Chemical composition and oxidative stability of Tunisian monovarietal
virgin olive oils with regard to fruit ripening

Olfa Baccouri a, Mokhtar Guerfel a, Bechir Baccouri a, Lorenzo Cerretani b,*,
Alessandra Bendini b, Giovanni Lercker b, Mokhtar Zarrouk a, Douja Daoud Ben Miled a,*

a Laboratoire Caractérisation et Qualité de l’Huile d’Olive, Centre de Biotechnologie de Borj-Cédria, 901-2050 Hammam-Lif, Tunisia
b Dipartimento di Scienze degli Alimenti, Università di Bologna, Piazza Goidanich 60, 47023 Cesena (FC), Italy

Received 12 November 2007; received in revised form 28 December 2007; accepted 18 January 2008

Abstract

The chemical composition of virgin olive oil may be influenced by genotype and different agronomic (i.e. fruit ripeness degree, water supply) and technological factors. This article reports the evaluation of the influence of the olive ripening stage on the quality indices, the major and the minor components and the oxidative stability of the two main monovarietal Tunisian cultivars (cvv. Chéoui and Chemlali) virgin olive oils. Moreover, the olives cv. Chéoui were tested in a rain-fed control and an irrigation regime. The oils sampled at five different ripeness stages were submitted to liquid chromatographic determination (HPLC-DAD/MSD) of their quali-quantitative phenolic and tocopherolic profiles. Moreover, the triacylglycerol and fatty acid compositions, and minor components such as squalene, pigments and their relation with the oil oxidative stability were evaluated. The tested oils showed very good correlation between the oxidative stability and the concentrations of total phenols, practically secoiridoids and α-tocopherol.

© 2008 Elsevier Ltd. All rights reserved.

Keywords: Virgin olive oil; Tunisian varieties; Composition; Oxidative stability; Antioxidants; Ripening stage; Irrigation

1. Introduction

Extra virgin olive oil (EVOO) is considered to be the best olive oil for its organoleptic characteristics, for its oxidative stability and its chemical composition. It is practically the only vegetable oil that can be consumed directly in its raw state and contains important nutritional elements (vitamins, antioxidants, etc.). Virgin olive oil is composed of triacylglycerols (around 97–98%), minor variable amounts of free fatty acids and minor glyceridic compounds – partial glycerides, phospholipids and oxidized triacylglycerols – and around 1% of unsaponifiable constituents of varied structure and polarity (Boskou, 1996). The oxidative stability, sensory quality and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition (Bendini et al., 2007). In fact, the high content of oleic acid in olive oil serves to slow down penetration of fatty acids into arterial walls (Charbonnier, 1982). Oils which are much higher in monounsaturated fatty acids (MUFAs) and lower in saturated fatty acids (SFAs) are preferred because of the proven beneficial effect of MUFAs on serum cholesterol levels. The biological properties of olive oil are also related to the presence of minor components, such as squalene and phytosterols, antioxidant compounds, such as tocopherols and particularly phenols (Owen et al., 2000). In particular, among the natural antioxidants, phenolic compounds, α-tocopherol and β-carotene, are reported to play a key role in preventing oxidation and have been already correlated to the storage stability of virgin olive oils (Rahmani & Saari-Csallany,
However, there is little available information on the effects of other components of the unsaponifiable fraction on the oxidative stability of oil. Some experiments carried out with the Rancimat test have shown that squalene, the most representative component of the olive oil unsaponifiable fraction, does not significantly affect the oil stability (Psomiadou & Tsimidou, 1999), despite its positive effects on human health (Owen et al., 2000).

EVOO composition determines its intrinsic quality and could be influenced by several factors. Cultivar, environment and agronomic practices affect the fruit physiology, whereas processing and storage conditions alter oil composition. Gutiérrez, Jiménez, Ruiz, and Albi (1999) and Caponio, Gomes, and Pasqualone (2001) found a decrease in many EVOO compounds (phenols, tocopherols, pigments) and also in oxidation stability as ripening proceeded and total phenols and induction times were particularly higher in oils from green olives than those from full-ripe fruits. Gomez-Rico et al. (2007) studied the influences of agronomic practices and found that ripening positively affected both fruit and oil quality. Several authors have already studied the influence of genetic and agronomic factors on some French (Amiot, Fleuriet, & Macheix, 1986), Spanish (Carrasco-Pancorbo et al., 2006; Salvador, Aranda, & Fregapane, 2001) and Italian (Cerretani et al., 2006; Romani, Mulinacci, Pinelli, Vincieri, & Cimato, 1999) cultivars. Nevertheless, there is scarce information available on the influence of the ripening degree of fruits and the irrigation of olives trees in the case of Tunisian cultivars (Baccouri et al., 2007). Tunisia is a very important country in the olive oil producing world, the largest African exporter and fourth worldwide after Spain, Italy and Greece with an annual average export over 10,000 metric tonnes (IOOC, 2004). The olive tree (*Olea europaea* L.) is present practically in every region of the country, up to the border of the southern desert. Many varieties occurred in Tunisia but they are two, which stand out: Chemlali, cultivar that occupies more than 2/3 of the total olive growing area, it is cultivated in the centre and the south of the country; the Chétoui variety is the second main variety cultivated in Tunisia. It is widespread in the north of the country, occurring in plains as well in mountain regions, and shows a high capacity of adaptation to various pedo-climatic conditions (Ben Témime et al., 2006b).

This is the first evaluation of chemical composition of Chétoui and Chemlali monovarietal EVOOs in relation to five stages of ripening degree (from October to January). Because of the importance of these two cultivars for Tunisian oil production, the aim of this work was to characterize Chétoui and Chemlali virgin olive oils based on the study of major (triacylglycerols and fatty acids) and minor compounds (squalene, tocopherols, chlorophylls, carotenoids and phenols) as well as on the oxidative stability. This work aimed also to study the effect of water supply in Chétoui oils at different stages of olive maturity. Considering the already noted factors, the ultimate goal was to define the optimum harvesting period in the tested oils.

