JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Effect of the Maturation Process on the Phenolic Fractions, Fatty Acids, and Antioxidant Activity of the Chétoui Olive Fruit Cultivar

NAHLA DAMAK,[†] MOHAMED BOUAZIZ,[†,‡] MOHAMED AYADI,[§] SAMI SAYADI,[‡] AND MOHAMED DAMAK*,[†]

Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences de Sfax, B.P. 802, 3038 Sfax, Tunisia, Laboratoire des Bioprocédés, Pôle d'Excellence Régionale AUF (PER-LBP), Centre de Biotechnologie de Sfax, B.P. K, 3038 Sfax, Tunisia, and Institut de l'Olivier de Sfax, 3029 Sfax, Tunisia

Maturity is one of the most important factors associated with the quality evaluation of fruit and vegetables. This work aims to investigate the effect of the maturation process of the olive fruit on the phenolic fraction and fatty acid of irrigated Chétoui cultivar. The phenolic composition was studied by using reverse-phase high-performance liquid chromatography followed by LC-MS and GC-MS analyses and fatty acids by GC. Oleuropein was the major phenolic compound at all stages of ripeness. Unexpectedly, both phenolic compounds hydroxytyrosol and oleuropein exhibited the same trends during maturation. Indeed, the oleuropein levels decreased during the ripening process and were not inversely correlated with the concentrations of hydroxytyrosol. The antioxidant capacity of olive extracts was evaluated by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl and the β-carotene linoleate model system. The IC_{50} and AAC values of the olive extracts decreased from 3.68 to 1.61 μg/mL and from 645 to 431, respectively. There was a correlation between the antioxidant activity and the oleuropein concentration. The fatty acid composition was quantified in olive fruit during maturation and showed that fatty acids were characterized by the highest level of oleic acid, which reached 65.2%.

KEYWORDS: Chétoui olive fruit; maturation; phenolics; fatty acids; antioxidant

INTRODUCTION

The olive tree (*Olea europaea*) is widely cultivated in many regions of the world where climatic conditions are as favorable as those prevailing in the Mediterranean countries. The Tunisian olive trees number about 57 million units covering 16000 ha. *O. europea* L. Chétoui is the second main variety, after Chemlali cultivar, cultivated in Tunisia. It covers an area of 176 ha and accounts for more than 20% of the olive oil produced in Tunisia (*1*). The fruit size is medium to large with a fat yield of about 20–30% of fresh weight.

The olive (*O. europaea*) is a source of several phenolic compounds with important antioxidant properties (*2*). The high concentration of phenolic compounds in olive and its derivative may contribute to the healthy nature of the mediterranean diet (*3*). These compounds act mainly as antioxidants and radical scavengers and could be used as sources of potentially safe natural antioxidants for the food industry (*4*). Olive oil extracted from olive fruits is known as the only stable oil during its storage and processing (*5*). The phenolic compounds increase the shelf life and improve the nutritional quality of the oil (*6*).

It is widely known that the composition of the phenolic fraction of oils depends on the cultivar, the climatic conditions during growth, the degree of maturation, and the agronomic practices related to the irrigation treatment (*7, 8*). Different studies have demonstrated the influence of these factors on the concentration of the phenolics and the quality of olive oil in terms of oxidative stability and sensory analysis. During the ripening cycle of olive fruits, many chemical transformations occur inside the drupes, which among others influence the phenol content and composition. Most of the works on the relationship between phenolics and olive development are concerned with oleuropein, which is known to be the most prominent individual phenolic component of olive fruits, responsible for their intense bitterness and can be hydrolyzed to generate hydroxytyrosol (*9*). The concentration of oleuropein varies with olive varieties and drops with the fruit physiological development (*10, 11*).

Most studies focused on the composition of phenolics in olive oil using high-resolution techniques and reported that during maturation oleuropein decreased whereas hydroxytyrosol in-

* Corresponding author. E-mail: Mohamed.Damak@fss.rnu.tn. Tel: 216 74 276 400. Fax: 216 74 274 437.
[†] Laboratoire de Chimie des Substances Naturelles.
[‡] Laboratoire des Bioprocédés.
[§] Institut de l'Olivier de Sfax.

10.1021/jf072273k CCC: $40.75 © 2008 American Chemical Society
Published on Web 02/08/2008

Table 1. Dates of Fruit Harvests and Dominant Fruit Color at the Respective Sampling Date Harvests

harvest	sampling date	fruit color
1	Sept 9, 2005	green
2	Sept 27, 2005	green
3	Oct 11, 2005	green
4	Oct 26, 2005	green
5	Nov 8, 2005	light green
6	Nov 23, 2005	small reddish spots
7	Dec 7, 2005	turn color
8	Dec 23, 2005	purple
9	Jan 26, 2006	black
10	Feb 20, 2006	black

creased. On the other hand, less information is available on the fatty acid evolution in olive fruits (*12*). The fatty acid composition is influenced also by the period of olive maturation, and some fatty acids determine the oil quality (*1, 9*).

The purpose of this study is to (i) identify and quantify the main phenolic compounds present in the methanol extract of the Chétoui olive cultivar at early and late maturation, (ii) evaluate the concentration of oleuropein and hydroxytyrosol as well as the antioxidant power at different stages of the fruit development, and (iii) determine the fatty acid concentration during maturation.

