
[c] Dipartimento di Ingegneria e Tecnologie Agro-Forestali, Università degli Studi di Palermo, Palermo, Italy

Preliminary chemical characterization of Tunisian monovarietal virgin olive oils and comparison with Sicilian ones

Work was carried out on the characterization of monovarietal virgin olive oils (VOO) from Tunisia and Sicily (Italy). The two main Tunisian VOO (cvv. Chétoui of the North and cv. Chemlali grown in the Center and some regions of the South) and three principal Sicilian VOO (cvv. Nocellara del Belice, Biancolilla and Cerasuola) were studied. Moreover, the Chétoui oils were tested in a rain-fed control and an irrigation regime. All olive samples were picked at three different stages of ripeness. Analyses of major components (fatty acids and triacylglycerols) and minor ones (squalene, tocopherols and phenolic compounds) were carried out. Chétoui oils had a higher level of phenolic compounds followed by Chemlali. Generally, in the Sicilian oils these natural antioxidant contents were lower. These preliminary results indicate that it was possible to classify the Tunisian and Sicilian oils tested in their original growing area based on their chemical composition.

Keywords: Virgin olive oil, composition, triacylglycerols, phenols, antioxidants, irrigation.

1 Introduction

The olive tree (*Olea europaea* L.) is one of the most important crops in the Mediterranean countries, especially in Spain, Italy, Greece and Tunisia. Virgin olive oil (VOO) is one of the edible fats most highly prized by the Mediterranean people and therefore it constitutes a fundament of their diet. The beneficial effect of the consumption of VOO on human health is well known and related to the characteristic fatty acid composition and the presence of minor components, such as squalene, phytosterols and antioxidant molecules as tocopherols and other phenolic compounds [1]. Moreover, it is widely known, that the quality characteristics of extra-virgin olive oils derive from concomitant action of various agronomical factors such as olive variety [2], climatic conditions during growth [3], degree of maturation and agronomic practices related to irrigation treatment [4–10]. The olive cultivar is one of the factors related to the oxidative stability of oil; in fact, it influences both fatty acid composition (in particular the ratio of oleic to linoleic acid) and phenolic content [11]. However, irrespective of the olive variety considered, the degree of olive ripeness may also directly or indirectly affect the quality of oil [4–6]. In fact, during the ripening process, important chemical changes related to the synthesis of organic substances, especially triacylglycerols (TAG) and other enzymatic activities occur inside the drupes [12], and this may affect VOO quality [13, 14]. Hence, the identification of the optimal stage of olive ripening is important to obtain impeccable oils and to improve the balance between the level of antioxidant substances responsible for oil stability and aromatic substances characterizing the oil flavor. Changes in the chemical composition of the fruit and the extracted oil, which take place during the ripening process, have been studied by several authors [4–6]. Some recent researches have shown differences in the chemical composition and the sensory characteristics of VOO from irrigated and rain-fed olive trees [7]. In fact, some authors reported that irrigation has a beneficial effect on TAG accumulation in olives [8, 15], whereas it has no effect on the fatty acids of TAG which are an important factor for oil conservation and organoleptic characteristics. Moreover, some studies reported that the chemical components most influenced by irrigation are the phenolic compounds [8–10]. These compounds are of great interest because they influence the quality and the palatability of the virgin olive oils and increase their shelf life by slowing the formation of polyunsaturated fatty acid hydroperoxides [11]. The phenolic compound levels show an inverse relationship with the amount of water applied to the olive trees [8–10]. Considering the previous, the aim of our work was to carry out

Correspondence: Lorenzo Cerretani, Dipartimento di Scienze degli Alimenti, Università di Bologna, Piazza Goidanich 60, 47023 Cesena (FC), Italy. Phone: +39-0547-338121, Fax: +39-0547-382348, e-mail: lorenzo.cerretani2@unibo.it

* Additional corresponding author: Douja Daoud Ben Miled, douja.daoud@cbbc.rnrt.tn

Research Paper

WILEY InterScience DISCOVER SOMETHING GREAT

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

the characterization of monovarietal VOO from Tunisia and Sicily (Italy) based on the study of major (TAG and fatty acids) and minor compounds (squalene, tocopherols and phenols). The two main Tunisian VOO (cvv. Chétoui of the north and Chemlali grown in the Center and some regions of the south) and three principal Sicilian VOO (cvv. Nocellara del Belice, Biancolilla and Cerasuola) were studied. Olives were picked at three different stages of ripeness based on the degree of skin and pulp pigmentation. These stages correspond to the traditional harvest dates. Moreover, the Chétoui cv. was tested in a rain-fed control and an irrigation regime, also at three different stages of ripeness. This is the first comparison between the chemical composition of Tunisian and Sicilian (Italy) monovarietal virgin olive oils.

2 Materials and methods

2.1 Plant material

The present work was carried out on monovarietal VOO from Tunisian and Sicilian (Italy) cultivars. The two main Tunisian VOO were cv Chétoui of the North and Chemlali grown in the Centre and some regions of the South, and the three principal Sicilian VOO were cv Nocellara del Belice, Biancolilla and Cerasuola. Moreover the Chétoui VOO was tested in a rain-fed control and an irrigation regime. The experimental irrigation treatment was carried out in the dry season of 2005 (June, July, August and September 2005) at the Centre of Biotechnology of Borj-Cédria (CBBC), Tunisia. The water requirements were calculated using a methodology based on the crop evapotranspiration (ET_c) proposed by the United Nations Food and Agriculture Organization [16]. Olive oil samples were collected at three maturity stages from the five cultivars (Chétoui, Chemlali, Nocellara del Belice, Biancolilla and Cerasuola). Chétoui and Chemlali olives were hand-picked, in triplicate in perfect sanitary conditions, at three different ripeness stages (RSI, December 5, 2005; RSII, January 2, 2006; RSIII, January 29, 2006) based on the degree of skin and pulp pigmentation according to the method developed by the Agronomic Station of Jaén [17]. After harvesting, the olives were washed and deleafed and then transported to the laboratory (Abencor system, MC" Ingenierias y sistemas, Sevilla, Spain) where they were immediately transformed. This extraction technology reproduced at laboratory scale the industrial process, following the same phases: milling, beating, centrifuging, and decanting. Olive samples from the Nocellara del Belice, Biancolilla and Cerasuola cultivars were also harvested in triplicate at three different ripeness stages (October 2005, November 2005 and December 2005) considering the fruit color in both skin and pulp [17]. All the oil samples were processed under the same conditions. The olives were crushed with a hammer crusher, and the paste was mixed at 25 °C for 40 min and treated with a three-phase decanter system. All the oils tested were not filtered.

2.2 Apparatus

All high-performance liquid chromatography (HPLC) analyses were performed using a HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump delivery system, a degasser, and an autosampler. Detection was performed with a diode array UV-Vis detector (DAD), a mass spectrometer detector (MSD), and an evaporative light scattering detector (ELSD) PL–ELS model 1000 Series (Polymer laboratories, Varian Inc., Amherst, MA, USA). All solvents were of HPLC grade and filtered through a 0.45-μm nylon filter disk (Lida Manufacturing Corp., Kenosha, WI, USA) prior to use. Spectrophotometric determinations were carried out using an UV-Vis 1601 instrument (Shimadzu Co., Kyoto, Japan), which had a six-slot shuttle and a system for temperature control of working conditions. Fatty acid methyl ester (FAME) and squalene analyses were performed using a GC Clarus 500 Perkin-Elmer (Wellesley, MA, USA) equipped with a flame ionization detector (FID).