### 2. Materials and methods

#### 2.1. Samples

The present work was carried on monovarietal virgin olive oils from the two main Tunisian cultivars (cvv. Chétoui and Chemlali). Chétoui and Chemlali olives were hand-picked, in triplicate in perfect sanitary conditions, at five different ripeness stages (RSI, October 3, 2005; RSII, November 1, 2005; RSIII, December 5, 2005; RSIV, January 2, 2006; RSV, January 29, 2006) based on the degree of skin and pulp pigmentation. Moreover, the olives from cv. Chétoui were tested in rain-fed control and an irrigation regime. The experimental field was carried out in the dry season of 2005 (June, July, August and September 2005) at the Centre of Biotechnology of Borig-Cédéria, in the north east of Tunisia. This experimental station has a collection of local and foreign varieties of 20-year-old trees, planted in square with spacing 7 × 7 m. The climate is Mediterranean type with hot and dry summers and mild winters, having an average annual rainfall of 250 mm. In the experimental irrigation treatment, the water requirements were calculated using a methodology based on the crop evapotranspiration (ETc) proposed by the United Nations Food and Agriculture Organization (Doorenbos & Pruitt., 1977). The orchard trees were irrigated daily with compensating drippers (4 L/h) placed around the trees. Olive trees were tested in a factorial combination with two irrigation levels: a rain-fed control (non-irrigated) and one treatment that received seasonal water amount equivalent to 100% of ETc (irrigated). After harvesting, the olives were washed and deleafed. Using an Abencor analyzer (MC2 Ingenierias y Sistemas, Sevilla, Spain), the fruits was crushed with a hammer crusher, and the paste mixed at 25 °C for 30 min, centrifuged without addition of warm water. Analyses were carried out after processing.

#### 2.2. Maturity index (MI)

The olive ripeness index was determined according to the method according to the method developed by the Agronomic Station of Jaén (Uceda & Hermoso, 1998) based on the evaluation of the olive skin and pulp colors. RI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

#### 2.3. Analytical indices

Free acidity, peroxide value and UV spectrophotometric indices (at 232 and 270 nm) were determined according to the European Communities official methods (EEC Reg. 2568/91, 1991). All parameters were determined in triplicate for each sample.
2.4. Chromatographic analysis of triglycerides

A 0.3 g oil sample was dissolved in 10 mL of 2-propanol/acetonitrile/n-hexane (2:2:1, v/v) and homogenized by stirring. HPLC analyses were performed using a HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump delivery system, degasser, and autosampler and an evaporative light scattering detector (ELSD) PL-ELS model 1000 Series (Polymer laboratories, Varian Inc., Amherst, MA, USA). Luna C18 (Phenomenex, Torrance, CA, USA) column, 5 μm particle size, 25 cm x 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. The eluent used was a gradient of 2-propanol as mobile phase A, and acetonitrile as mobile phase B of the solvent system. Elution was performed at a solvent flow rate of 0.7 mL min⁻¹ with a linear gradient as follows: from 0 to 2 min 52% B, up to 4 min 58% B, 25 min 58% B, 30 min 10% B, 37 min 52% B. Peak assignment was carried out by comparison with chromatograms reported in the literature (Perrin & Prevot, 1986) and with several pure standards in particular PPO, SOO were from Matreya (Pleasant Gap, PA, USA) and OOL, POO, OOO from Supelec (St. Louis, MO, USA). 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⁻¹.

2.5. Fatty acids and squalene analysis

FAMEs (fatty acid methyl ester) and squalene analysis was performed using a GC Clarus 500 Perkin–Elmer (Wellesley, MA, USA) equipped with a flame ionization detector (FID). The analysis were carried out after alkaline treatment; this was obtained by mixing 0.05 g of oil dissolved in 1 mL of n-hexane with 1 mL of 2 N potassium hydroxide in methanol according to Christie (1998). In order to quantify the squalene content in studied samples an internal standard (C₁₉₀₀ g from Sigma–Aldrich, St. Louis, MO, USA) of known concentration was added. One microliter of the upper phase was injected into a split 1:20 GC port set at 240 °C; a fused silica capillary column (50 m length, 0.25 mm i.d.), coated with CPSil88 (0.25 μm film thickness, Varian), was utilized. A flow rate of 1.25 mL min⁻¹ of helium as a 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⁻¹ and maintained for 4 min. Peaks were identified by comparison to their retention times with those of authentic reference compounds (MIX 463 from Nucheck Prep. Inc., Elyssian, MN, USA). The fatty acid composition was expressed as relative percentages of each fatty acid, calculated by internal normalization of the chromatographic peak area, whereas the squalene amount in EVOO was estimated based on internal standard and expressed as gram per kilogram of oil. The average was calculated by three replications for each sample.

2.6. Extraction of the phenolic fraction

The LLE was carried out with the same extraction conditions reported by Pirisi, Cabras, Falqui Cao, Migliorini, and Mugelli (2000). Briefly, 4 g of the oil sample was added to 2 mL of n-hexane and 4 mL of a methanol/water (60:40, v/v) solution in a 20 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 4 mL of methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 4 mL of n-hexane to remove the residual oil, then concentrated and dried by evaporative centrifuge (Mivac Duo of Genevac Inc., Valley Cottage, NY, USA) in vacuum at 35 °C. For HPLC analysis, 100 μL of 3,4-dihydroxyphenylacetic acid solution (0.1 mg mL⁻¹) as internal standard was added to 4 g of oil, then after extraction procedure dry extracts were solut-ed in 0.5 mL of a methanol/water (50:50, v/v) solution. Extractions were performed in three replicates (n = 3). Unless otherwise stated extracts were stored at −18 °C before use in order to analyze by HPLC-DAD/MSD.