MATERIALS AND METHODS

Samples. The Chétoui variety is widespread in the north of Tunisia. The samples used in this study were harvested from olive trees grown under linear irrigation treatment in the region of Sfax (south of Tunisia). The olive fruits were crops in the 2005–2006 harvest season (from September to February) and the 2006–2007 harvest season (from October to March). The olives (150 g) were classified on the basis of their color. The color varies from green (G), light green (LG), small reddish spots (SRS), turning color (TC), purple (P), to black (B) (**Table 1**). Samples of 20 olives for each harvest were weighed to determine the growth curve.

Extraction. The olives were washed, deleafed, and then crushed with a hammer crusher. The olive homogeneous paste (150 g) was extracted according to the procedure described by Bouaziz et al. (*10, 11*). The olive paste was extracted using a mixture of methanol and water (8:2 v/v). The solution was kept under agitation at room temperature for 24 h. The extract was filtered using GF/F filter paper and washed three times with hexane (3 × 70 mL) to remove oil using a separatory funnel (*12*).

Both extracts (methanol/water, hexane) were concentrated in vacuo at 40 °C. The residue of the methanol extract was redissolved in 5 mL of methanol and stored at 0 °C in glass vials and in darkness for the chromatographic analysis. Special care was taken to avoid chemical and enzymatic modifications and to ensure that the extraction was properly conducted (*13*).

Reagents and Standards. Phenolic compounds including caffeic acid, *p*-coumaric acid, ferulic acid, tyrosol, and vanillic acid were obtained from Sigma-Aldrich (Chemie Gmbh, Steinhein, Germany). Oleuropein was purchased from Extrasynthèse (Genay, France). Hydrxytyrosol was prepared as described previously by Bouaziz et al. (*14*). The phenolic compounds were dissolved in a mixture of methanol/water (8:2 v/v). Pure HPLC solvents were used in all cases. Tween 40, linoleic acid, β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT) were bought from Sigma.

High-Performance Liquid Chromatography. The identification and quantification of phenolic monomers were carried out by HPLC analysis (*10*). The assays were performed on a Shimadzu apparatus composed of an LC-10ATvp pump and an SPD-10Avp detector. Eluates were detected at 280 nm. The column was 4.6 × 250 mm (Shim-pack VP-ODS), and its temperature was maintained at 40 °C. The flow rate was 0.6 mL/min. The mobile phase was 0.1% phosphoric acid in water (A) versus 70% acetonitrile in water (B) for a total running time of 50 min. The elution conditions applied for monomeric phenols were as follows: 0–25 min, 10–25% B; 25–35 min, 25–80% B; 35–37 min, 80–100% B; 37–40 min, 100% B. Finally, the column was subjected to washing and reconditioning steps for 40–50 min with a linear gradient of 100–10% B.

LC-MS Analyses. The LC-MS/MS experiments were carried out with an Agilent 1100 LC system consisting of a degasser, a binary pump, an auto sampler, and a column heater. The column outlet was coupled with an Agilent MSD ion trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out on a personal computer with Data Analysis software (Chemstations). For the chromatographic separation a Zorbax 300 Å Extend-C-18 column (2.1 × 150 mm) was used. The column was held at 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in ACN) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, and then it was kept for 4 min with 100% B. Finally, the elution was achieved with a linear gradient from 100% B to 5% B in 2 min. The flow rate was 200 μL min^{-1} and the injection volume 5 μL. The following parameters were employed throughout all MS experiments: for electrospray ionization with positive ion polarity, the capillary voltage was set to 3.5 kV, the drying temperature to 350 °C, the nebulizer pressure to 40 psi, and the drying gas flow to 10 L min^{-1}. The maximum accumulation time was 50 ms, the scan speed was 26000 m z^{-1} s^{-1} (ultra scan mode), and the fragmentation time was 30 ms.

Fatty Acid Methylation and Analyses. Methyl esters were prepared in compliance with the standard method (*15*) and analyzed by gas chromatography to determine the fatty acid composition (*16*). Their relative composition was subsequently determined as the percentage of total fatty acids using a Shimadzu GC-17A gas chromatograph, which is equipped with a flame ionization detector (FID). A polyethylene glycol fused silica capillary column, Carbowax (15 m × 0.25 mm × 0.25 μm film thickness), was employed. Azote was used as a carrier gas with a column flow of 1.2 mL/min. The temperatures of the detector, injector, and column were set at 250, 180, and 230 °C, respectively.

GC-MS Analysis. GC-MS analysis was performed with an HP model 5975B inert MSD, equipped with a capillary DB-5MS column (30 m length; 0.25 mm i.d.; 0.25 μm film thickness; Agilent Technologies, J&W Scientific Products). The carrier gas was helium used with a 1 mL min^{-1} flow rate. The oven temperature program was as follows: 1 min at 100 °C, ranging from 100 to 260 at 4 °C min^{-1}, and 10 min at 260 °C. The chromatograph was equipped with a split/splitless injector used in the split mode. The split ratio was 100:1. Bis(trimethylsilyl)acetamide (BSTFA) (100 μL) was added to 100 μL of the ethyl acetate extract of the OMW sample. The obtained solution was incubated for 60 min at 80 °C.

DPPH Radical Scavenging Assay. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging effect was evaluated according to the method employed by Bouaziz et al. (*11, 17*). Four milliliters of a methanolic solution of varying sample concentrations (25, 500, 100, and 150 μg/mL) was added to 10 mL of DPPH methanol solution (1.5 × 10^{-4} M). The solutions prepared were mixed and left for 30 min at room temperature. The optical density was measured at 520 nm using an Anadéo UV spectrophotometer. Test samples and positive control BHT were tested over the same range of sample concentrations. The antioxidant activities of each test sample and BHT were expressed in terms of concentrations required to inhibit 50% DPPH radical formation (IC_{50} μg/mL) and calculated from the log-dose inhibition curve.