2.3 Standard, reagents and solvents

The standard used for spectrophotometric phenol quantification (gallic acid) and the Folin-Ciocalteau reagent were obtained from Merck (Darmstadt, Germany); the standard for HPLC quantification (3,4-dihydroxyphenylacetic acid) and for the evaluation of the antioxidant capacity of phenolic extracts (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox), such as the reagent 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS, diammonium salt), were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the quantification of squalene, $C_{19:0}$ was bought from Sigma–Aldrich, and for the identification of fatty acids, a mix (MIX 463) of FAME standards was purchased from Nu-check Prep. Inc. (Elysian, MN, USA). All solvents used were of analytical or HPLC grade (Merck).

The standards for the HPLC identification (tyrosol, vanillic and coumaric acids) were from Sigma Chemicals Co. (St. Louis, MO, USA); PPO, SOO were from Matreya (Pleasant Gap, PA, USA) and OOL, POO, OOO from Supelco (St.-Louis, MO, USA). Hydroxytyrosol for qualitative analysis was obtained as previously described [4].

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

2.4 Fatty acid and squalene analysis

The fatty acid composition of the oil samples was determined as methyl esters by capillary gas chromatography (GC) analysis after alkaline treatment; this was obtained by mixing 0.05 g of oil dissolved in 2 mL *n*-hexane with 1 mL 2 N potassium hydroxide in methanol, according to Christie [18]. In order to quantify the squalene content in the studied samples, an internal standard ($C_{19:0}$) of known concentration was added. Of the upper phase, 1 μL was injected into a split 1 : 20 GC port set at 240 °C; a fused-silica capillary column (50 m length, 0.25 mm ID), coated with CPSil-88 (0.25 μm film thickness; Varian, Palo Alto, CA, USA), was utilized. A flow rate of 1.25 mL/min of helium as the carrier gas was used. The FID detector was at 240 °C. The initial oven temperature was kept at 120 °C for 1 min and raised to 240 °C at a rate of 4.0 °C/min and maintained for 4 min. Peaks were identified by comparison of their retention times with those of authentic reference compounds (MIX 463).

The fatty acid composition was expressed as relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area, whereas the squalene amount in VOO was estimated based on the internal standard and expressed as g/kg of oil. The average was calculated by three replications for each sample.

2.5 Chromatographic analysis of triglycerides

A 0.3-g oil sample was dissolved in 10 mL 2-propanol/acetonitrile/*n*-hexane (2 : 2 : 1 vol/vol) mixture and homogenized by stirring. A Luna™ C18 (Phenomenex, Torrance, CA, USA) column, 5 μm particle size, 250 mm × 3.00 mm ID, with a C18 precolumn filter (Phenomenex) was used at room temperature. All solvents were filtered through a 0.45-μm nylon filter disk (Lida Manufacturing Corp., Kenosha, WI, USA) prior to use. The injection volume was 10 μL. 2-Propanol and acetonitrile were mobile phase A and B, respectively. Elution was performed at a solvent flow rate of 0.7 mL/min with the following gradient: from 0 to 2 min 52% B, up to 4 min 58% B maintained until 25 min, then at 30 min carried to 10% B and finally returned to 52% B at 37 min. Peak assignment was carried out by comparison with several pure standards and chromatograms reported in the literature [19]. The effluent was monitored with an ELSD, with the following settings: evaporator temperature, 70 °C; nebulizer, 30 °C; transfer line, 30; and gas flow rate, 1.0 L/min.

2.6 Extraction of the phenolic fraction

According to Pirisi et al. [20], 2 g of the oil sample was added to 1 mL *n*-hexane and 2 mL of a methanol/water (60 : 40, vol/vol) solution in a 10-mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min at 1490 × g. The hydroalcoholic phase was collected, and the hexanic phase was re-extracted twice with 2 mL methanol/water (60 : 40, vol/vol) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 2 mL *n*-hexane to remove the residual oil, then concentrated and evaporated in vacuum at 35 °C. For spectrophotometric and radical-scavenging activity analysis, the dry extracts were resuspended in 5 mL of a methanol/water (50 : 50, vol/vol) solution. However, for HPLC analysis, 100 μL of 3,4-dihydroxyphenylacetic acid solution (0.1 mg/mL) as internal standard was added to 4 g of oil; then, after the extraction procedure, dry extracts were solved in 0.5 mL of a methanol/water (50 : 50, vol/vol) solution and filtered through a 0.2-μm nylon filter (Whatman Inc., Clifton, NJ, USA). Extractions were performed in three replicates ($n = 3$). Unless otherwise stated, extracts were stored at −18 °C before analysis by HPLC-DAD/MSD, by UV-vis spectrometry and by radical-scavenging activity.

2.7 Spectrophotometric determination of total phenols

The total phenols (TP) content of the extracts was determined according to the Folin-Ciocalteu spectrophotometric method at 750 nm [21], using a gallic acid calibration curve ($r^2 = 0.998$). The results were expressed as mg gallic acid/kg of oil. The spectrophotometric analysis was repeated three times for each type of extract.

2.8 Determination of *o*-diphenols

A 0.5-mL sample of each phenolic extract was dissolved in 5 mL methanol/water (50 : 50, vol/vol), and 4 mL of the resulting solution was added to 1 mL of a 5% solution of sodium molybdate dihydrate in ethanol/water (50 : 50, vol/vol) and shaken vigorously. After 10 min, they were centrifuged for 3 min at 1490 × g; the absorbance at 370 nm of the upper layer was measured using the calibration curve of gallic acid ($r^2 = 0.996$). The results were expressed in mg gallic acid/kg of oil. The spectrophotometric analysis was repeated three times for each type of extract.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

2.9 Radical-scavenging activity of phenolic extracts

The radical-scavenging capability of phenolic extracts was evaluated by $ABTS^+$ radical cation assay according to the Re et al. [22] method with detection at 734 nm. Results were expressed as mmol Trolox/kg of oil using its calibration curve (r^2 = 0.981).

2.10 Chromatographic analysis of phenols by HPLC-DAD/MSD

A Luna C18 column (Phenomenex) of 5 μm particle size and 250 mm length, 3.00 mm ID was used. The mobile phase flow rate was 0.5 mL/min. The wavelength was set at 280 nm for phenolic acids, phenyl ethyl alcohols and secoiridoids. The injection volume was 10 μL. Analyses were carried out at room temperature. The gradient elution [23] was carried out using water/formic acid (99.5 : 0.5, vol/vol) as mobile phase A and acetonitrile as mobile phase B of the solvent system. The total run time was 75 min. Phenolic compounds were quantified using a calibration curve made with 3,4-dihydroxyphenylacetic acid (Sigma-Aldrich) (r^2 = 0.999). The average was calculated by three replications for each sample.

The MS analyses were carried out using an electrospray (API-ES) interface operating in positive mode using the following conditions: drying gas flow, 9 L/min; nebulizer pressure, 50 psi; gas drying temperature, 350 °C. Phenolic compounds were identified (Fig. 1) comparing retention times (by spiking attempts) and UV and MS spectra of the detected peaks with those of commercial (tyrosol, vanillic and coumaric acids) and synthesized compounds (hydroxytyrosol) when available. Decarboxy-methyl oleuropein aglycon (DAOA) and lignan (acetoxypinoresinol) were identified by analyzing and comparing their MS spectra with those reported in the literature [4].

2.11 Chromatographic analysis of tocopherols

Of oil sample, 1 g was dissolved in 10 mL *n*-hexane and extracts were filtered through a 0.45-μm nylon filter. α-, β- and γ-tocopherols (α-toc, β-toc, and γ-toc, respectively) were determined by HPLC equipped with a photodiode detector array set at 295 nm. The used column was a Phenomenex, Luna, CN 100A (150 mm, 4.6 mm ID) under isocratic conditions with *n*-hexane/dichloromethane (95 : 5, vol/vol) as the mobile phase at a flow rate of 1 mL/min. The injection volume was 20 μL. Analyses were carried out at room temperature. The total run time was 10 min. Three calibration curves were constructed with standard solutions of each compound (α-, β- and γ-toc, r^2 = 0.999, 0.986 and 0.999, respectively) and used for quantification. The results are expressed in mg α,β,γ-toc/kg of oil.