2.7. Chromatographic analysis of phenols by HPLC-DAD/MSD

Quanti-quantitative phenolic analysis was carried out by HPLC HP 1100 Series instrument equipped with a diode array UV–Vis detector (DAD), mass spectrometer detector (MSD). A column Luna C18 (Phenomenex) of 5 μm particle size and 250 mm, 3.00 mm ID was used. The mobile phase flow rate was 0.5 mL min⁻¹. The wavelength of DAD 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 (Rotondi et al., 2004) was carried out using water/formic acid (99.5:0.5, v/v) 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) (r² = 0.999). The average was calculated by tree 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, 9.0 L min⁻¹; nebulizer pressure, 50 psi; gas drying temperature, 350 °C. Phenolic compounds were identified comparing retention times (by spiking attempts) and UV and MS spectra of the detected peaks with those of commercial (tyrosol, vanillic and o-coumaric acids from Sigma–Aldrich) and synthesized compounds (hydroxytyrosol prepared by chemical reduction of 3,4-dihydroxy-phenylacetic acid, according to Baraldi, Simon, Manfredini, and Menziani (1983)). Decarboxymethyl oleuropein aglycon (DAOA) and (+)-leucidic acid were detected.
2.8. Chromatographic analysis of tocopherols

One gram of oil sample was dissolved in 10 mL of n-hexane, extracts were filtered through a 0.45 μm nylon filter. α-toc, β-toc, γ-toc and δ-toc, respectively, were determined by HPLC equipped with a DAD set at 295 nm. The used column was a Phenomenex, Luna, CN 100A, (150 mm, 4.6 mm ID) in isocratic conditions with n-hexane: dichloromethane (95:5, v/v), as the mobile phase, at a flow rate of 1.0 mL min⁻¹. 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, r² = 0.999, 0.986 and 0.999, respectively) and used for quantification. Results are given in mg of α or β or γ tocopherol per kg of oil.

2.9. Carotenoids and chlorophylls determination

The pigment composition was determined modifying the procedures described by (Mingué-Mosquera, Rejano, Gandul, Sanchez, & Garrido, 1991): 1 g oil was dissolved with iso-octane until 10 mL solution. Spectrophotometric determinations were carried out using an UV–Vis 1601 instrument (Shimadzu Co., Kyoto, Japan). The absorbance of this solution was read at 448 and 475 nm for carotenoids and at 670 nm for chlorophylls. Total carotenoids and chlorophylls were determined by calibration with known standard solutions of standard β-carotene (Fluka) and chlorophyll a (Carlo Erba, Milan, Italy) (r² = 0.986 and 0.999, respectively). The results are expressed in mg of β-carotene or chlorophyll a per kg of oil. All experiments and analytical determinations were carried out in triplicate.

2.10. Evaluation of oxidative stability under forced conditions

Oxidative stability was measured with the Rancimat 743 apparatus (Metrohm Ω, Basel, Switzerland) according to Gutierrez (1989). Stability was expressed as the oxidation induction time (h), using an oil sample of 3.6 g warmed to 101.6 °C and an air flow of 10 L/h.

2.11. Statistical analysis

The results reported in this study are the averages of at least three repetitions (n = 3), unless otherwise stated. Chemical data were analysed by the SPSS r.11.0.0 statistical software (SPSS Inc., Chicago, IL, USA). The significance of differences at a 5% level between averages was determined by one-way ANOVA using Tukey’s test.

3. Results and discussion

3.1. Maturity index

At the first harvesting date, olive ripening index (RI) of cv. Chètouri cultivated in rain-fed conditions on October 3, was 0.5. On the three subsequent dates (II, III and IV), the ripening index markedly increased at 4. Then at the last harvesting date (V), ripening index increased slightly. With the regard to the second variety of olives (Chemlali) on average the maturation of olives reached the black stage perfectly in December, whereas, in Chétoui olives this stage of maturation was observed in January. All this collected RI data confirm how the genetic traits influence the ripening process. In fact, these two Tunisian olive varieties were characterized by a different trend of ripening process; Chétoui ripped earlier than Chemlali olives. Moreover, in the case of Chétoui variety, the RI was apparently not affected by the irrigation regime, in fact, no statistically significant differences were observed (Table 1).

3.2. Free acidity, peroxide value, UV spectrophotometric indices

Parameters considered in this work (Table 1) of all studied olive oils were widely within estimated limits of EEC Reg. 2568/91 (1991), so oils could be classified in the category of extra virgin olive oil. It is obvious that the oils produced from olive fruits, harvested with the mentioned ripening indexes and processed immediately after harvest,
were not exposed to serious hydrolytic and oxidative damage. Table 1 showed that there were no statistically significant differences in free acidity values between the two studied varieties. Whereas, in all tested EVOO, a slight rise in free acidity values was observed as ripening progress. These results are in agreement with those of other authors (Salvador et al., 2001). Olives at a later stage of ripening give oils with higher levels of free acidity since they undergo an increase in enzymatic activity, especially by lipopolit enzymes (Martinez Suarez, 1973), and are more sensitive to pathogenic infections and mechanical damage. The relatively lower free acidity observed in tested samples, is due to the use of only healthy fruits and typically small scale of system used for the processing.

The changes in peroxide index were similar in the two varieties: a decrease during ripening process (Table 1). The monovarietal EVOO obtained from olives at more advanced stages of maturity showed lower peroxide values. This behaviour can be explained by a decrease in the activity of the enzyme lipoygenase in both varieties. These results are in accord with those of other authors (Gutierrez et al., 1999; Salvador et al., 2001). Moreover, these two indices of oil quality were not influenced by the irrigation of Chetoui trees, since no statistically significant differences between oils obtained from rain-fed and irrigation treatments were observed. This was also obtained by Tovar, Motilva, and Romero (2001) in virgin olive oils from Arbequina cultivar.

Measurements of absorbance at specific wavelengths in the UV region are used to provide information on the quality of olive oil. EVOO is required to have extinction coefficients at 232 and 270 nm, respectively, of less than 2.50 and 0.20, the respective limits for “extra virgin olive oil” category (Table 1).