Antioxidant Assay Using the β-Carotene Linoleate Model System. The antioxidant activity of the extracts was assayed on the basis of the β-carotene bleaching method developed by Chevolleau et al. (*18*). BHT was used as the standard. β-Carotene (0.1 mg in 1 mL of chloroform), linoleic acid (20 mg), and Tween 40 (200 mg) were mixed. Chloroform was eliminated with nitrogen. Distilled water (50 mL) saturated with oxygen was added to the residue and then shaken vigorously to form an emulsion. Five milliliter aliquots of the emulsion were pipetted into a series of glass vials containing 0.2 mL (2 mg/mL) of each extract or standard (15 mg/mL) or methanol (as control). The vials were placed at 50 °C in an incubator, and the absorbance at 470 nm was taken at zero time ($t = 0$).

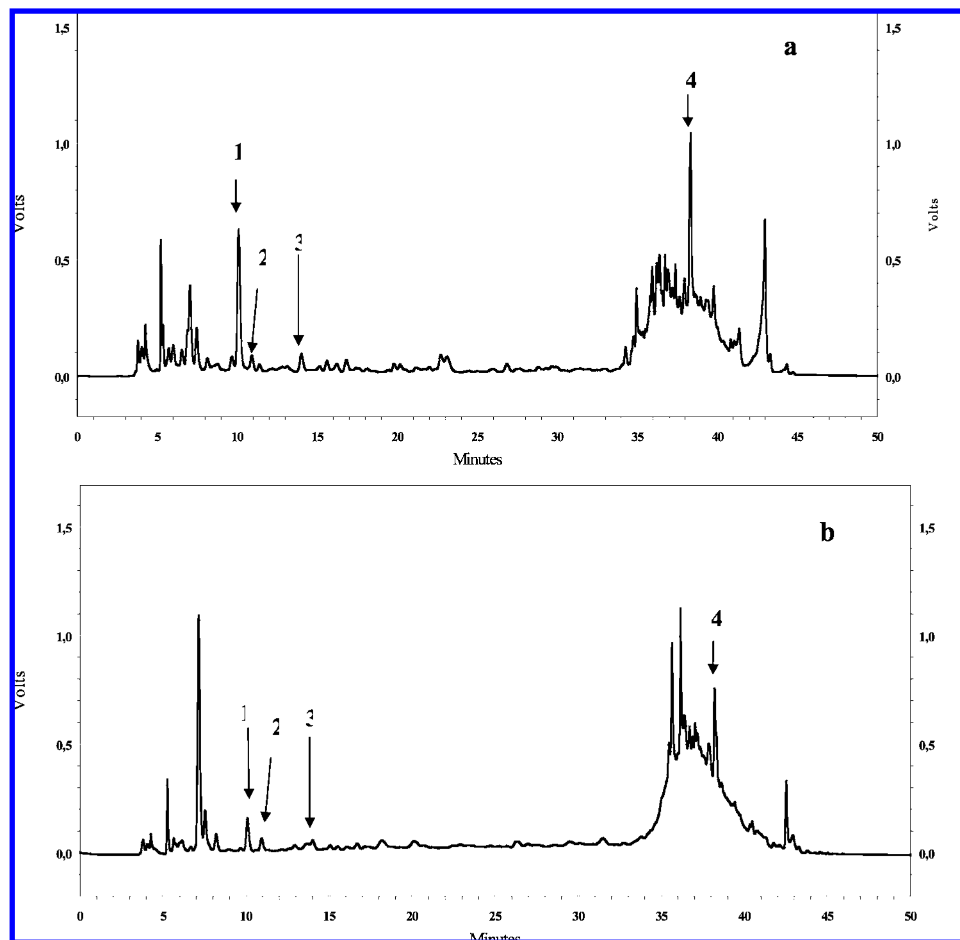

Figure 1. HPLC chromatogram profile at 280 nm of Chétoui olive extract. (**a**) Olive fruit harvested in September 2005. (**b**) Olive fruit harvested in February 2006. Peaks: 1, hydroxytyrosol; 2, 3,4-dihydroxyphenylacetic acid; 3, tyrosol; 4, oleuropein.

The measurement of absorbance was continued until the color of β-carotene disppeared in the control vials ($t = 120$ min) at an interval of 15 min. Absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant, they retained their color, and thus absorbance, for a longer time. The antioxidant activity coefficient (AAC) of the extracts was evaluated using the formula AAC $= 1000[A^t_{\text{extract or standard}} - A^t_{\text{control}}]/[A^0_{\text{control}} - A^t_{\text{control}}]$, where A^t and A^0 are the absorbance values measured after incubation for 120 min and at zero time, respectively.