2.12 Statistical analysis

The results reported in this study are the averages of at least three repetitions (n = 3), unless otherwise stated. Chemical data were analyzed using the Statistica 6.0 (Statsoft Inc., Tulsa, OK, USA) statistical software. The significance of differences at a 5% level between averages was determined by one-way ANOVA using Tukey's test. Data were also analyzed by principal component and classification analysis, to determine the correlation between the analyses and the differences between the samples.

3 Results and discussion

3.1 Fatty acid composition and squalene amount

The difference between the five cultivars regarding their fatty acid composition was shown in oleic (18:1) and linoleic (18:2) acid contents. In fact, it can be seen that the oleic acid contents of the Tunisian olive oils were lower than for the Sicilian samples. However, the linoleic acid content of the former was significantly higher than that of the latter. Hence, the ratio between oleic and linoleic acid (C18:1/C18:2) in the Tunisian olive oils was lower than in the Sicilian ones (Tab. 1). Moreover, our results showed that the oleic, linoleic and linolenic acid contents varied during the maturation process. In fact, in the two Tunisian and in Cerasuola VOO, from the first harvest to the last one, the oleic/linoleic acid ratio decreased during olive maturation. These results coincide with those obtained by other authors [5, 6]. However, this trend was not observed in the other varieties. In fact, the oleic/linoleic acid ratio increased from 10.4 to 15.4% in the Nocellara del Belice variety and remained almost unchanged in the Biancolilla cultivar. Moreover, irrigation treatment apparently did not affect the fatty acid composition in the Chétoui oils since no statistically significant differences were observed in the present study (Tab. 1). In contrast, other authors [7, 24] observed that olive oils of irrigated trees had higher contents of palmitic and linoleic acids, but these changes were very slight and did not have any nutritional relevance. Hence, our results confirmed that mainly the variety and the ripening degree of the olives influence the acidic composition of the studied olive oils, whereas, the impact of irrigation is less relevant.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Tab. 1. Fatty acid, TAG composition and squalene content of the studied olive oils.

Variety	Stage of ripening	C18:1[§]	C18:2[§]	C18:1/ C18:2[§]	Squalene[$]	PLL[#]	OLL[#]	POLn + EeOL[#]	OOL[#]	POL[#]	OOO[#]	POO[#]	PPO[#]	SOO[#]
NB	I	73.04$^{b;w}$	6.99$^{a;x}$	10.43$^{b;w}$	9.67$^{a;v}$	0.00$^{a;z}$	1.29$^{a;w}$	0.89$^{a;yz}$	11.15$^{a;z}$	2.65$^{a;y}$	45.56$^{a;x}$	36.26$^{a;v}$	0.87$^{a;w}$	1.32$^{a;vw}$
	II	74.74$^{ab;v}$	6.81$^{a;x}$	10.97$^{b;w}$	8.32$^{b;w}$	0.00$^{a;z}$	1.20$^{a;w}$	0.45$^{b;z}$	10.81$^{a;z}$	1.92$^{ab;yz}$	51.58$^{a;wx}$	31.30$^{b;w}$	0.85$^{a;w}$	1.89$^{a;v}$
	III	76.38$^{a;v}$	5.05$^{b;x}$	15.41$^{a;v}$	6.27$^{c;wx}$	0.00$^{a;z}$	0.56$^{b;xy}$	0.24$^{c;z}$	10.70$^{a;z}$	1.20$^{ab;z}$	55.52$^{a;w}$	29.91$^{b;wx}$	0.42$^{b;x}$	1.45$^{a;vw}$
BA	I	73.12$^{ab;w}$	7.49$^{ab;x}$	9.75$^{a;x}$	5.18$^{c;wx}$	0.00$^{c;z}$	0.88$^{a;x}$	0.46$^{b;z}$	9.22$^{a;z}$	1.46$^{a;z}$	56.99$^{a;w}$	29.25$^{a;wx}$	0.73$^{a;w}$	1.02$^{a;w}$
	II	70.68$^{ab;x}$	9.06$^{a;x}$	7.80$^{b;y}$	8.95$^{a;v}$	0.15$^{a;x}$	1.09$^{a;wx}$	0.27$^{c;z}$	10.09$^{a;z}$	1.97$^{a;yz}$	56.09$^{a;w}$	29.28$^{a;wx}$	0.54$^{a;x}$	0.53$^{b;x}$
	III	74.34$^{a;v}$	7.82$^{ab;x}$	9.50$^{a;x}$	7.25$^{b;w}$	0.05$^{b;y}$	1.03$^{a;wx}$	0.82$^{a;yz}$	12.35$^{a;yz}$	1.22$^{a;z}$	57.60$^{a;w}$	25.61$^{b;x}$	0.00$^{b;y}$	1.32$^{a;vw}$
CE	I	76.51$^{a;v}$	7.46$^{c;x}$	10.28$^{a;w}$	4.01$^{a;x}$	0.07$^{a;x}$	1.06$^{a;wx}$	0.62$^{a;yz}$	15.32$^{b;y}$	1.18$^{b;z}$	58.32$^{b;w}$	21.71$^{b;x}$	0.50$^{a;x}$	1.24$^{a;vw}$
	II	70.68$^{b;x}$	9.06$^{b;x}$	7.80$^{b;y}$	2.08$^{b;z}$	0.10$^{a;x}$	1.34$^{a;w}$	0.62$^{a;yz}$	12.65$^{c;yz}$	1.61$^{b;yz}$	63.15$^{a;v}$	19.76$^{b;x}$	0.70$^{a;wx}$	0.06$^{b;y}$
	III	75.57$^{a;v}$	10.01$^{a;x}$	7.56$^{b;y}$	1.04$^{c;z}$	0.04$^{a;y}$	0.43$^{b;xy}$	0.65$^{a;yz}$	19.60$^{a;x}$	2.93$^{a;y}$	47.42$^{c;x}$	27.01$^{a;wx}$	0.27$^{b;x}$	1.66$^{a;vw}$
CT RF	I	71.72$^{a;wx}$	13.65$^{ab;w}$	5.26$^{a;z}$	7.94$^{b;w}$	0.00$^{c;z}$	0.23$^{c;y}$	1.25$^{c;y}$	22.20$^{c;wx}$	4.34$^{ab;x}$	49.20$^{ab;wx}$	21.36$^{a;x}$	0.76$^{a;wx}$	0.66$^{b;x}$
	II	70.71$^{a;x}$	15.23$^{a;v}$	4.65$^{ab;z}$	8.27$^{a;v}$	0.23$^{b;w}$	0.46$^{b;xy}$	5.37$^{b;w}$	29.79$^{b;w}$	5.49$^{a;x}$	44.10$^{b;x}$	13.21$^{b;y}$	0.00$^{b;y}$	1.34$^{a;vw}$
	III	70.20$^{a;x}$	14.87$^{ab;vw}$	4.72$^{ab;z}$	5.79$^{c;wx}$	0.45$^{a;v}$	3.04$^{a;v}$	7.20$^{a;v}$	36.59$^{a;v}$	4.61$^{ab;x}$	43.01$^{b;x}$	5.08$^{c;z}$	0.00$^{b;y}$	0.00$^{c;z}$
CT IR	I	68.72$^{a;x}$	16.13$^{b;v}$	6.52$^{a;yz}$	4.26$^{a;x}$	0.04$^{a;y}$	0.31$^{b;y}$	4.56$^{b;w}$	22.37$^{b;wx}$	5.46$^{b;x}$	40.30$^{ab;xy}$	23.31$^{ab;x}$	0.80$^{ab;w}$	2.86$^{a;v}$
	II	68.28$^{a;x}$	17.22$^{a;v}$	6.35$^{a;yz}$	3.97$^{a;y}$	0.15$^{a;x}$	1.22$^{a;wx}$	1.63$^{c;y}$	15.42$^{c;y}$	4.90$^{b;x}$	49.01$^{a;wx}$	25.74$^{a;x}$	1.29$^{a;w}$	0.64$^{c;x}$
	III	65.92$^{ab;x}$	18.41$^{a;v}$	3.60$^{b;z}$	3.58$^{a;y}$	0.06$^{a;y}$	0.37$^{b;y}$	5.93$^{a;w}$	25.79$^{a;wx}$	9.13$^{a;w}$	35.01$^{c;y}$	22.00$^{ab;x}$	0.30$^{b;x}$	1.41$^{b;vw}$
CM	I	61.57$^{a;y}$	14.27$^{b;vw}$	4.32$^{a;z}$	6.47$^{a;wx}$	0.00$^{b;z}$	0.30$^{b;y}$	3.36$^{a;x}$	14.52$^{c;y}$	12.67$^{b;vw}$	30.77$^{b;y}$	35.42$^{a;v}$	2.54$^{a;v}$	0.41$^{a;x}$
	II	61.46$^{a;y}$	15.79$^{a;v}$	3.90$^{ab;z}$	4.77$^{b;x}$	0.21$^{a;w}$	0.48$^{a;xy}$	3.29$^{a;x}$	18.65$^{b;x}$	17.42$^{a;v}$	24.57$^{c;z}$	32.87$^{b;w}$	2.20$^{a;v}$	0.31$^{a;x}$
	III	59.25$^{b;y}$	16.38$^{a;v}$	3.63$^{ab;z}$	2.00$^{c;z}$	0.00$^{b;z}$	0.20$^{b;y}$	3.68$^{a;x}$	22.80$^{a;wx}$	13.17$^{b;vw}$	32.60$^{b;y}$	26.26$^{c;x}$	1.02$^{b;w}$	0.27$^{b;x}$