### 3.3. Major compounds composition

#### 3.3.1. Fatty acids

Identified fatty acids were: palmitic (C16:0), palmitoleic (C17:0), margaric (C17:1), margaroleic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), gadoleic (C20:1), behenic (C22:0) and lignoceric (C24:0) acids (Table 2). Palmitic, stearic, oleic and linoleic acids were major fatty acids. Palmitoleic, linolenic and arachidic acids were also determined in small amounts in all the samples. Margaric, margaroleic, behenic, gadoleic and lignoceric were present at less than 0.2% in studied monovarietal olive oils. In all the samples, the oleic acid is always the most abundant compound, never less than 59% of the total fatty acid. It can be seen that with the exception of palmitic, stearic linoleic, and linolenic acids, the fatty acid content did not vary during maturation process. In fact, the palmitic acid content...
slightly decreased as fruit ripened, from 12.44% down to 9.42% and from 12.97% to 10.57%, in Chétoui oils obtained in a rain-fed and an irrigation regime, respectively. The palmitic acid level fell during ripening process, possibly as a result of a dilution effect (Gutierrez et al., 1999). However, in Chemlali EVOO, the percentage of this fatty acid did not show a trend as much clear throughout the maturity process. In the case of stearic acid, the two varieties behaved differently. In fact, the content of this fatty acid Chétoui samples (from irrigated and non irrigated trees) increased slightly at the last stage of ripening. Whereas, in Chemlali oils, the stearic acid content decreased from 3.19% down to 1.99%.

In the course of fruit ripening, oleic and linoleic acids showed an opposite trend in studied samples. In fact, oleic acid contents decreased gradually, whereas, linoleic acid levels increased as fruit ripened. Hence, changes observed from first harvest to last harvest in the oleic/linoleic acid ratio show a decreasing trend during maturity process, which was also confirmed by a good negative correlation ($r^2 = 0.812$, 0.949, $p < 0.001$) between this ratio and the RI of Chétoui oils obtained in rain-fed and irrigation conditions, respectively. In the case of Chemlali oils, this correlation was $r^2 = 0.513$, $p < 0.001$. Therefore, in these EVOO, the ratio between monounsaturated and polyunsaturated fatty acids tended to decrease during the olive maturation process (Table 2). This behaviour could be explained by the activity of the enzyme oleate desaturase transforming oleic acid into linoleic (Gutierrez et al., 1999). With the exception of the fist stage of ripening, Chétoui EVOO obtained in rain-fed conditions showed a statistically significant higher content of oleic acid, whereas EVOO from irrigated trees had higher content of linoleic acid. Consequently, the MUFA/PUFA ratio was significantly higher in oils obtained in rain-fed conditions, in line with the results obtained by other authors (Gomez-Rico et al., 2007).

### 3.3.2. Triacylglycerols

HPLC–ELSD analysis of triacylglycerols permitted the identification and the quantification of 10 triacylglycerols (TAGs), 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. Triacylglycerol contents, expressed in percentage of total triacylglycerols (Table 3) showed variations between samples from different cultivars throughout the olive ripening.