RESULTS AND DISCUSSION

Identification of Phenolic Compounds. A representative chromatogram of HPLC analysis of olive drupe from the Chétoui cultivars harvested in the 2005–2006 season and in the 2006–2007 season is given in **Figures 1** and **2**, respectively. The identification was based on comparisons of the chromatographic retention time and UV absorbance spectra of compounds in olive extracts with those of an authentic standard. These chromatograms show that the olive extract is made up of hydroxytyrosol, 3,4-dihydroxyphenylacetic acid, tyrosol, coumaric acid, and oleuropein, which was confirmed by GC-MS analysis (**Table 2**). In addition, another phenolic compound, *p*-hydroxyphenylacetic acid was identified by GC-MS analysis. The obtained mass fragments agreed with those described previously (*19*). To elucidate the structures of the high molecular mass phenolic compounds in chétoui olive extract, a new sample harvested in March 2007 was monitored by liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (APCI-LC-MS) in the negative mode. **Table 3** lists each of the identified phenolic compounds in an elution order. The structure assignment was based on a systematic search for molecular ions using extracted ion mass chromatograms and comparing those with data in the literature (*14, 20–23*). For example, the spectra generated for compound **3**, verbascoside, in the negative mode gave the deprotonated molecule $[M - H]^-$ at m/z 632. The ion fragment at m/z 461 is due to the loss of the glucose group. Compound **4** exhibited a base peak $[M - H]^-$ at m/z 609 and also an intermediate ion at m/z 463 and an aglycon ion at m/z 301. The loss of 146 amu from the pseudomolecular ion represents the sugar rhamnose, and the loss of 162 amu from the intermediate ion is due to the loss of glucose. The obtained MS spectra suggest that compound **4** was rutin. Compound **5** had a deprotonated molecule $[M - H]^-$ at m/z 447, and a strong fragment at m/z 285 suggests that this compound was the luteolin 7-*O*-glucoside. In the case of compound **8** the mass spectrum showed a strong peak at m/z 551 corresponding to the $[M - H]^-$ species. The principal fragment was originated by the loss of 44 amu, giving rise to the intense signal at m/z 507, the ionic species at m/z 389 representing the oleoside structure. Its formation was accomplished by the loss of a hexose moiety (162 amu), suggesting that the compound was a hexoside derivative of oleoside. Moreover, the presence of a fragment at m/z 341, which corresponded to a disaccharide, indicated that this hexose molecule should be linked to the sugar moiety of the oleoside. This suggested that **8** might be an oleoside derivative, and the obtained scheme of fragmentation of compound **8** in the negative mode agreed with those described previously (*24*). According to the above results compound **8** was a 6′-β-hexopyranosyl-

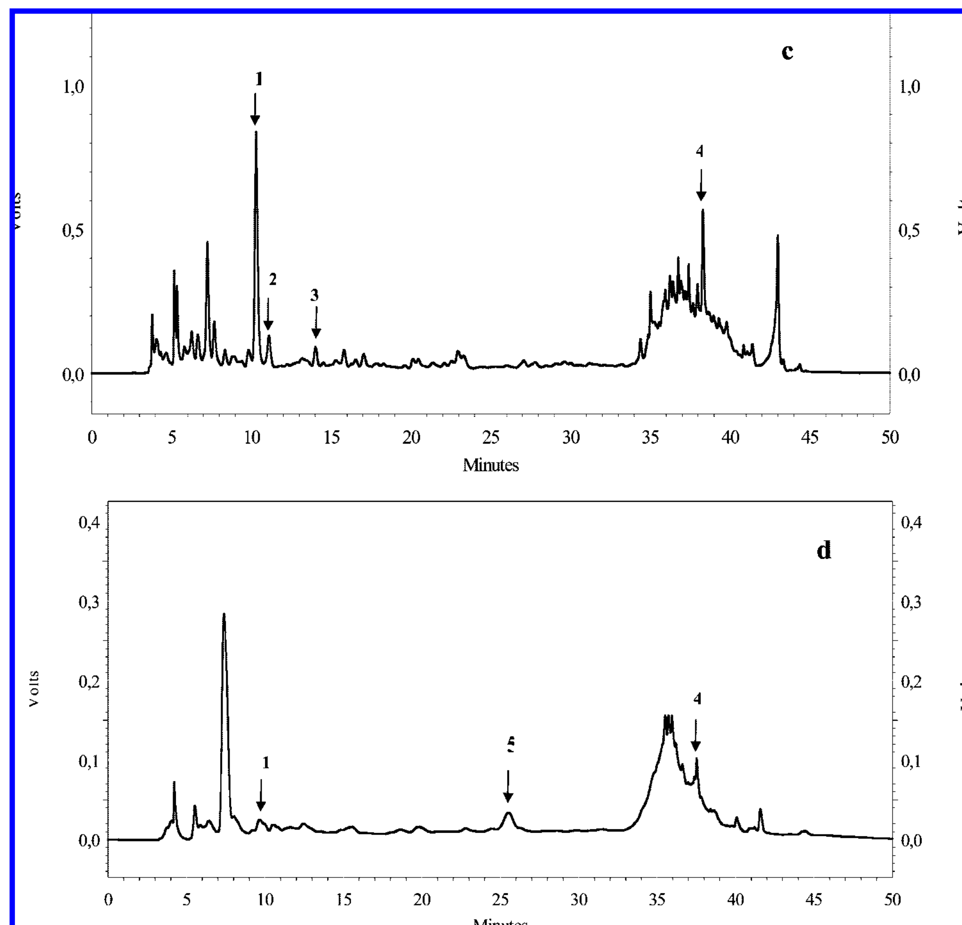

Figure 2. HPLC chromatogram profile at 280 nm of Chétoui olive extract. (**c**) Olive fruit harvested in October 2006. (**d**) Olive fruit harvested in March 2007. Peaks: 1, hydroxytyrosol; 2, 3,4-dihydroxyphenylacetic acid; 3, tyrosol; 4, oleuropein; 5, *p*-coumaric acid.