NB: Nocellara del Belice; BA: Biancolilla; CE: Cerasuola; CT RF: Chétoui rain-fed; CT IR: Chétoui irrigated; CM: Chemlali. P: palmitic; S: stearic; O: oleic; L: linoleic; Ln: linolenic; Ee: elaidinic acids.
[a–c] Different letters in the same column concerning the same cultivar indicate significantly different values ($p < 0.05$).
[v–z] Different letters in the same column concerning all studied samples indicate significantly different values ($p < 0.05$).
[§] As percentage of total fatty acids.
[$] In g/kg of oil.
[#] As percentage of total TAG.

Squalene is the major olive oil hydrocarbon; in fact, this molecule makes up more than 90% of the hydrocarbon fraction [1], ranging from 0.2 to 7.5 g/kg oil [25] or even higher (0.8–12 g/kg oil) [26]. Cerasuola samples present a considerably lower content of squalene with respect to Biancolilla, Chétoui, Nocellara del Belice and Chemlali samples (Tab. 1). These results confirmed other studies showing that the squalene content depends on the olive cultivar [25]. The changes in squalene amount during the olive maturation process were similar in the Nocellara del Belice, Cerasuola and Chemlali varieties: a slight decrease during ripening (Tab. 1). However, in the Biancolilla variety, the squalene content increased to reach a maximum of 8.95 g/kg at the second stage of olive ripening, and then a decrease was observed. A similar trend was also found in the Chétoui samples under the rain-fed regime. Nevertheless, under the irrigation treatment, the squalene amount in the Chétoui samples decreased slightly during fruit maturation. These results could not be confirmed by the literature because there has been no other study about this topic.

3.2 TAG composition

TAG contents, expressed in percentage of total TAG (Tab. 1) showed variations between samples from different cultivars. HPLC-ELSD analysis of TAG permitted the identification and quantification of ten TAG. Among these, POLn and EeOL overlapped. PLL, OLL, PPO, SOO and POLn + EeOL were present in low percentages, whereas OOL, POL, OOO and POO accounted for more than 89% of the total area of peaks in the chromatographic profile.

In relation to the main TAG (OOL, POL, OOO and POO), the level of triolein (OOO), was remarkably high, ranging from 24.6 to 49.2% and from 45.6 to 63.2% in the Tunisian and Sicilian oils, respectively. Among the studied olive oil samples, cv. Chemlali registered the lowest percentages of triolein. The second peak in order of quantitative importance among the studied olive oils corresponded to the POO. The Tunisian oils had higher levels of POL (4.3–17.4%), whereas this level did not exceed 2.9% in the Sicilian oils. The OOL content ranged from 14.5 to 36.6% in the Tunisian samples, whereas it did not exceed 12.3%

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

in the Nocellara del Belice and Biancolilla varieties; nevertheless, in Cerasuola, this content reached 19.6%. Similar to fatty acids, the composition of TAG of the Chétoui cultivar varied widely according to the ripening stage of the fruit. The major change in the TAG profile concerned lowering of OOO of the Chétoui cultivar, and this trend coincides with that concerning the fatty acid composition. In fact, in the Chétoui olive oil, the oleic acid percentage decreased during the olive ripening, while linoleic acid levels increased (data not shown), probably due to oleate desaturase activity during TAG biosynthesis [27].

A clear decrease was observed for the POO percentages in Chétoui cultivated in the rain-fed condition and in Chemlali, whereas for the same varieties the OOL content increased remarkably during fruit maturation. The OOO percentage in Chétoui olive oils cultivated under the irrigation regime showed a decreasing trend during olive maturation, similar to the same cultivar under rain-fed conditions but this last starting at a higher percentage. The POL fraction increases from the first olive harvest to the last one in the irrigated Chétoui olive oils.

3.3 Change in natural antioxidant content

Tab. 2 illustrates the evolution of the TP content of the Tunisian and Sicilian oils throughout the olive maturation process, determined by spectrophotometry.

Chétoui oils had higher levels of these compounds (fluctuating from 222.4 to 852.9 mg/kg of oil) followed by Chémlali (varying from 96.4 to 190.1 mg/kg of oil). Generally, in the Sicilian oils these natural antioxidants contents were lower (ranging from 25.2 to 183.7 mg/kg of oil, from 65.7 to 178.6 mg/kg of oil and from 71.9 to 124 mg/kg of oil in Nocellara del Belice, Biancolilla and Cerasuola, respectively). Therefore, different responses to the har-

Tab. 2. Minor compound content in olive oil samples.