In relation to the main TAGs (OOL, POL, OOO and POO), the level of triolein (OOO), was remarkably high, ranging from 33.5% to 56.3% and from 24.6% to 50.4% in Chétoui and Chemlali oils, respectively. Among the two studied EVOO samples, cv. Chemlali registered the lowest percentages of triolein. The second peak in order of quantitative importance in studied EVOO corresponded to the POO which ranged from 5.1% to 30% and from 26.3% to 37.3% in Chétoui and Chemlali VOO, respectively. Chemlali oils had higher amounts of POL (4–17.42%) and lower levels of OOL (10.85–22.80%). Such as for fatty acids, the composition of triacylglycerols of Chétoui and Chemlali oils, showed variations depending on the ripening stage of the fruit. The major change about TGs profile regards to a strong lowering of OOO. The percentage on this TG fell drastically during the olive ripening, this finding was corroborated by the negative correlations with the RI ($r^2 = 0.932$, 0.776, $p < 0.001$) in Chétoui samples obtained under rain-fed and irrigation conditions.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Ripening index</th>
<th>PLL</th>
<th>OLL</th>
<th>POLn + EeOL</th>
<th>OOL</th>
<th>POL</th>
<th>OOO</th>
<th>POO</th>
<th>PPO</th>
<th>SOO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT RF</td>
<td>0.5$^{a}$</td>
<td>0.10$^{a}$</td>
<td>0.07$^{b}$</td>
<td>0.41$^{b}$</td>
<td>12.23$^{a}$</td>
<td>3.21$^{b}$</td>
<td>56.32$^{a}$</td>
<td>25.93$^{b}$</td>
<td>0.53$^{a}$</td>
<td>1.20$^{a}$</td>
</tr>
<tr>
<td></td>
<td>1.3$^{a}$</td>
<td>0.00$^{a}$</td>
<td>0.43$^{b}$</td>
<td>1.60$^{a}$</td>
<td>19.16$^{a}$</td>
<td>4.27$^{b}$</td>
<td>51.58$^{a}$</td>
<td>21.09$^{a}$</td>
<td>0.76$^{b}$</td>
<td>1.13$^{a}$</td>
</tr>
<tr>
<td></td>
<td>2.9$^{a}$</td>
<td>0.00$^{a}$</td>
<td>0.23$^{b}$</td>
<td>1.25$^{a}$</td>
<td>22.20$^{a}$</td>
<td>4.34$^{b}$</td>
<td>49.20$^{a}$</td>
<td>21.36$^{a}$</td>
<td>0.76$^{b}$</td>
<td>0.66$^{b}$</td>
</tr>
<tr>
<td></td>
<td>4.1$^{a}$</td>
<td>0.23$^{b}$</td>
<td>0.46$^{b}$</td>
<td>5.37$^{a}$</td>
<td>29.79$^{b}$</td>
<td>5.49$^{b}$</td>
<td>44.10$^{b}$</td>
<td>13.21$^{b}$</td>
<td>0.00$^{a}$</td>
<td>1.34$^{b}$</td>
</tr>
<tr>
<td></td>
<td>5.3$^{a}$</td>
<td>0.45$^{a}$</td>
<td>3.04$^{a}$</td>
<td>7.20$^{a}$</td>
<td>36.59$^{a}$</td>
<td>4.61$^{a}$</td>
<td>43.01$^{a}$</td>
<td>5.08$^{a}$</td>
<td>0.00$^{a}$</td>
<td>0.00$^{a}$</td>
</tr>
<tr>
<td>CT IR</td>
<td>0.7$^{a}$</td>
<td>0.15$^{a}$</td>
<td>1.22$^{a}$</td>
<td>1.60$^{a}$</td>
<td>15.40$^{a}$</td>
<td>4.39$^{b}$</td>
<td>49.18$^{a}$</td>
<td>26.51$^{b}$</td>
<td>1.29$^{a}$</td>
<td>0.64$^{a}$</td>
</tr>
<tr>
<td></td>
<td>1.5$^{a}$</td>
<td>0.05$^{a}$</td>
<td>0.35$^{b}$</td>
<td>1.79$^{a}$</td>
<td>19.70$^{b}$</td>
<td>5.50$^{a}$</td>
<td>40.50$^{b}$</td>
<td>30.02$^{a}$</td>
<td>0.44$^{b}$</td>
<td>1.65$^{b}$</td>
</tr>
<tr>
<td></td>
<td>3.0$^{a}$</td>
<td>0.04$^{a}$</td>
<td>0.31$^{b}$</td>
<td>4.56$^{a}$</td>
<td>22.37$^{a}$</td>
<td>5.46$^{a}$</td>
<td>40.30$^{a}$</td>
<td>23.31$^{b}$</td>
<td>0.80$^{b}$</td>
<td>2.86$^{b}$</td>
</tr>
<tr>
<td></td>
<td>4.5$^{a}$</td>
<td>0.15$^{a}$</td>
<td>0.22$^{b}$</td>
<td>7.51$^{a}$</td>
<td>23.80$^{b}$</td>
<td>8.73$^{a}$</td>
<td>33.54$^{a}$</td>
<td>22.21$^{b}$</td>
<td>1.00$^{b}$</td>
<td>0.64$^{a}$</td>
</tr>
<tr>
<td></td>
<td>5.5$^{a}$</td>
<td>0.06$^{a}$</td>
<td>0.33$^{b}$</td>
<td>5.93$^{a}$</td>
<td>25.79$^{a}$</td>
<td>9.13$^{a}$</td>
<td>35.01$^{a}$</td>
<td>22.00$^{b}$</td>
<td>0.30$^{b}$</td>
<td>1.41$^{b}$</td>
</tr>
<tr>
<td>CM</td>
<td>1.9$^{a}$</td>
<td>0.00$^{b}$</td>
<td>0.60$^{b}$</td>
<td>0.39$^{a}$</td>
<td>10.85$^{a}$</td>
<td>4.00$^{a}$</td>
<td>50.42$^{a}$</td>
<td>31.95$^{a}$</td>
<td>0.68$^{a}$</td>
<td>1.11$^{a}$</td>
</tr>
<tr>
<td></td>
<td>2.8$^{a}$</td>
<td>0.00$^{b}$</td>
<td>0.30$^{b}$</td>
<td>2.47$^{a}$</td>
<td>13.88$^{a}$</td>
<td>11.80$^{b}$</td>
<td>31.04$^{b}$</td>
<td>37.34$^{a}$</td>
<td>2.49$^{a}$</td>
<td>0.68$^{a}$</td>
</tr>
<tr>
<td></td>
<td>3.5$^{a}$</td>
<td>0.00$^{b}$</td>
<td>0.30$^{b}$</td>
<td>3.36$^{a}$</td>
<td>14.52$^{a}$</td>
<td>12.62$^{b}$</td>
<td>30.79$^{a}$</td>
<td>35.45$^{b}$</td>
<td>2.54$^{b}$</td>
<td>0.41$^{b}$</td>
</tr>
<tr>
<td></td>
<td>4.7$^{a}$</td>
<td>0.21$^{b}$</td>
<td>0.45$^{b}$</td>
<td>3.29$^{a}$</td>
<td>18.65$^{b}$</td>
<td>17.42$^{a}$</td>
<td>24.57$^{a}$</td>
<td>32.87$^{b}$</td>
<td>2.20$^{b}$</td>
<td>0.31$^{b}$</td>
</tr>
<tr>
<td></td>
<td>6.7$^{a}$</td>
<td>0.00$^{b}$</td>
<td>0.20$^{b}$</td>
<td>3.68$^{a}$</td>
<td>22.80$^{a}$</td>
<td>13.17$^{b}$</td>
<td>32.60$^{b}$</td>
<td>26.26$^{a}$</td>
<td>1.02$^{a}$</td>
<td>0.27$^{b}$</td>
</tr>
</tbody>
</table>

Note: P: palmitic; S: stearic; O: oleic; L: linoleic; Ln: linolenic; E: elaidinic acids. *Different letters in the same column concerning the same cultivar indicate significantly different values ($p < 0.05$). **Different letters in the same column concerning all samples indicate significantly different values ($p < 0.05$).

As percentage of total triacylglycerols.
respectively. Moreover, Chétoui oils obtained in rain-fed conditions presented a high negative correlation between POO percentages and the RI ($r^2 = 0.888$, $p < 0.001$). This trend coincides with those concerning fatty acid composition: in fact, as previously observed, the oleic acid percentage decreased during the olive ripening, at the same time as quantity of linoleic acid increased, due to oleate desaturase activity during triacylglycerol biosynthesis (Sanchez & Harwood, 2002). In the case of Chemlali oils these correlations if less remarkable, were evidenced. The OOL content was positively correlated with RI ($r^2 = 0.970$, 0.987, $p < 0.001$) in the rain-fed Chétoui and Chemlali samples, respectively.