Table 2. Abbreviated Mass Spectra of Major Phenolic Monomers Identified in Chétoui Olive Extract

TMS derivatives of	mass spectra (*m/z* and % of base peak)
hydroxytyrosol	370 (M^+, 39), 267 (90), 193 (25), 179 (12), 73 (100)
tyrosol	282 (M^+, 18), 267 (13), 193 (15), 179 (100), 73 (42)
3,4-dihydroxyphenylacetic acid	384 (M^+, 68), 369 (66), 311 (42), 296 (85), 281 (67), 252 (30), 179 (31), 164 (28), 147 (12), 73 (100)
p-coumaric acid	308 (M^+, 81), 293 (100), 249 (44), 219 (82), 179 (13), 73 (51)
p-hydroxyphenylacetic acid	296 (M^+, 74), 281 (57), 252 (51), 179 (86), 164 (40), 147 (14), 133 (11), 73 (100)

oleoside. The mass spectrum of compound **9** showed a high-intensity ion at *m/z* 535. The ESI-MS^2 spectrum of that ion (**Table 3**) showed some similarities to the spectral profile of compound **8**. The main fragment was obtained by the loss of 44 amu (ion at 491), and an ionic species corresponding to the oleoside ion (*m/z* 309 and *m/z* 325) was also detected. The loss of 146 and 162 amu from the pseudomolecular ion can be justified by the elimination of two sugars, rhamnose and glucose, respectively. This compound was previously detected in olive leaves and fruit by the use of ESI-MS and was identified as 6′-deoxyhexopyranosyloleoside (*25*).

Compound **11** exhibited a pseudomolecular ion $[M - H]^-$ at *m/z* 1613. These *m/z* values suggest the presence of oleuropein trimers. The product ion scan spectrum showed the *m/z* 539 (oleuropein molecular ion). The *m/z* 1075 can be explained by the loss of an oleuropein molecule, another intense peak at 377 indicative of oleuropein aglycon fragments. The ESI-MS^2 spectrum of the molecular ion at *m/z* 1613 showed a fragment ion at *m/z* 823. It could be justified by a loss of 790 caused by the release of the 11-methyloleoside (*m/z* 404) and hexose plus the secoiridoid part of an oleuropein molecule (*m/z* 386). The ionic species at *m/z* 701 could be justified by the oleuropein molecule and its aglycon fragment loss. These results are in accordance with the mass interpretation reported by Cardoso et al. (*26*), who detected the same compound in olive fruit Verdial cultivar.

The comparison of the ESI-MS data with literature data was possible for compounds **1** (oleoside), **2** (11-methyloleoside), **3** (verbascoside), **6** (luteolin 7-*O*-rutinoside), **7** (oleuropein glucoside), and **10** (oleuropein). Their fragmentation scheme was in agreement with that previously detected in *O. europaea* leaves and fruits (*21, 22, 25, 27*).

Oleuropein and Hydroxytyrosol Quantification. Several factors are known to affect the quantitative phenolic profiles of olive fruits. Among these factors, the degree of ripeness, the geographical origin, the irrigation treatment, and the nature of the cultivar are certainly those having a pronounced influence on the phenolic composition. Oleuropein is the main phenolic compound in olive drupes during the fruit ripening, having their maximum concentration in green olive and the minimum one in black olive. During this period, oleuropein concentrations decreased as the sampling period progressed. A high concentration of oleuropein was obtained during the green maturation sampling. It attained 17 and 8.18 g/kg (all data are reported on a fresh mass basis) for the sample harvested at the beginning

Table 3. Phenolic Compounds Detected in Chétoui Olive Fruit (Harvested in March 2007) with Their HPLC Retention Time and Mass Spectral Data

no.	compound	retention time (min)	predominant negative ion $[M - H]^-$	main fragments by $ESI-MS^2$
1	oleoside	4	389	370, 345, 209, 165
2	11-methyloleoside	5.5	403	371, 333, 223
3	verbascoside	6.8	623	461, 315, 135, 161, 297
4	rutin	9.9	609	463, 301
5	luteolin 7-*O*-glucoside	10.3	447	285
6	luteolin 7-*O*-rutinoside	10.4	593	447, 285
7	oleuropein glucoside	10.8	701	539, 377, 307
8	6′-β-hexopyranosyloleoside	10.9	551	533, 507, 389, 341, 323, 489
9	6′-deoxyhexopyranosyloleoside	11.4	535	517, 491, 390, 345, 325, 265
10	oleuropein	11.5	539	377, 197, 153
11	oleuropein trimers	12	1613	539, 1075, 823, 377, 701
12	oleuropein aglycon	13.1	377	225, 153, 197

of September 2005 and October 2006, respectively. Oleuropein concentration showed a progressive decrease in the two harvest seasons. During maturation it reached 7.45 g/kg in February 2006 and 2.25 g/kg in March 2007.

The second observed major phenolic compound was hydroxytyrosol. Unexpectedly, in our investigation, hydroxytyrosol decreased during the sampling period (**Figure 3**). Its concentration ranged from 1 g/kg to 500 mg/kg in fresh olive in the 2005–2006 harvest season and from 430 mg/kg to 195 mg/kg in the 2006–2007 harvest season.

Several studies reported a decrease in the oleuropein content of olive pulp with maturation of the fruit and an increase of its derivatives (*28–31*). Some of those results described an increase in the amount of oleuropein degradation products such as elenoic acid, demethyloleuropein, and hydroxytyrosol. Thus the olive pomace used in our study was produced from olives which were under linear irrigation and were not from its native geographical origin. The disappearance of oleuropein in the pulp of the fruit could be related to the formation of phenolic oligomers as recently described by Cardoso et al. (*26*). These authors reported that the polymerization of oleuropein explained the decrease of its concentration with the formation of phenolic oligomers and the decrease of the hydroxytyrosol concentration which was the principal product of its degradation. Indeed, our study confirms this hypothesis by the detection of oleuropein trimers in Chétoui olive extract at late harvest (March 2007). On the other hand, our results are in line with those previously found by Morello et al. (*8, 32*), who showed that the major phenolic compounds in olive drupe (hydroxytyrosol and oleuropein) followed the same decreasing trend during maturation. Our results clearly suggest that the irrigation treatment and the geographical origin would have a great influence on the maturity of olive fruits, which further influenced the phenol content, as was previously reported (*33, 34*).