Variety	Stage of ripening	TPC[§]	*o*-diph[§§]	α-toc[$]	T toc[¤]	ABTS•−[#]	Htyr[‡]	Tyr[‡]	DAOA[‡]	DLigAgl + AcPin[‡]	OlAgl[‡]	LigAgl[‡]	VA[‡]	*o*-CA[‡]	SID[‡]
NB	I	183.73$^{a;xy}$	88.10$^{a;xy}$	498.65$^{a;v}$	561.17$^{a;v}$	0.39$^{a;y}$	13.14$^{a;y}$	7.18$^{ab;x}$	29.05$^{a;y}$	24.80$^{a;xy}$	11.01$^{a;z}$	2.27$^{a;y}$	1.98$^{a;v}$	18.12$^{a;v}$	42.34$^{a;x}$
	II	63.99$^{b;yz}$	50.74$^{b;y}$	185.64$^{b;x}$	213.34$^{b;xy}$	0.27$^{b;y}$	3.37$^{b;z}$	8.94$^{a;x}$	5.22$^{b;z}$	15.36$^{b;yz}$	3.81$^{b;z}$	2.09$^{a;y}$	1.39$^{ab;v}$	18.00$^{a;v}$	11.12$^{b;z}$
	III	25.21$^{c;z}$	28.30$^{c;z}$	164.27$^{b;x}$	195.93$^{b;xy}$	0.20$^{c;y}$	3.04$^{b;z}$	8.76$^{a;x}$	2.91$^{c;z}$	15.34$^{b;yz}$	3.54$^{b;z}$	1.80$^{a;yz}$	0.95$^{b;w}$	17.10$^{a;v}$	8.25$^{c;z}$
BA	I	178.56$^{a;xy}$	66.06$^{a;xy}$	242.17$^{ab;w}$	287.12$^{a;x}$	0.53$^{a;y}$	7.31$^{a;yz}$	6.63$^{a;x}$	26.60$^{a;y}$	27.42$^{a;xy}$	14.61$^{a;z}$	1.64$^{a;z}$	0.52$^{a;w}$	2.02$^{a;y}$	42.85$^{a;x}$
	II	82.49$^{b;y}$	42.52$^{b;yz}$	263.08$^{a;w}$	294.25$^{a;x}$	0.21$^{b;y}$	1.5$^{c;z}$	3.70$^{b;y}$	12.66$^{b;yz}$	12.85$^{b;z}$	5.25$^{b;z}$	2.38$^{a;y}$	0.27$^{b;x}$	2.29$^{a;y}$	10.29$^{c;z}$
	III	65.69$^{c;yz}$	35.98$^{c;yz}$	201.47$^{b;w}$	233.09$^{b;x}$	0.18$^{c;z}$	3.37$^{b;z}$	4.45$^{b;y}$	12.20$^{b;yz}$	13.96$^{b;z}$	6.11$^{b;z}$	2.75$^{a;y}$	0.38$^{b;x}$	1.97$^{a;y}$	21.06$^{b;y}$
CE	I	123.98$^{a;y}$	53.32$^{a;y}$	238.92$^{a;w}$	268.70$^{a;x}$	0.42$^{a;y}$	6.36$^{a;yz}$	18.21$^{a;w}$	6.09$^{b;z}$	35.65$^{a;x}$	8.70$^{a;z}$	1.80$^{a;yz}$	0.42$^{ab;w}$	3.31$^{a;x}$	16.59$^{a;y}$
	II	97.35$^{b;y}$	41.89$^{b;yz}$	224.52$^{ab;w}$	252.59$^{a;x}$	0.36$^{b;y}$	3.31$^{b;z}$	6.94$^{c;x}$	10.04$^{a;yz}$	38.36$^{a;x}$	6.72$^{a;z}$	1.73$^{a;yz}$	0.60$^{ab;w}$	4.02$^{a;wx}$	18.49$^{a;y}$
	III	71.93$^{c;yz}$	38.57$^{b;yz}$	224.81$^{ab;w}$	250.69$^{a;x}$	0.15$^{c;z}$	3.69$^{b;z}$	10.05$^{b;wx}$	10.60$^{a;yz}$	19.79$^{b;y}$	6.10$^{a;z}$	1.94$^{a;yz}$	0.31$^{ab;x}$	2.68$^{b;y}$	18.64$^{a;y}$
CT RF	I	656.12$^{b;w}$	287.38$^{b;w}$	273.51$^{a;w}$	311.82$^{a;x}$	1.52$^{c;w}$	75.62$^{a;v}$	30.88$^{a;v}$	151.63$^{a;v}$	51.71$^{a;w}$	266.77$^{a;v}$	24.96$^{a;v}$	0.20$^{ab;x}$	1.04$^{b;z}$	443.36$^{a;v}$
	II	852.87$^{a;v}$	348.91$^{a;v}$	250.03$^{a;w}$	283.55$^{a;x}$	2.33$^{a;v}$	29.29$^{b;x}$	24.85$^{ab;vw}$	85.44$^{c;x}$	22.73$^{c;xy}$	243.96$^{bc;w}$	15.75$^{b;w}$	0.24$^{ab;x}$	1.29$^{b;z}$	345.12$^{b;w}$
	III	646.69$^{b;w}$	295.51$^{b;w}$	278.14$^{a;w}$	315.63$^{a;x}$	1.82$^{b;w}$	16.77$^{c;y}$	27.3$^{ab;v}$	131.38$^{b;v}$	32.07$^{b;x}$	200.15$^{bc;w}$	12.31$^{b;w}$	0.16$^{c;y}$	2.54$^{ab;y}$	343.84$^{b;w}$
CT IR	I	294.01$^{a;x}$	206.46$^{a;x}$	478.38$^{ab;v}$	511.84$^{a;w}$	0.89$^{a;x}$	58.21$^{a;w}$	35.61$^{a;v}$	65.80$^{c;x}$	24.46$^{c;xy}$	227.93$^{a;w}$	6.37$^{a;x}$	0.66$^{b;w}$	2.72$^{b;y}$	300.10$^{a;w}$
	II	258.99$^{b;x}$	193.89$^{ab;x}$	261.44$^{c;w}$	295.45$^{c;x}$	0.46$^{b;xy}$	10.28$^{b;y}$	7.77$^{c;x}$	85.99$^{b;x}$	35.14$^{b;x}$	202.31$^{b;w}$	7.04$^{a;x}$	1.19$^{ab;v}$	6.58$^{a;w}$	295.33$^{a;w}$
	III	222.37$^{c;x}$	155.70$^{b;x}$	418.32$^{ab;v}$	458.48$^{b;w}$	0.26$^{c;y}$	13.05$^{b;y}$	18.32$^{b;w}$	109.45$^{a;w}$	60.94$^{a;v}$	182.67$^{bc;x}$	6.54$^{a;x}$	1.71$^{ab;v}$	4.04$^{ab;wx}$	298.66$^{a;w}$
CM	I	190.06$^{a;xy}$	59.61$^{a;y}$	259.83$^{a;w}$	300.51$^{a;x}$	0.25$^{a;y}$	4.21$^{a;z}$	9.54$^{a;x}$	12.64$^{a;yz}$	31.25$^{a;x}$	32.78$^{a;y}$	19.75$^{a;w}$	1.29$^{ab;v}$	3.27$^{a;x}$	65.18$^{a;x}$
	II	171.91$^{b;xy}$	59.21$^{a;y}$	259.66$^{a;w}$	295.75$^{a;x}$	0.24$^{a;y}$	1.22$^{b;z}$	3.54$^{b;y}$	1.15$^{b;z}$	19.30$^{c;y}$	13.31$^{b;z}$	7.66$^{b;x}$	1.06$^{ab;v}$	3.00$^{a;x}$	22.13$^{b;y}$
	III	96.39$^{c;y}$	54.31$^{ab;y}$	235.21$^{a;w}$	266.14$^{ab;x}$	0.19$^{ab;z}$	1.65$^{b;z}$	1.50$^{d;x}$	1.78$^{b;z}$	25.08$^{b;xy}$	11.11$^{b;z}$	3.56$^{c;y}$	1.84$^{a;v}$	3.68$^{a;x}$	16.46$^{a;yz}$

TPC: Total phenols determined by spectrophotometry; *o*-diph: *o*-diphenols determined by spectrophotometry; α-toc: α-tocopherols; T toc: total tocopherols; ABTS•−: antioxidant power; Htyr: hydroxytyrosol; Tyr: tyrosol; DAOA: decarboxymethyl oleuropein aglycone; AcPin: (+)-1-acetoxypinoresinol; DLigAgl: deacetoxy ligstroside aglycone; OlAgl: oleuropein aglycone; LigAgl: ligstroside aglycone; VA: vanillic acid; *o*-CA: *o*-coumaric acid; SID: secoiridoids.