Concerning Chétoui EVOO subjected of the irrigation regime: small changes occurred in POO and OOL levels. Moreover, the OOO percentage in irrigation regime such as in oils obtained rain-fed conditions, Chétoui oils showed during olive maturation, a decreasing trend but starting at a lower percentage. The POL percentages showed a more important positive correlation with RI in Chétoui oils obtained in irrigation conditions ($r^2 = 0.874$, $p < 0.001$). Whereas, in rain-fed conditions POL content vs. RI was $r^2 = 0.581$ ($p < 0.001$).

### 3.4. Minor unsaponifiable compounds

#### 3.4.1. Squalene

Squalene is the major olive oil hydrocarbon. In fact, this terpenoid hydrocarbon makes up more than 90% of the hydrocarbon fraction (Owen et al., 2000). Chemlali samples present the highest content of squalene reaching 10.48 g kg$^{-1}$ of oil. During the maturity process, this level, deceased remarkably down to 2 g kg$^{-1}$ of oil (Table 4). The same behaviour was observed in Chétoui oils obtained under irrigation regime, in fact, the squalene content fell progressively from 5.99 down to 3.58 g kg$^{-1}$ as ripening progressed. However, in Chétoui oils obtained in rain-fed conditions, this content increased until it reached a maximum of 8.27 g kg$^{-1}$ at the fourth stage of olive ripening, then, decreased. These results could not be confirmed in the literature because there has been no other study on the topic.

#### 3.4.2. Tocopherols

In EVOO, tocopherols usually described are those which were also detected in this work: α-, β- and γ-tocopherols (Beltran, Aguilera, Del Rio, Sanchez, & Martinez, 2005). Together with polar phenolic compounds they are responsible by the oxidative stability of olive oil and, therefore, for its shelf life with special emphasis for α-tocopherol (Mateos, Dominguez, Espartero, & Cert, 2003).

Table 4 reported the tocopherol composition of the studied oils. In all tested samples, and as expected for EVOO (Beltran et al., 2005), α-tocopherol is by far the most abundant isof orm of vitamin E. The two varieties behaved differently in response to maturity process. In Chemlali EVOO, α-tocopherol content remained practically constant until RI 3.5 and then fell in the last stages. In Chétoui, samples obtained in rain-fed system the levels of α-tocopherol do not evidence an univocal trend during the olive ripening. Whereas, in Chétoui EVOO subjected of the irrigation regime, the content of α-tocopherol decreased slightly along the ripening. The content of β and γ isof orm did not show a trend as much clear as maturity progressed. This behaviour has already been found for other cultivars (Gutiérrez et al., 1999).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Ripening index</th>
<th>Tocopherols (mg kg$^{-1}$)</th>
<th>Chlorophyll (mg kg$^{-1}$)</th>
<th>Carotenoid (mg kg$^{-1}$)</th>
<th>Squalene (g kg$^{-1}$ of oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT RF</td>
<td>0.5$^{a,c}$</td>
<td>120.55$^{b,x}$, 4.52$^{a,b}$, 12.63$^{b,w}$, 137.72$^{a,xy}$</td>
<td>17.35$^{a,wx}$, 7.45$^{c,xy}$, 5.63$^{b,x}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.3$^{b,y}$</td>
<td>399.12$^{b,x}$, 6.73$^{a,w}$, 11.71$^{b,x}$, 417.58$^{a,wx}$</td>
<td>12.57$^{a,wx}$, 5.57$^{c,xy}$, 7.92$^{a,x}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.9$^{a,x}$</td>
<td>273.51$^{a,x}$, 4.54$^{a,w}$, 14.02$^{a,wx}$, 292.07$^{c,xy}$</td>
<td>5.46$^{a,xy}$, 2.38$^{b,wx}$, 7.94$^{a,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1$^{b,w}$</td>
<td>478.13$^{c,xy}$, 6.11$^{a,w}$, 11.67$^{b,wx}$, 495.92$^{c,xy}$</td>
<td>2.90$^{a,wy}$, 2.02$^{b,wx}$, 8.27$^{a,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.3$^{c,w}$</td>
<td>250.03$^{c,wx}$, 3.37$^{a,wx}$, 9.17$^{c,xy}$, 262.58$^{c,xy}$</td>
<td>1.88$^{d,wx}$, 1.66$^{d,wx}$, 5.79$^{a,x}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT IR</td>
<td>0.7$^{a,c}$</td>
<td>421.66$^{a,wx}$, 7.37$^{a,wx}$, 13.83$^{b,wx}$, 442.87$^{a,wx}$</td>
<td>21.40$^{a,xy}$, 9.12$^{a,wx}$, 5.99$^{a,x}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5$^{b,y}$</td>
<td>372.34$^{b,wx}$, 7.02$^{a,x}$, 14.91$^{a,wx}$, 400.33$^{a,wx}$</td>
<td>14.19$^{b,wx}$, 7.6$^{a,wx}$, 5.64$^{a,x}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0$^{b,x}$</td>
<td>378.38$^{b,wx}$, 7.02$^{a,x}$, 14.91$^{b,wx}$, 400.33$^{a,wx}$</td>
<td>10.7$^{c,y}$, 6.04$^{c,x}$, 4.26$^{b,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5$^{a,w}$</td>
<td>318.39$^{b,wx}$, 6.97$^{a,x}$, 18.15$^{c,xy}$, 343.45$^{c,wx}$</td>
<td>8.8$^{d,wx}$, 4.06$^{b,wx}$, 3.97$^{b,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5$^{c,w}$</td>
<td>261.44$^{a,wx}$, 5.50$^{a,wx}$, 9.77$^{b,wx}$, 276.72$^{a,wx}$</td>
<td>5.49$^{y}$, 2.1$^{a,wx}$, 3.58$^{c,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM</td>
<td>1.9$^{a,y}$</td>
<td>321.19$^{a,wx}$, 2.88$^{a,xy}$, 6.76$^{a,wx}$, 330.84$^{a,wx}$</td>
<td>20.66$^{a,xy}$, 11.33$^{a,xy}$, 10.48$^{a,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.8$^{b,x}$</td>
<td>325.81$^{b,wx}$, 12.83$^{a,x}$, 11.66$^{b,wx}$, 350.32$^{b,wx}$</td>
<td>17.3$^{b,wx}$, 7.58$^{b,wx}$, 6.67$^{b,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.5$^{c,x}$</td>
<td>329.69$^{a,wx}$, 10.43$^{a,xy}$, 4.83$^{c,xy}$, 344.28$^{b,wx}$</td>
<td>14.4$^{c,xy}$, 4.56$^{b,wx}$, 6.47$^{b,wx}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.7$^{b,w}$</td>
<td>259.66$^{a,wx}$, 2.20$^{a,xy}$, 6.21$^{b,wx}$, 268.08$^{b,wx}$</td>
<td>6.8$^{c,xy}$, 2.33$^{c,xy}$, 4.77$^{c,xy}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.7$^{c,w}$</td>
<td>214.00$^{a,wx}$, 2.93$^{a,xy}$, 6.63$^{b,wx}$, 223.78$^{a,wx}$</td>
<td>3.6$^{c,xy}$, 1.23$^{c,xy}$, 2.00$^{c,xy}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. α-toco: α-tocopherols; β-toco: β-tocopherols; γ-toco: γ-tocopherols; Total: total tocopherols. * Different letters in the same column indicate significantly different values ($p < 0.05$). ** Different letters in the same column concerning all studied samples indicate significantly different values ($p < 0.05$).
3.4.3. Pigments (chlorophylls and carotenoids)