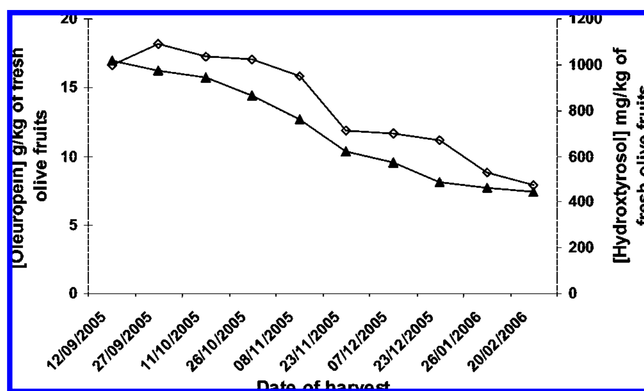

Figure 3. Oleuropein and hydroxytyrosol levels during the maturation of Chétoui olive variety: (▲) oleuropein; (◇) hydroxytyrosol. Each point represents the mean of two determinations and two independent experiments (SD <12% of the mean).

Fatty Acid Composition. The effect of the degree of ripeness on the main fatty acid composition in the fruit of Chétoui olive cultivar is shown in **Table 4**. Arachidic, palmitoleic, and linoleinic acids were found in small concentrations at all harvests. During maturation, as fruit ripened, the content of stearic and palmitic acids slightly decreased from 2.3 ± 0.28% to 1.97 ± 0.12% and from 16.23 ± 0.28% to 9.35 ± 0.65%, respectively. On the other hand, oleic and linoleic acids showed an opposite trend; the linoleic acid varied from 15.53 ± 0.28% to 22.98 ± 0.51% and the oleic acid content varied from 56.95 ± 0.48% to 65.15 ± 1.56%. The increase in linoleic acid content could be due to the transformation of oleic acid into linoleic acid by the oleate desaturase activity which is active during the triacylglycerol biosynthesis (*35*).

Furthermore, it is well-known that, in addition to the maturity stage, fatty acid composition could be affected by environmental factors such as rainfall and geographical origin. In this context, according to Montedoro et al. (*36*), the amount of oleic acid was affected by autumn temperatures, relative humidity, and rainfall during the whole year. On the other hand, our results are in agreement with the findings of Ben Temim et al. (*1*), who observed that the variations in oleic and linoleic acid

Table 4. Fatty Acid Composition of the Olive Fruit Hexane Extract from the Chétoui Cultivar[a]

fatty acid (%)	9/12/05	9/27/05	10/11/05	10/26/05	11/23/05	12/7/05	12/23/005	1/26/06	2/20/06
palmitic (C16:0)	16.23 ± 0.25	15.20 ± 0.25	15.87 ± 0.21	10.94 ± 0.5	14.75 ± 0.25	11.74 ± 0.25	10.70 ± 0.3	9.9 ± 0.50	9.35 ± 0.40
palmitoleic (C16:1)	0.51 ± 0.05	0.50 ± 0.06	1.32 ± 0.10	0.50 ± 0.05	1.18 ± 0.20	0.50 ± 0.20	0.40 ± 0.15	0.43 ± 0.05	0.04 ± 0.01
stearic (C18:0)	2.30 ± 0.25	2.40 ± 0.30	2.75 ± 0.20	2.11 ± 0.35	2.48 ± 0.30	2.84 ± 0.20	2.90 ± 0.21	2.50 ± 0.40	1.97 ± 0.02
oleic (C18:1)	63.07 ± 0.41	65.15 ± 0.61	56.95 ± 1	63.36 ± 0.60	58.46 ± 0.50	62.43 ± 0.50	64.24 ± 0.25	63.2 ± 0.25	64.48 ± 0.29
linoleic (C18:2)	15.53 ± 0.50	15 ± 0.45	18.40 ± 0.50	20.72 ± 0.25	19.05 ± 0.45	20.76 ± 0.25	20.23 ± 0.25	22.25 ± 0.30	22.98 ± 0.30
linoleinic (C18:3)	1.56 ± 0.05	1.04 ± 0.15	1.53 ± 0.07	1.24 ± 0.25	1.97 ± 0.02	0.92 ± 0.10	0.64 ± 0.05	0.73 ± 0.04	0.55 ± 0.15
arachidic (C20:0)	0.37 ± 0.05	0.31 ± 0.03	0.18 ± 0.01	0.32 ± 0,04	0.89 ± 0.10	0.34 ± 0.02	0.39 ± 0.01	0.39 ± 0.02	0.23 ± 0.02

[a] Each value in the table is represented as the mean ± SE ($n = 3$).

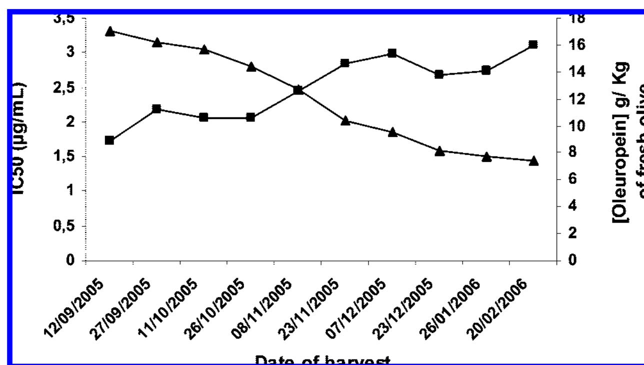

Figure 4. Correlation between oleuropein concentration and DPPH radical scavenging effect (IC_{50}) of the extracts during maturation of Chétoui fruits cultivar: (▲) oleuropein concentrations; (■) IC_{50}. Each point represents the mean of two determinations and two independent experiments (SD < 10% of the mean).