[a–c] Different letters in the same column indicate significantly different values ($p < 0.05$).

[v–z] Different letters in the same column concerning all studied samples indicate significantly different values ($p < 0.05$).

[§,§§] mg of gallic acid/kg of oil

[$,¤] mg of tocopherols/kg of oil

[#] mol of Trolox/kg of oil

[‡] mg of 3,4-dihydroxyphenylacetic acid/kg of oil

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

vesting time were observed for each monovarietal oil tested. A negative effect of the fruit ripening stage on the phenolic concentration of VOO is particularly clear. The TP content showed the lowest concentration in oils obtained from over-ripened olives. In fact, at stage III of olive ripening, the decrease of the TP content reached 86.3, 63.2, 49.3 and 42% in Nocellara del Belice, Biancolilla, Chemlali and Cerasuola, respectively, in agreement with the results obtained by other authors [5, 23]. However, in the rain-fed Chétoui variety, the TP contents increased to reach a maximum level at stage II of fruit ripening; then, the TP content decreased. These results coincide with those obtained by others authors [6, 28] showing that, during olive ripening, the concentration of phenolic compounds increased to reach a maximum at the spotted and purple pigmentation stage. The increase in TP at the last stage of maturation could be due to the reduction in water content (olive fruit humidity) observed during ripening. This can affect the extraction of partially soluble compounds [6]. Tab. 2 shows that the TP of Chétoui oils were significantly affected by the irrigation regime. In fact, oils obtained from irrigated trees had lower levels of TP that non-irrigated ones. With regard to water availability, it is generally agreed that the level of phenolic compounds is higher in oils obtained from drought-stressed crops than in those from irrigated crops [8, 9]. Previous studies [8] showed that phenolic compounds, both in the fruit and in the corresponding oil, were negatively affected by the water supply. The observed differences in phenolic concentration in the oils could be a consequence of the different water stress levels of olives from rain-fed and irrigation conditions; this condition can involve changes in the activity of enzymes responsible for phenolic compound synthesis, such as L-phenylalanine ammonium-lyase, taking into account that the activity of this enzyme is greater under higher water stress conditions [8, 9].

The change in the *o*-diphenol contents in the Sicilian olive oils was parallel to that in the TP. However, the Tunisian samples cultivated under rain-fed conditions did not show evident losses in *o*-diphenols during ripening. Nevertheless, in Chétoui oils obtained from irrigated trees, *o*-diphenols decreased slightly at the last harvesting date.

Tab. 2 shows the change in tocopherols content of the samples studied. The five varieties behaved differently. In Nocellara del Belice, there was a drastic decrease of this molecule from the first to the second olive ripening degree, and then its amount did not change until the last harvest. In Biancolilla, Cerasuola, Chétoui and Chémlali, the α-toc levels did not decrease significantly, coinciding with the results obtained by other authors regarding Spanish varieties [5]. Moreover, in Chétoui oils obtained after irrigation, the variation in the concentration of this antioxidant did not show a univocal trend during the olive maturation process. The data available in the present study confirmed previous findings [7].

3.4 Identification of phenolic compounds by HPLC-MSD

Fig. 1 shows the HPLC phenolic composition of the cv. Chétoui and Nocellara del Belice at the first stage of olive ripening in monovarietal VOO extracts, recorded at 280 nm. HPLC-MS analysis showed that the VOO samples contain low amounts of phenyl acids and phenyl alcohols and high concentrations of secoiridoid derivatives (SID) such as oleuropein and ligstroside aglycons and DAOA which originate from the oleuropein [29, 30].

The HPLC phenolic profile evidenced also the occurrence, in small amounts, of phenolic acids such as vanillic and *o*-coumaric acid, as observed by others authors [30]. The vanillic acid amounts did not exceed 2 mg/kg of oil in all studied samples (Tab. 2). The levels of *o*-coumaric acid in the Tunisian oils and in Biancolilla and Cerasuola olive oils ranged from 1 to 6.6 mg/kg of oil; however, in Nocellara del Belice oils it reached 18.1 mg/kg of oil. It seems that neither the olive ripening degree nor the irrigation of the trees had a clear influence on the levels of phenolic acids (Tab. 2).

The Chétoui cv. oil extract showed high concentrations of the secoiridoid derivative DAOA and oleuropein aglycon ranging from 85.4 to 151.6 and from 182.7 to 266.8 mg/kg of oil, respectively (Tab. 2). However, in the other studied samples, the DAOA concentration did not exceed 29 mg/kg and the oleuropein aglycon levels did not surpass 32.8 mg/kg. In the analyzed extra VOO extracts, other secoiridoids were present. The content of ligstroside aglycon ranged from 3.6 to 25 and from 1.6 to 2.7 mg/kg in the Tunisian and Sicilian varieties, respectively. The lignan acetoxypinoresinol was detected together with the decarboxy-methyl ligstroside aglycon, this last known also as oleocanthal [31]; their levels ranged from 19.3 to 61 and from 12.8 to 38.4 mg/kg of oil in the Tunisian and Sicilian VOO, respectively. Hence, as reported by different authors, the qualitative and quantitative phenolic composition of VOO is affected by the cultivar and/or the growing area [4, 6, 7].

3.5 Changes in phenolic compound composition

In the most studied oils samples, the SID levels decreased as ripening progressed, as shown by the spectrophotometric values found through the TP content.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

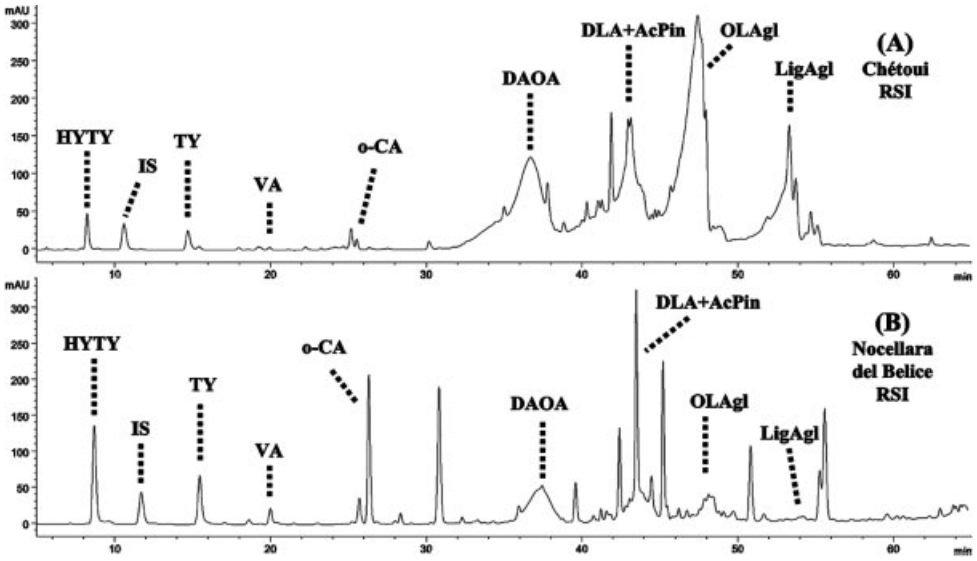

Fig. 1. HPLC chromatograms (detection at 280 nm) of the extracts from cv. Chétoui (A) and Nocellara del Belice (B) olive oils at the first stage of ripening. Htyr: hydroxytyrosol; IS: internal standard; Tyr: tyrosol; VA: vanilic acid; *o*-CA: *o*-coumaric acid; DAOA: decarboxy-methyl oleuropein aglycone; AcPin: (+)-1-acetoxypinoresinol; DLA: decarboxy-methyl ligstroside aglycone; OlAgl: oleuropein aglycone; LigAgl: ligstroside aglycone.