With the spectrophotometric method used in the present research, in any of the samples, a drastic decrease in the chlorophyll content was detected, irrespective of the initial concentration in EVOO. In fact, statistical data analysis for all analysed samples evidenced negative correlations between the chlorophyll concentrations and the RI ($r^2 = 0.926$, 0.908, 0.946, $p < 0.001$) in Chétoui obtained under rain-fed and irrigation conditions and Chemlali oils, respectively. The concentrations of carotenoid pigments, decreased also markedly during ripening in the studied EVOO. In fact, all the tested oils presented a high negative correlation between carotenoid pigment levels and RI ($r^2 = 0.875$, 0.990, 0.841, $p < 0.001$) in Chétoui (rain-fed and irrigation conditions) and Chemlali oils, respectively. The color change in olive oils during maturity process was explained not only by the reduction of the pigment concentration but also the formation of other colored compounds, such as anthocyanins (Vazquez, Maestro, & Graciani, 1971). These results showing the loss of pigments as ripening progress are in accord with those of others authors (Salvador et al., 2001). In general, during fruit ripening, the chlorophylls, which are present in all unripe fruit, break down following the transformation of chloroplasts into chromoplasts, and carotenogenesis takes over, mainly in the tomato, which is one of the most important carotenogenic fruits (Kozukue & Friedman, 2003). The olive fruit, *O. europaea*, a well-known and widespread species of the Oleaceae family is a green, fleshy, edible drupe. During the ripening process, it darkens to purple-black at the same time as the oil content increases. As ripening progresses, photosynthetic activity decreases and the concentrations of both chlorophylls and carotenoids decrease progressively. At the end of the maturation process, the violet or purple colour of the olive fruit is due to the formation of anthocyanins (Roca & Minguez-Mosquera, 2001). Moreover, the water supply to Chétoui trees affected pigment contents in the obtained virgin olive oils. In fact, oils obtained in irrigation regime had higher levels of chlorophyll pigments that non-irrigated one. However, the content of carotenoids was less affected by the water supply. Other studies showed that there were no differences between irrigation treatments in the chlorophyll and carotenoid contents of the Spanish oils (Tovar, Romero, Alegre, Girona, & Motilva, 2003).

3.4.4. Phenolic compounds

The amount of phenolic compounds in EVOO is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation, and to certain extent, are responsible for its sharp bitter taste (Bendini et al., 2007).

Chétoui oils had higher levels of TPs-HPLC at all the stages of maturity (Table 5). This behaviour may be attributed to its markedly late maturation compared to Chemlali variety. Therefore, the same response to the maturity process was observed in the two varieties: the concentration of...
phenolic compounds progressively increased until to reaches a maximum at the “reddish” and “black” pigmentation stage (ripening index between 3 and 4), after which it decreased. These results coincide with those observed in Cornicabra variety (Salvador et al., 2001). Total phenols levels (TPs-HPLC), in Chétoui oils, were significantly affected by the irrigation regime. In fact, with the exception of the first ripening index, oils obtained from irrigated trees had lower phenol contents that non-irrigated ones. Many authors reported similar behavior (Patumi et al., 2002; Tovar et al., 2003). 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 (Gomez-Rico et al., 2007; Patumi et al., 2002). Different hypothesis 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 (Allogio & Caponio, 1997) and a different effectiveness in the release of phenolic compounds during crushing and malaxation linked to polysaccharides of the cell wall (Tovar et al., 2001) and the water stress suffered by the trees that could imply a greater synthesis of phenolic compounds in the fruit and so in the oil obtained from them. In fact the activity of enzymes responsible for phenolic compound synthesis, such as L-phenylalanine ammonia-lyase whose activity is greater under higher water stress conditions (Morello, Romero, Ramo, & Motilva, 2005).

HPLC–MS analysis showed that extra virgin olive oil samples contain low amounts of phenyl acids and phenyl alcohols and high concentrations of secoiridoid derivatives (SID) such as oleuropein aglycon (OlAgl), ligstroside aglycon (LigAgl) and decarboxymethyl oleuropein aglycon (DAOA) which originate from the oleuropein (Tasioula-alcohols and high concentrations of secoiridoid derivatives samples contain low amounts of phenyl acids and phenyl/C0

The HPLC analysis of phenolic extracts obtained from Chétoui EVOO 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 trees throughout the ripening process (Table 5). In fact, in samples obtained under the irrigation system, at the difference of the rain-fed conditions, SIDS content decreased slightly as ripening progressed. In general, our results agree with those found by other authors (Caponio et al., 2001). This behaviour was not observed in Chemlali samples. The acetoxypinoresinol, was detected together with the decarboxymethyl ligstroside aglycon, this last known also as oleocanthal (Beauchamp et al., 2005). Their concentrations ranged from 24.5 to 80.3 mg kg⁻¹ and from 15.1 to 31.3 mg kg⁻¹ in Chétoui and Chemlali samples, respectively.

The HPLC analysis of phenolic extracts obtained from Chétoui EVOO 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 trees throughout the ripening process (Table 5). In fact, in samples obtained under the irrigation system, at the difference of the rain-fed conditions, SIDS content decreased slightly as ripening progressed. In general, our results agree with those found by other authors (Artajo, Romero, Tovar, & Motilva, 2006; Bonoli et al., 2004). Others studies have explained these findings by the fact that secoiridoids are produced from the secondary metabolism of terpenes and are usually derived from the oleic side type of glucosides oleosides, which are characterized by a combination of elenolic acid and a glucosidic residue. It could be stated that these three compounds proceed from the same biogenetic route, the acetate/mevalonate pathway, and consequently respond similarly to water stress: their concentration increases (Artajo et al., 2006).
The simple phenol levels were also affected by the irrigation regime throughout the olive maturity; its increased markedly at the three first RI until to reach a maximum 109.4 mg kg⁻¹, then decreased down to 48.3 mg kg⁻¹. Whereas, the content of the sum of acetoxy pinosylvinol and decarboxymethyl ligstroside aglycon do not evidence an univocal variation in the irrigation regime.

3.5.5. Oxidative stability

Stability to oxidation, measured by Rancimat method, of the EVOO at different stages of ripening is shown in Table 4. The most stable analysed samples were Chètoui oils (57.1–75.5 h). These results were in accord with published data on Tunisian varieties (Abaza et al., 2005; Ben Témime et al., 2006b) showing that Chètoui and Sayali are the two Tunisian olive varieties whose oils are most stable to oxidation. It is well known that the oxidative stability variations were affected by some minor compounds such as phenols and tocopherols (Velasco & Dobarganes, 2002). Hence, as observed in total phenol content, the values of oxidative stability of Chètoui and Chemlali EVOO increased progressively until it reached a maximum at the RI 4.1 and 3.5, respectively, after which it decreased. On all the tested oils, stability decreased in the last stages of ripening. This trend is explained by the loss of natural antioxidants (phenols and tocopherols), as shown previously. These results are in accord with those of other authors (Salvador et al., 2001).

In fact, statistical data analysis, evidenced positive correlations between the values of oxidative stability and the TPs-HPLC contents ($r^2 = 0.786, 0.948, p < 0.001$) of Table 5, in Chètoui obtained under rain-fed conditions and Chemlali oils, respectively. A high positive correlations were obtained between oxidative stability and SIDs ($r^2 = 0.940, 0.898, p < 0.001$) in Chètoui obtained under rain-fed conditions and Chemlali oils, respectively. Hydroxytyrosol content is low (with respect to the other compounds) and for this reason is very poorly correlated with oxidative stability. Other positive correlations, also if less remarkable ($r^2 = 0.760, 0.661, 0.777, p < 0.001$) were evidenced between oxidative stability and α-tocopherol contents in Chètoui (rain-fed and irrigation regime) and Chemlali EVOO, respectively. These results agree with the studies by Ben Témime et al. (2006a) in Chètoui oils, which showed a clear influence of total phenol contents on olive oil stability and a much lower contribution of α-tocopherol compounds. Other studies on the relationship between tocopherols and stability revealed that α-tocopherol seems to have a synergistic effect in association with some phenolic compounds with significant antioxidant activity and the concentration of α-tocopherol becomes effective when the activity of the polar phenolic fraction is reduced and primary products of autoxidation reach a critical concentration (Deina et al., 2002).

In other works, Aparicio, Roda, Albi, and Gutierrez (1999) using statistical analysis of data relative to 79 VOO of olives cv. Hojiblanca and Picual, measured correlations between oxidative stability (valued by Rancimat) and several compositional variables. Their results showed that the phenols ($R^2 = 0.87$), α-diphenols ($R^2 = 0.77$), and the oleic/linoleic ratio ($R^2 = 0.71$) had the highest values, followed by chlorophylls ($R^2 = 0.68$), total tocopherols ($R^2 = 0.65$) and carotenoids ($R^2 = 0.59$). Principal components analysis confirmed that phenols, oleic/linoleic ratio, and tocopherols had the maximum correlation with oxidative stability.

4. Conclusion

The chemical data discussed in this work can be considered useful in providing information about the presence in the oil of major and minor compounds of the two main Tunisian cultivars for what concerns the increase of olive ripening. The analytical parameters studied (free acidity, peroxide index and spectrophotometric absorption K₂₃₂ and K₂₇₀) remained widely within estimated limits of the regulation throughout the maturity process. Nevertheless, this research evidenced that the oleic/linoleic acid ratio shows a decreasing trend as the olives ripened. With regard to phenolic compounds, Chètoui VOO had highest level on oleuropein aglycon and consequently the highest stability value. Finally, the present work shows in Chètoui EVOO, the anticipate harvest-date (RI between 3 and 4), gave the best results in terms of all the parameters considered. Instead, for the cv. Chemlali, the data clearly evidenced higher values of oxidative stability, phenolic and tocopherolic contents between the second and the third harvest-date (RI between 2.8 and 4.5). All that data could be used to define the ideal ripening degree to confer a good balance between chemical profiles, together with the best oxidative shelf-life of the EVOO. Additional works will be addressed to assess how these cultivars can be grown in other olive growing districts in order to separate the genetic effect from the environmental factor.

Acknowledgments

This work has been done as part of a National research project. We thank the Ministry of Scientific Research, Technology and Competency Development for supporting financially this project. Part of this work was carried out at the Dipartimento di Scienze degli Alimenti di Università di Bologna at Campus of Cesena, Italy.

References