Table 5. Coefficient of Antioxidant Activity of the Olive Fruit Extract from the Chétoui Cultivar and Positive Control (BHT) in the β-Carotene/Linoleic Acid Assay[a]

date of harvest	AAC of extracts	date of harvest	AAC of extracts
9/12/2005	645 ± 4.5	12/7/2005	750 ± 7.0
9/27/2005	520 ± 5.0	12/23/2005	690 ± 5.7
10/11/2005	643 ± 6.5	1/26/2006	580 ± 5.1
10/26/2005	662 ± 4.0	2/20/2006	431 ± 5.5
11/23/2005	799 ± 5.0	BHT	874 ± 6.0

[a] Each value in the table is represented as the mean ± SE (n = 3).

contents in olive oil samples obtained from the Chétoui cultivar were probably related to both genetic factors and environmental conditions during the fruit development and maturity.

Antioxidant Potential of Crude Extracts during Maturation. The DPPH radical scavenging effect for all of the methanol extracts exhibited antioxidant activity (**Figure 4**) which showed a correlation between the phenolic compound content and DPPH radical-scavenging activity over all stages of fruit development. The extracts show similar trends of activity in both the linoleate model and the DPPH radical-scavenging assays (**Figure 4**, **Table 5**).

The highest IC_{50} (3.68 μg/mL) value and the lowest antioxidant activity coefficient (AAC = 431), which indicated a lower antioxidant potential, were observed for the samples harvested at the end of February (black olive extract). This is probably due to the lower inhibition of the radical formed by a low concentration of *O*-diphenol such as hydroxytyrosol and oleuropein. The extract obtained from the first harvest has the highest oleuropein level and the highest antioxidant activity with an IC_{50} value of 1.6 μg/mL. It was reported that the concentration of phenolics such as hydroxytyrosol and oleuropein was in relationship with the percent of radical inhibition in the measurement of the antioxidant activity (*18*). In addition, the antioxidative action of the extract may be partly the result of the number of hydroxyl substituents in the aromatic ring (*37*).

Conclusion. This work is the first report on the identification and characterization of phenolic compounds and fatty acids in the Chétoui olive fruit cultivar in Tunisia. Many phenolic compounds have been identified such as flavonoids and oleuropein derivatives. The concentrations of oleuropein and hydroxytyrosol compounds showed the same trend during maturation. The disappearance of oleuropein in the fruit is not due to its degradation but could be related to the formation of phenolic oligomers. These changes may be due to the varietal origin and climatic and agronomical conditions, where the olive trees are growing. This investigation reported a low variation of fatty acids during maturation and showed that antioxidant activity of olive extracts was closely related to the oleuropein concentration.

ACKNOWLEDGMENT

We thank Adel Gargoubi for HPLC analyses and Hedi Aouissaoui and Loubna Jlil for help with LC-MS analyses.

LITERATURE CITED

(1) Ben Temim, S.; Campeol, E.; Luigi Cioni, P.; Daoud, D.; Zarrouk, M. Volatile compounds from Chétoui olive oil and variations induced by growing area. *Food Chem.* **2006**, *99*, 315–325.

(2) Ryan, D.; Antolovich, M.; Prenzler, P.; Robards, K.; Lavee, S. Biotransformations of phenolic compounds in *Olea europaea*. *Sci. Hortic.* **2002**, *92*, 147–176.

(3) Francesco, C.; Vincenzo, A.; Tommaso, G. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem.* **1999**, *64*, 203–209.

(4) Andrés, M.; Jose, M. C.; Daniel, F.; Manuel, D.; Jorge, S.; Herminia, D.; Maria, J. N.; Carlos, P. J. Natural antioxidants from residual sources. *Food Chem.* **2001**, *72*, 145–171.

(5) Visioli, F.; Galli, C. Antiatherogenic components of olive oil. *Curr. Atheroscler. Rep.* **2001**, *3*, 64–67.

(6) Visioli, F.; Galli, C. Oleuropein protect low-density lipoproteins from oxidation. *Life Sci.* **1994**, *55*, 1965–1971.

(7) Morello, J. R.; Motilva, M. J.; Ramo, T.; Romero, M. P. Effect of freeze injuries in olive fruit on virgin olive oil composition. *Food Chem.* **2003**, *81*, 547–553.

(8) Morello, J. R.; Romero, M. P.; Motilva, M. J. Effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga and Morrut cultivars. *J. Agric. Food Chem.* **2004**, *52*, 6002–6009.

(9) Dhifi, W.; Hamrouni, I.; Ayachi, S.; Chahed, T.; Saidani, M.; Marzouk, B. Biochemical characterization of some Tunisian olives oils. *J. Food Lipids* **2004**, *11*, 287–296.

(10) Bouaziz, M.; Damak, M.; Sayadi, S. Influence of fruit ripening process on the natural antioxidant content of Chemlali olive cultivar. *J. Soc. Chim. Tunis.* **2006**, *8*, 11–20.

(11) Bouaziz, M.; Chamkha, M.; Sayadi, S. Comparative study on phenolic content and antioxidant activity during maturation of the olive cultivar Chemlali from Tunisia. *J. Agric. Food Chem.* **2004**, *52*, 5476–5481.

(12) Cevdet, N.; Yesim, E. Compositional variation of olive fruit during ripening. *Food Chem.* **2000**, *69*, 55–59.

(13) Ryan, D.; Robards, K.; Lavee, S. Changes in phenolic content of olive during maturation. *Int. J. Sci. Food. Technol.* **1999**, *34*, 265–274.

(14) Bouaziz, M.; Sayadi, S. Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. *Eur. J. Lipid Sci. Technol.* **2005**, *107*, 118–125.

(15) International standard of animal and vegetable oils and fats. Preparation of methyl esters of fatty acids; Iso: Geneva, Switzerland, 2000; p 5509.

(16) International standard of animal and vegetable oils and fats. Analysis of methyl esters of fatty acids; Iso: Geneva, Switzerland, 1990; p 5508.

(17) Bouaziz, M.; Grayer, R. J.; Simmonds, M. S. J.; Damak, M.; Sayadi, S. Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar Chemlali growing in Tunisia. *J. Agric. Food Chem.* **2005**, *53*, 236–241.

(18) Chevolleau, S.; Debal, A.; Ucciani, E. Détermination de l'activité antioxydante d'extraits végétaux. *Rev. Fr. Corps Gras.* **1992**, *1/2*, 3–8.

(19) Allouche, N.; Fki, I.; Sayadi, S. Toward a High Yield Recovery of Antioxidants and Purified Hydroxytyrosol from Olive Mill Wastewaters. *J. Agric. Food Chem.* **2004**, *52*, 267–273.

(20) Romero, C.; García, P.; Brenes, M.; García, A.; Garrido, A. Phenolic compounds in natural black Spanish olive varieties. *Eur. Food Res. Technol.* **2002**, *215*, 489–496.

(21) De Nino, A.; Lombardo, N.; Perri, E.; Procopio, A.; Raffaelli, A.; Sindona, G. Direct identification of phenolic glucosides from olive leaf extracts by atmospheric pressure ionization tandem mass spectrometry. *J. Mass Spectrom.* **1997**, *32*, 533–541.

(22) Ryan, D.; Robards, K.; Prenzler, P.; Jardine, D.; Herlt, T.; Antolovich, M. Liquid chromatography with electrospray ionisation mass spectrometric detection of phenolic compounds from *Olea europaea*. *J. Chromatogr. A* **1999**, *855*, 529–537.

(23) Ryan, D.; Robards, K.; Lavee, S. Determination of phenolic compounds in olives by reversed-phase chromatography and mass spectrometry. *J. Chromatogr. A* **1999**, *832*, 87–96.

(24) Cardoso, S. M.; Guyot, S.; Marnet, N.; Lopes-da-silva, J. A.; Renard, C. M. G. C.; Coimbra, M. A. Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. *J. Sci. Food Agric.* **2005**, *85*, 21–32.

(25) De Nino, A.; Mazzotti, F.; Morrone, S. P.; Perri, E.; Raffaelli, A.; Sindona, G. Characterization of cassanese olive cultivar through the identification of new trace components by ion spray tandem mass spectrometry. *J. Mass Spectrom.* **1999**, *34*, 10–16.

(26) Cardoso, S. M.; Guyot, S.; Marnet, N.; Lopes-da-silva, J. A.; Silva, M. S. A.; Renard, C. M. G. C.; Coimbra, M. A. Identification of oleuropein oligomers in olive pulp and pomace. *J. Sci. Food Agric.* **2006**, *86*, 1495–1502.

(27) Çalis, D.; Hosny, M.; Khalifa, T.; Nishibe, S. Secoiridoids from *Fraxinus angustifolia*. *Phytochemistry* **1993**, *33*, 1453–1456.

(28) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Importance and evolution of phenolic compouds in olive during growth and maturation. *J. Agric. Food Chem.* **1986**, *34*, 823–826.

(29) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Accumulation of oleuropein derivatives during olive maturation. *Phytochemistry* **1989**, *28*, 67–69.

(30) Briante, R.; Patumi, M.; Limongelli, S.; Ferdinando, F.; Vaccaro, C.; Di Salla, A.; La Cara, F.; Nucci, R. Changes in phenolic and enzymatic activities content during fruit ripening in two Italian Cultivars of *Olea europaea* L. *Plant Sci.* **2002**, *162*, 791–798.

(31) Ryan, D.; Robards, K. Phenolic compounds in olive. *Analyst* **1998**, *123*, 31R–44R.

(32) Morello, J. R.; Romero, M. P.; Ramo, T.; Motilva, M. J. Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe (*Olea europaea* L.) from fruit setting period to harvesting time. *Plant Sci.* **2005**, *168*, 65–72.

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

(34) Vinha, A. F.; Ferreres, F.; Silva, B. M.; Valentao, P.; Gonçalves, A.; Pereira, J. A.; Oliveira, M. B.; Seabra, R. M.; Andrade, P. B. Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influence of cultivar and geographical origin. *Food Chem.* **2005**, *89*, 561–568.

(35) Sanchez, J.; Harwood, J. L. Biosynthesis of triacylglycerols and volatiles in olives. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 564–573.

(36) Montedoro, G.; Servili, M.; Baldioli, M.; Miniati, E. Simple and hydrolysable compounds in virgin olive oil. 3. Spectroscopic characterization of secoridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228–2234.

(37) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933–956.

Received for review July 28, 2007. Revised manuscript received November 30, 2007. Accepted December 5, 2007.

JF072273K