In fact, in the Chétoui oils obtained under rain-fed conditions, the SID amounts decreased slightly from 443.4 down to 343.8 mg/kg of oil. This trend to decrease as ripening progressed was more pronounced in Chemlali, Nocellara del Belice and Biancolilla VOO (from 65.2 down to 16.5, 42.3 down to 8.2 and 42.8 down to 21.1 mg/kg of oil, respectively). Such observations are in conformity with other findings showing that the SID amount decreases strongly in over-ripened olives [4, 32, 33]. Thus, during maturation, oleuropein aglycone, the major secoiridoid identified in all analyzed olive oils, undergoes hydrolysis and yields several simpler molecules that build up the well-known olive oil complex taste [32, 33]. SID levels in the Cerasuola oils remained practically unchanged throughout the olive maturation process. Regarding the amount of simple phenols (sum of tyrosol and hydroxytyrosol), the Tunisian and Sicilian oils (Tab. 2) showed an inverse relationship between the amount of simple phenols and the maturation degree of the olives, as previously reported by other authors [4, 23]. The level of the sum of acetoxypinoresinol and decarboxy-methyl ligstroside aglycon did not show significant differences during the maturity process in the tested oils.

The HPLC analysis of phenolic extracts obtained from Chétoui VOO under irrigation yielded HPLC profiles containing the same chromatographic peaks. Identified and quantified secoiridoids were affected by the irrigation regime applied to the Chétoui olive tree throughout the ripening process (Tab. 2). In fact, for samples obtained under irrigation conditions, the SID content remained practically constant as ripening progressed, whereas in oils obtained in rain-fed conditions these levels decreased slightly. In general, our results agree with those found by other authors [9]. Other studies explained these findings by the fact that secoiridoids are produced from the secondary metabolism of terpenes and are usually derived from the oleoside type of glucoside oleosides, which are characterized by a combination of elenolic acid and a glucosidic residue. It could be stated that these compounds originate from the same biogenetic route, the acetate/mevalonate pathway, and consequently respond similarly to water stress. Different hypotheses have been developed to explain the differences found in the phenol content of oils under irrigation: the different water content of the pastes that could imply a different solubilization of phenols which are more soluble in water than in oil [34] and a different effectiveness in the release of phenolic compounds during crushing and malaxation linked to polysaccharides of the cell wall [10].

3.6 Statistical correlation of olive oil composition and antioxidant activity

The antioxidant activity of the phenolic fraction of VOO is mainly due to radical scavenging; this was confirmed by investigating the ability of the polar extracts to scavenge

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

$ABTS^+$ radicals [28]. It is affected by different factors. It is used to evaluate oil and fat quality as it gives a good estimation of their susceptibility to oxidative degradation. In fact, an interesting positive Pearson's correlation between spectrophotometric indices and the evaluation of antioxidant activity were also found: TP *vs. o*-diphenols ($r^2 = 0.9329$, $p < 0.001$), TP *vs.* ABTS activity ($r^2 = 0.9630$, $p < 0.001$) and *o*-diphenols *vs.* ABTS activity ($r^2 = 0.9226$, $p < 0.001$). The correlations recorded could be explained by the antioxidant activity of the phenolic compounds that contributes to the resistance of extra-virgin olive oils to oxidation processes [22].

3.7 Chemometrics

Principal component analysis (PCA) is used in exploratory analysis. It gives graphical representations of inter-sample and inter-variable relationships and provides a way to reduce the complexity of the data. PCA (Fig. 2) was carried out using 15 variables selected between the chemical profiles reported in Tab. 1 and 2, using a factor analysis as extraction variables technique. PCA revealed that the variables linked to phenols provide similar information because the individual vectors had the same direction and intensity with respect to the principal component 1 (PC 1). The explained total variance was high (around 84%). Fig. 1 showed that the samples obtained from olives grown under similar pedo-climatic conditions, such as the three Sicilian cultivars (the first PCA quadrant) and Chétoui oils, can be grouped together. The PCA did distinguish neither between VOO obtained from olive orchards under rain-fed or irrigation conditions nor among different ripening stages. Moreover, at the bottom of Fig. 1, a cluster is shown, formed only by oil samples from the cultivar Chemlali. In fact, the last olive variety, typical of the south of Tunisia, was characterized by the lowest percentages of triolein.

In conclusion, the characterization of some Tunisian and Sicilian monovarietal VOO tested in their original cultivation area allowed the detection of significant differences in the chemical composition, practically in relation to olive ripening stages and some agronomic techniques such as irrigation. The contents of minor components of the studied monovarietal VOO were especially affected by the agronomic conditions of production.

These preliminary results indicate that it was possible to classify the Tunisian and Sicilian oils tested in their original growing area based on their chemical composition. However, the study of a larger number of samples from various years of production would lend support to the results obtained by this first screening. Additional work will be addressed to evaluate the behavior of these local cultivars in different growing districts, in order to separate the genetic effect from the environmental factor.

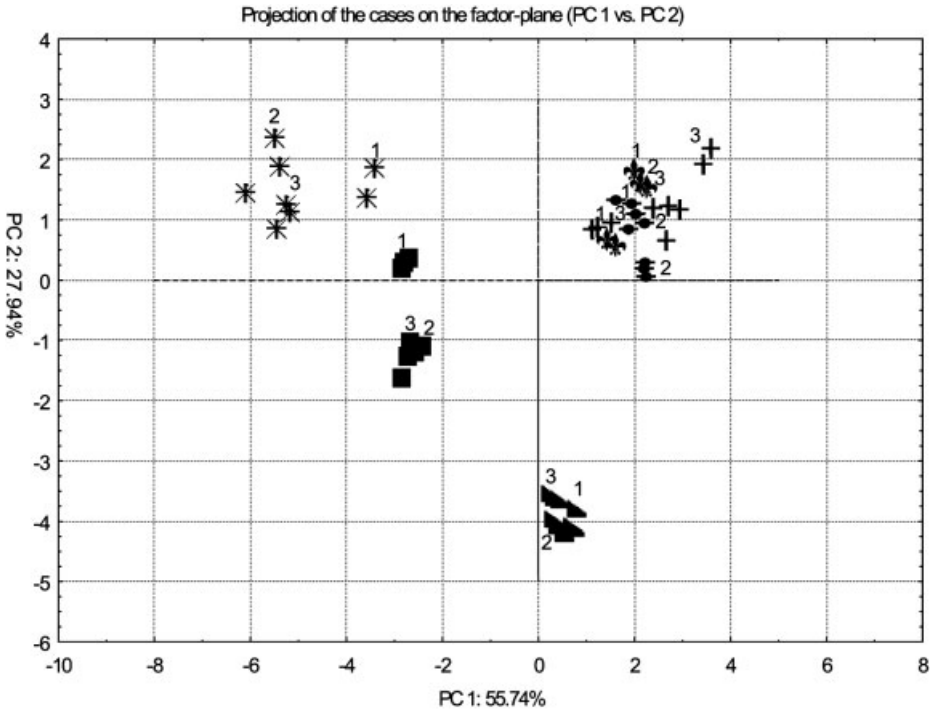

Fig. 2. PCA of Tunisian and Sicilian olive oils. (◣) Chemlali, (✳) Chétoui cv. in rain-fed control, (■) Chétoui cv. under irrigation regime, (+) Nocellara del Belice, (-●-) Biancolilla, (✤) Cerasuola. (1, 2, 3): The three different ripeness stages of the olive samples.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

References

[1] R. W. Owen, W. Mier, A. Giacosa, W. E. Hull, B. Spiegelhalder, H. Bartsch: Phenolic compounds and squalene in olive oils: The concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food Chem Toxicol.* 2000, **38**, 647–659.

[2] L. Abaza, M. Msallem, D. Daoud, M. Zarrouk: Caractérisation des huiles de sept variétés d'olivier tunisiennes. *Oléagineux Corps Gras Lipides* 2002, **9**, 174–179.

[3] S. Ben Témime, W. Taamalli, B. Baccouri, L. Abaza, D. Daoud, M. Zarrouk: Changes in olive oil quality of Chétoui variety according to origin of plantation. *J Food Lipids* 2006, **13**, 88–99.

[4] M. Bonoli, A. Bendini, L. Cerretani, G. Lercker, T. Gallina Toschi: Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. *J Agric Food Chem.* 2004, **52**, 7026–7032.

[5] F. Guitérrez, B. Jimenz, A. Ruiz, M. A. Albi: Effect of ripeness on oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and the different components involved. *J Agric Food Chem.* 1999, **47**, 121–127.

[6] M. D. Salvador, F. Aranda, G. Fregapane: Influence of fruit ripening on Cornicabra virgin olive oil quality: A study of four crop seasons. *Food Chem.* 2001, **73**, 45–53.

[7] A. Gomez-Rico, M. D. Salvador, A. Moriana, D. Perez, N. Olmedilla, F. Ribas, G. Fregapane: Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food Chem.* 2006, **2**, 568–578.

[8] M. Patumi, R. d'Andria, G. Marsilio, G. Fontanazza, G. Morelli, B. Lanza: Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. *Food Chem.* 2002, **77**, 27–34.

[9] M. J. Tovar, M. P. Romero, M. Girona, M. J. Motilva: L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing fruit of olive tree (*Olea europaea* L. cv. Arbequina) grown under different irrigation regimes. *J Sci Food Agric.* 2002, **82**, 892–898.

[10] L. S. Artajo, M. P. Romero, M. J. Tovar, M. J. Motilva: Effect of irrigation to olive trees (*Olea europaea* L.) on phenolic compounds transfer during olive oil extraction. *Eur J Lipid Sci Technol.* 2006, **108**, 19–27.

[11] L. Abaza, W. Taamalli, S. Ben Temime, D. Daoud, F. Gutiérrez, M. Zarrouk: Natural antioxidant composition as correlated to stability of some Tunisian virgin olive oils. *Riv Ital Sost Grasse* 2005, **82**, 12–18.

[12] D. Boskow: *Olive Oil. Chemistry and Technology*. AOCS Press, Champaign, IL (USA) 1999.

[13] G. F. Montedoro, L. Garafolo, M. Bertolucci: Factors sharing in the quality characteristics of an olive oil. *Ind Aliment Italy* 1986, **25**, 549–555.

[14] E. Monteleone, G. Caporale, A. Carulucci, E. Pagliarini: Optimisation of the extra virgin olive oil quality. *J Sci Food Agric.* 1998, **77**, 31–37.

[15] G. Bini, L. Sapia, C. Briccoli Bati, P. Toscano: Irrigazione dell'olivo con sistemi a microportata. *Informatore Agrario* 1997, **22**, 37–44.

[16] J. Doorenbos, W. O. Pruitt: Guidelines for predicting crop water requirements. *Irrigation and drainage paper n° 24*. Food and Organisation of the United Nations, Rome (Italy) 1977.

[17] M. Uceda, M. Hermoso: La calidad del aceite de oliva. In: *El Cultivo del Olivo.* Eds. D. Barranco, R. Fernandez-Escobar, L. Rallo, Junta de Andalucıa Ediciones Mundi-Prensa, Madrid (Spain) 1998, pp 547–572.

[18] W. W. Christie: The preparation of derivatives of fatty acids. In: *Gas Chromatography and Lipids*. The Oily Press, Ayr (UK) 1998, pp 64–84.

[19] J. L. Perrin, A. Prevot: Analyse des triglycérides des huiles. *Rev Franc Corps Gras* 1986, **11**, 437–445.

[20] F. M. Pirisi, P. Cabras, C. Falqui Cao, M. Migliorini, M. Mugelli: Phenolic compounds in virgin olive oil. 2. Reappraisal of the extraction, HPLC separation, and quantification procedures. *J Agric Food Chem.* 2000, **48**, 1191–1196.

[21] L. Cerretani, A. Bendini, B. Biguzzi, G. Lercker, T. Gallina Toschi: Valutazione della stabilità ossidativa di oli extravergini di oliva, ottenuti con diversi impianti tecnologici, in relazione ad alcuni parametri qualitativi. *Ind Aliment Italy* 2003, **427**, 706–711.

[22] E. Re, N. Pellegrini, N. Proteggente, A. Pannala, M. Yang, C. Rice-Evans: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* 1999, **26**, 1231–1237.

[23] A. Rotondi, A. Bendini, L. Cerretani, M. Mari, G. Lercker, T. Gallina Toschi: Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. *J Agric Food Chem.* 2004, **52**, 3649–3654.

[24] J. Salas, M. Pastor, J. Castro, V. Vega: Influencia del riego sobre la composicion y caracterısticas del aceite de oliva. *Grasas Aceites* 1997, **48**, 74–82.

[25] A. Lanzon, T. Albi, A. Cert, J. Gracian: The hydrocarbon fraction of virgin olive oil and changes resulting from refining. *J Am Oil Chem Soc.* 1994, **71**, 285–291.

[26] A. De Leonardis, V. Macciola, M. De Felice: Rapid determination of squalene in virgin olive oils using gas-liquid chromatography. *Ital J Food Sci.* 1998, **1**, 75–80.

[27] J. Sanchez, J. L. Harwood: Biosynthesis of triacylglycerols and volatiles in olives. *Eur J Lipid Sci Technol.* 2002, **104**, 564–573.

[28] J. L. Perrin: Minor components and natural antioxidants of olives and olive oils. *Rev Fr Corps Gras* 1992, **39**, 25–32.

[29] M. Tasioula-Margari, O. Okogeri: Isolation and characterization of virgin olive oil phenolic compounds by HPLC/UV and GC/MS. *J Food Sci.* 2001, **66**, 530–534.

[30] F. Pirisi, A. Angioni, P. Cabras, V. L. Garau, M. L. Sanjust di Teulada, M. Kaim dos Santos, G. Bandino: Phenolic compounds in virgin olive oils. I. Low-wavelength quantitative determination of complex phenols by high-performance liquid chromatography under isocratic elution. *J Chromatogr A* 1997, **768**, 207–213.

[31] G. K. Beauchamp, R. S. J. Keast, D. Morel, J. Lin, J. Pika, Q. Han, C. Lee, A. B. Smith, P. A. S. Breslin: Ibuprofen-like activity in extra-virgin olive oil. *Nature* 2005, **437**, 45–46.

[32] M. J. Amiot, A. Fleuriet, J. J. Macheix: Importance and evolution of phenolic compounds in olive during growth and maturation. *J Agric Food Chem.* 1996, **34**, 823–826.

[33] T. Gallina Toschi, L. Cerretani, A. Bendini, M. Bonoli-Carbognin, G. Lercker: Oxidative stability and phenolic content of virgin olive oil: An analytical approach by traditional and high resolution techniques. *J Sep Sci.* 2005, **28**, 859–870.

[34] V. Allogio, F. Caponio: The influence of olive paste preparation techniques on the quality of olive oil. II. Evolution of phenolic substances and some quality parameters referred to the ripening of drupes in virgin olive oil from the Coratina cv. *Riv Ital Sost Grasse* 1997, **74**, 443–447.

[Received: May 11, 2007; accepted: July 16, 2007]

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim