JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

MATTEO BONOLI, ALESSANDRA BENDINI, LORENZO CERRETANI,* GIOVANNI LERCKER, AND TULLIA GALLINA TOSCHI

Dipartimento di Scienze degli Alimenti, Università di Bologna, Piazza Goidanich 60, 47023 Cesena (FC), Italy

Capillary electrophoresis (CE) can be effectively used as a fast screening tool to obtain qualitative and semiquantitative information about simple and complex phenolic compounds of extra virgin olive oil. Three simple phenols (tyrosol, hydroxytyrosol, and vanillic acid), a secoiridoid derivative (deacetoxy oleuropein aglycon), and two lignans (pinoresinol and acetoxypinoresinol) were detected as the main compounds in extra virgin olive oils by high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). Spectrophotometric indices, radical scavenging activity, and oxidative stability of extra virgin olive oil samples obtained from olives hand-picked at different ripening degrees were statistically correlated with the CZE and HPLC quantification. The concentration of phenols in extra virgin olive oil decreased with ripeness of olive fruits. The high correlations found between CZE and the other analytical results indicate that CE can be applied as a rapid and reliable tool to routinely determine phenolic compounds in extra virgin olive oils.

KEYWORDS: Capillary zone electrophoresis (CZE); high-performance liquid chromatography−mass spectrometry detection (HPLC-MSD); phenolic compounds; lignans; virgin olive oil

INTRODUCTION

The beneficial effect of the consumption of virgin olive oils on human health is well-known and related to the characteristic fatty acid composition, the presence of minor components, such as squalene and phytosterols, and the antioxidant properties of phenolic compounds (*1*−*3*). The most representative phenolic compounds in extra virgin olive oils are simple phenols (phenylethyl alcohol, such as tyrosol and hydroxytyrosol, and other phenolic acids, namely, cinnamic or benzoic acid derivatives), secoiridoids (the aglycons of oleuropein and ligstroside and their respective decarboxylated derivatives), and lignans, (+)-pinoresinol and (+)-1-acetoxypinoresinol (*1*−*4*).

Many works in the literature determine the total phenolic amount of olive oils by spectrophotometric analysis and characterize their phenolic patterns by capillary gas chromatography (CGC) (*2*−*5*) and, mainly, by reverse phase high-performance liquid chromatography (RP-HPLC) (*2*−*13*). Even if the characterization and quantification of phenolic compounds from olive oils have been successfully carried out by HPLC, this analytical technique needs accurate sample preparation and, generally, is time-consuming due to the complexity of the matrix. Actually, in the worst case the whole HPLC analysis of olive oil's phenols requires up to 90 min.

* Corresponding author (e-mail lorenzo.cerretani2@unibo.it; fax +39-0547-382348; telephone +39-0547-338121).

Therefore, the use of faster analytical techniques and screening tools, allowing a rapid screening of phenolic compounds of olive oils, is strongly recommended. Capillary electrophoresis (CE) might represent a good compromise between analysis time and satisfactory characterization for some classes of phenolic compounds of virgin olive oils (*9*, *14*). Unfortunately, to date, references about the capillary electrophoretic separation of phenolic compounds of olive oil and their evolution with the ripening degree are still scattered and incomplete, whereas several authors have reported HPLC and spectrophotometric studies about this topic (*15*−*18*).

In this work, the identification of extra virgin olive oil phenols was realized by spiking attempts or by interpretation of the HPLC-MS and UV spectra. A semiquantitative study of the phenolic compounds was performed by capillary zone electrophoresis (CZE), HPLC, and spectrophotometry. Moreover, the scavenging activity and the oxidative stability of the extra virgin olive oil samples were assayed. The evolution and the trend of phenolic compounds during the ripeness of the olive fruits were also verified and discussed.

Thus, the main purpose of this work was to investigate if HPLC and, particularly, CZE could effectively give reliable information about the tendency of the amount of phenolic compounds in virgin olive oil during the ripeness of olives, especially when the results obtained by these two analytical techniques are compared with usual spectrophotometric (total

10.1021/jf048868m CCC: $27.50 © 2004 American Chemical Society
Published on Web 10/19/2004

Table 1. HPLC/ESI-MSD Data (*m/z* Ions) of Deacetoxy Oleuropein Aglycon and Lignans

phenolic compound	ESI-MSD polarity	ESI-MSD major fragment ions, *m/z*
deacetoxy oleuropein aglycon	negative	639 $[2M - H]^-$, 319 $[M - H]^-$, 301 $[M - H_2O]^-$, 249, 195, 163
(+)-pinoresinol	positive	739 $[2M + Na]^+$, 427 $[M + HCOOH + Na]^+$, 397 $[M + K]^+$, 381 $[M + Na]^+$, 359 $[M + H]^+$, 341 $[M - H_2O + H]^+$, 323 $[M - 2H_2O + H]^+$
(+)-1-acetoxypinoresinol	positive	463 $[M + HCOOH + H]^+$, 455 $[M + K]^+$, 439 $[M + Na]^+$, 417 $[M + H]^+$, 357 $[M - CH_3COOH + H]^+$, 233 $[M - CH_3COOH - phenylOCH_3 + H]^+$

phenols by the Folin−Ciocalteu method and *o*-diphenols) and antioxidant activity indices (scavenging property and oxidative stability).

The ability of CZE for the fast screening of the amount of virgin olive oil phenols belonging to different classes (simple phenols, lignans, and secoiridoids) is also discussed.

MATERIALS AND METHODS

Chemicals. Tyrosol, gallic acid, 3,4-dihydroxyphenylacetic acid, vanillic acid, and diphenylpicrylhydrazyl (DPPH) were from Sigma Chemicals Co. (St. Louis, MO). Hydroxytyrosol for qualitative analysis was synthesized as previously described (*9*, *14*). Unless otherwise stated, all solvents were of pro analysis grade and from Merck (Darmstadt, Germany). Sodium molybdate dihydrate was from Carlo Erba (Rodano, Milano, Italy). Na_2CO_3 was from BDH AnalaR (Poole, U.K.). Folin−Ciocalteu reagent was purchased from Merck. Sodium tetraborate and water for HPCE were from Fluka (Buchs, Switzerland).

Extra Virgin Olive Oil Samples. Samples were extra virgin olive oils obtained from fruits of *Olea europaea* L. (cv. Nostrana di Brisighella, cv. Peranzana, and cv. Ghiacciolo). Nostrana di Brisighella olives were hand-picked at four different ripeness stages (RS_I, October 22, 2002; RS_{II}, November 7, 2002; RS_{III}, November 19, 2002; RS_{IV}, December 3, 2002), based on the degree of skin and pulp pigmentation, and processed as reported by Rotondi et al. (*15*). Lignans were collected from fresh Ghiacciolo olives (Brisighella, Ravenna, Italy) because this cv. is particularly rich in these compounds.

Oxidative Stability of Oil Samples. The oxidative stability of the samples was evaluated by the oxidative stability index (OSI), using an eight-channel oxidative stability instrument (Omnion, Decatur, IL). The instrumental conditions were set following the analytical protocol described by Jebe et al. (*19*). The OSI index (or OSI time) was expressed in hours ($n = 4$).

Liquid−Liquid Extraction of Phenolic Compounds from Olive Oils. The extraction was performed following the Pirisi et al. (*13*) protocol, modified according to that of Rotondi et al. (*15*). Unless otherwise stated, extractions were replicated three times ($n = 3$). The extracts were stored at −18 °C before use.

Solid−Liquid Extraction of Phenolic Compounds from Olive Fruits. Cv. Ghiacciolo olives were divided in three fractions, such as pulp, bark, and seed. Similarly to the extraction of the phenolic fraction from oils, 10 mL of *n*-hexane and 40 mL of 60:40 methanol/water (v/v) were added to 20 g of crushed bark, 20 g of mashed pulp, and 5 g of ground seed. The mixture was stirred for 2 min by vortex, sonicated for 10 min, and, finally, centrifuged at 3000 rpm for 5 min. The hydroalcoholic phase was collected, and the hexane phase was re-extracted twice with 40 mL of 60:40 methanol/water (v/v). Finally, the three fractions were combined and evaporated to dryness under reduced pressure and low temperature (<35 °C). Phenolic residues were dissolved in 5 mL of 1:1 methanol/water (v/v) and filtered through a 0.45 μm nylon filter for high-performance analysis.

Spectrophotometric Assays: Total Phenolic Compounds (TPCs) and *o*-Diphenols. The TPCs and *o*-diphenols of the virgin olive oil extracts were determined using a UV−Vis 1204 Shimadzu spectrophometer (Kyoto, Japan). TPCs and *o*-diphenols were evaluated according to the methods of Singleton et al. (*20*) and Mateos et al. (*6*), respectively. TPCs and *o*-diphenols were detected at 750 and 370 nm, respectively, and quantified using gallic acid calibration curves ($r^2 = 0.996$ and $r^2 = 0.998$, respectively). Data were expressed as milligrams of gallic acid per kilogram of oil ($n = 3$).

Radical Scavenging Activity of Phenolic Extracts. The DPPH radical scavenging activity of the extracts was evaluated following the Parejo et al. (*21*) and Brand-Williams et al. (*22*) analytical protocols, modified according to that of Rotondi et al. (*15*).

High-Performance Liquid Chromatography Analysis. HPLC analyses were performed with an HP 1100 series (Agilent Technologies, Palo Alto, CA), equipped with a binary pump delivery system, a degasser, an autosampler, a diode array UV−Vis detector (DAD), and a mass spectrometer detector (MSD). A C18 Luna column, 5 μm, 25 cm × 3.00 mm i.d. (Phenomenex, Torrance, CA), with a C18 precolumn (Phenomenex) filter was used. All solvents were of HPLC grade and filtered through a 0.45 μm filter disk. The mobile phase flow rate was 0.5 mL/min. The wavelengths were set at 280 nm. The injection volume was 10 μL. All of the analyses were carried out at room temperature. The gradient elution was carried out using HPLC grade water/formic acid (99.5:0.5, v/v) as mobile phase A and HPLC grade acetonitrile as mobile phase B of the solvent system, as described by Rotondi et al. (*15*). MS analyses were carried out using an electrospray (ESI) interface operating both in positive and in negative mode using the following conditions: drying gas flow, 9.0 L/min; nebulizer pressure, 35 psig; gas drying temperature, 350 °C; capillary voltage, 3000 V; fragmentor voltage, 60 V. The average was calculated by four replications for each sample and expressed as area in mAU.

Phenolic compounds were identified comparing retention times (by spiking attempts) and UV and MS spectra of the detected peaks with those of commercial (tyrosol and vanillic acid) and synthesized compounds (hydroxytyrosol) when available. Deacetoxy oleuropein aglycon (DAOA) and lignans (acetoxypinoresinol and pinoresinol) were identified by analyzing and comparing their MS spectra with those reported in the literature (see also **Table 1**).

Capillary Zone Electrophoresis Analysis. CZE analyses were performed with a Beckman P/ACE 5500 model equipped with a UV−vis diode array detector (Beckman Instruments, Inc., Fullerton, CA) according to the method of Bendini et al. (*9*). The capillary cartridge contained a fused silica capillary (50 μm i.d. × 375 μm o.d.), supplied from Beckman. Total capillary length was 47 cm, whereas effective length was 40 cm. The running buffer was 45 mM sodium tetraborate (pH 9.6), prepared by dissolving an appropriate amount of solid salt in HPLC grade water. The buffer was sonicated and filtered through a 0.2 μm syringe filter. Samples were injected hydrodynamically in the anodic end at low-pressure mode (0.5 psi), for 3 s. Each electrophoretic run was carried out at 27 kV with the capillary temperature maintained at 30 °C, resulting in a current of ∼110 μA. After each electrophoretic run, the capillary was rinsed with HPCE grade water for 2 min; between each run the capillary was rinsed at high pressure (20 psi) consecutively with a 0.1 M NaOH solution for 2 min and HPCE grade water for 2 min and then re-equilibrated with running buffer, for 2 min. All steps of washing were performed at 30 °C. The running buffer was changed after each run. UV detection was performed at 200 nm, rise time was set at 0.17 s, and data rate was 10 Hz.

Phenolic compounds were identified by comparing retention times (by spiking attempts) and UV spectra (see **Figure 3**) of the detected peaks with those of commercial (tyrosol and vanillic acid), synthesized (hydroxytyrosol), and HPLC-collected (acetoxypinoresinol) compounds. DAOA was identified to be the highest peak of the cv. Peranzana extract (see **Figure 1**) by comparing the UV spectra recorded by HPLC and CE (**Figure 3E**,**I**, respectively).

Statistical Analysis. The results reported in this study are the averages of at least three repetitions ($n = 3$), unless otherwise stated. Tukey's honest significant difference (HSD) multiple comparison (one-

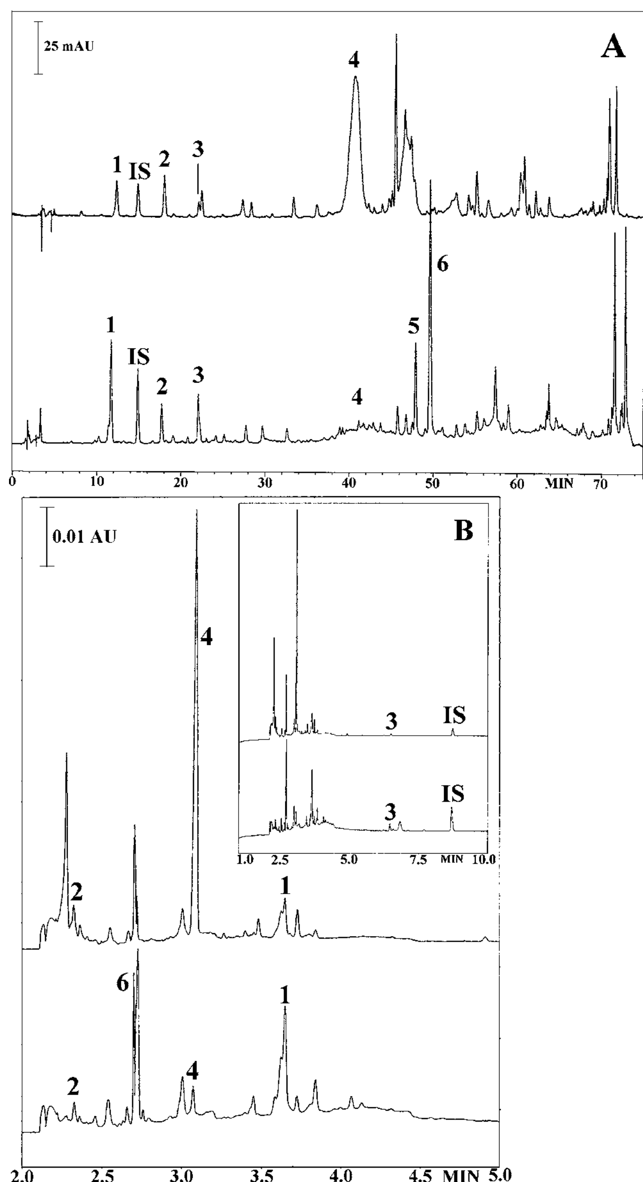

Figure 1. HPLC chromatograms (**A**, detection at 280 nm) and CZE electropherograms (**B**, detection at 200 nm) of the extracts from cv. Peranzana (upper trace) and cv. Nostrana di Brisighella (lower trace) extra virgin olive oils. The inset in panel **B** is the 1–10 min electrophoretic separation showing peak 3 and IS. Peak identification: 1, hydroxytyrosol; 2, tyrosol; 3, vanillic acid; 4, deacetoxy oleuropein aglycon zone; 5, (+)-pinoresinol; 6, (+)-1-acetoxypinoresinol; IS, internal standard (3,4-dihydroxyphenylacetic acid) added for graphical clarity. Other analytical conditions are given under Materials and Methods.

way ANOVA) and Pearson's linear correlations, both at $p < 0.05$, were evaluated using Statistica 6.0 (2001, StatSoft, Tulsa, OK). Data were also analyzed by principal components and classification analysis (using Statistica 6.0) to evidence the correlation between the analyses and the differences in the samples. The analytical data were arranged in a matrix with the rows corresponding to the samples (objects) and the columns corresponding to the analytical indices (variables).

RESULTS AND DISCUSSION

Identification of Phenolic Compounds by HPLC-MSD and CZE-DAD. Figure 1A shows the HPLC phenolic composition of the cv. Peranzana and cv. Nostrana di Brisighella extra virgin olive oil extracts, recorded at 280 nm. In both samples three simple phenols were detected, namely, tyrosol, hydroxytyrosol,

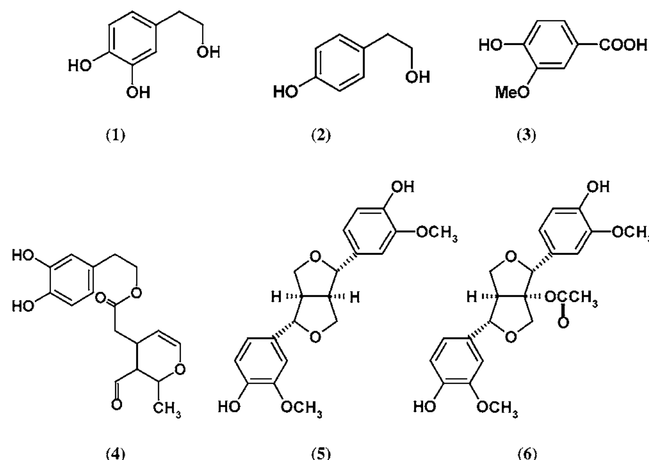

Figure 2. Structures of the phenolic compounds quantified in this work: (**1**) hydroxytyrosol; (**2**) tyrosol; (**3**) vanillic acid; (**4**) deacetoxy oleuropein aglycon (DAOA); (**5**) (+)-pinoresinol; (**6**) (+)-1-acetoxypinoresinol.

and vanillic acid, and identified as reported under Materials and Methods. The cv. Peranzana oil extract showed a high concentration of the secoiridoid derivative DAOA, whereas the cv. Nostrana di Brisighella sample showed two lignans, (+)-pinoresinol and (+)-1-acetoxypinoresinol. These phenolic compounds were identified by HPLC-MSD by comparing the MS spectra with those available in the literature (*4*, *7*). **Table 1** lists the major fragment ions (*m/z*) found by HPLC-MSD analysis of the DAOA and lignans, as reported previously (*23*). **Figure 2** shows the chemical structures of the detected compounds.

Figure 1B shows the cv. Peranzana and cv. Nostrana di Brisighella phenolic profiles recorded by CZE. In this case, the migration order of phenols, according to the different separation principle, was different from the HPLC one. Tyrosol, hydroxytyrosol, and vanillic acid were recognized in both extracts by spiking attempts (as reported under Materials and Methods). Similarly to the chromatographic analysis, DAOA was found to be the highest peak of the Peranzana extract, and it was particularly sharp and baseline-resolved from the other peaks, as well as for the Nostrana di Brisighella extract. The DAOA UV spectra recorded by HPLC and by CZE showed the same profile, with a shoulder around 230 nm higher than that at 280 nm (see **Figure 3E**,**I**, respectively).

To recognize lignans in the electropherograms, (+)-1-acetoxypinoresinol and (+)-pinoresinol were collected by HPLC from the Nostrana di Brisighella extract and from the extract of bark of cv. Ghiacciolo olive fruits, respectively. Actually, (+)-pinoresinol was particularly abundant in the extract of bark of olive fruits, whereas only a slight amount of (+)-1-acetoxypinoresinol was detected (data not shown). These two lignans were not detected in extracts of seed and pulp, as reported by other authors (*24*, *25*). Panels **A** and **B** of **Figure 3** represent the HPLC-UV spectra of (+)-pinoresinol and (+)-1-acetoxypinoresinol, respectively. The UV spectra exhibited two absorption maxima at around 230 and 280 nm, the latter being due to the hydroxyphenyl groups. The shapes of UV spectra of (+)-pinoresinol and (+)-1-acetoxypinoresinol are similar to those of hydroxytyrosol (**Figure 3C**) and tyrosol (**Figure 3D**), respectively, where the 230/280 nm ratio of (+)-1-acetoxypinoresinol and tyrosol is higher than those for the other two compounds, as reported by Brenes et al. (*7*).

After some spiking attempts, the (+)-1-acetoxypinoresinol was recognized in the Nostrana di Brisighella CZE electropherogram (**Figure 1B**). **Figure 3F** shows the CZE-UV spectrum of (+)-1-acetoxypinoresinol. Unfortunately, (+)-

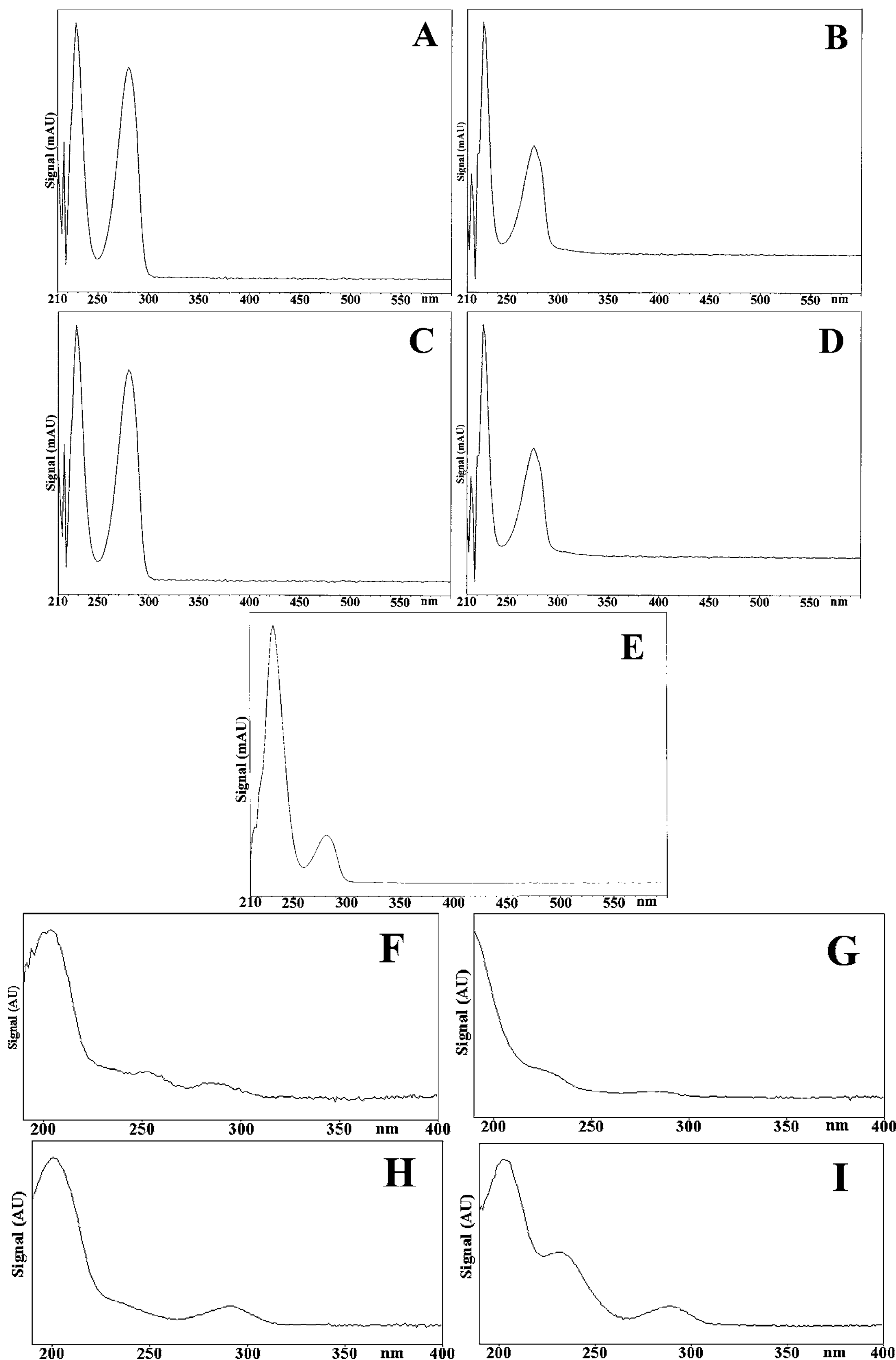

Figure 3. HPLC (210–600 nm range) and CZE (190–400 nm range) on-line UV spectra of (+)-pinoresinol (**A**, HPLC), (+)-1-acetoxypinoresinol (**B**, HPLC; **F**, CZE), hydroxytyrosol (**C**, HPLC; **H**, CZE), tyrosol (**D**, HPLC; **G**, CZE), deacetoxy oleuropein aglycon (**E**, HPLC; **I**, CZE).

pinoresinol, collected and confirmed by HPLC, showed a very low absorbance at the selected CZE conditions, probably due to the lack of the acetate group or interactions occurring between this molecule and the running buffer; thus, the CZE-UV spectra were highly disturbed and impossible to record. However, probably (+)-pinoresinol migrated just before (+)-1-acetoxypinoresinol. Lignans were not detected by CZE in the cv. Peranzana oil extract, as they were by HPLC.

The shape of the CZE-UV spectrum of (+)-1-acetoxypinoresinol was similar to that of tyrosol (**Figure 3G**), where

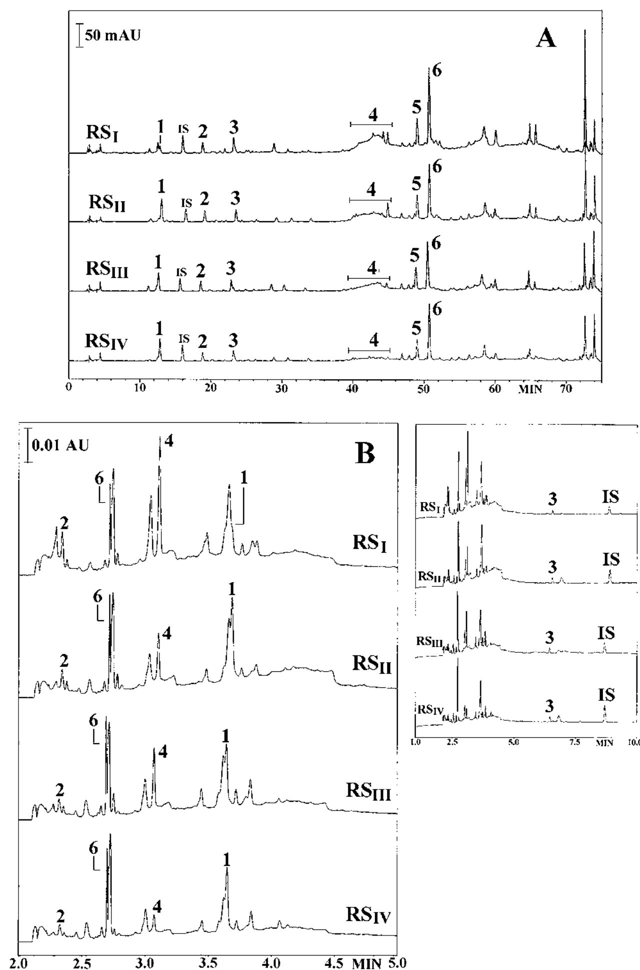

Figure 4. HPLC chromatograms (**A**, detection at 280 nm) and CZE electropherograms (**B**, detection at 200 nm) of the extracts from cv. Nostrana di Brisighella extra virgin olive oils at four ripeness stages (RS_{I-IV}). The inset in panel B is the 1–10 min electrophoretic separation showing peak 3 and IS. Peak identification: 1, hydroxytyrosol; 2, tyrosol; 3, vanillic acid; 4, deacetoxy oleuropein aglycon zone; 5, (+)-pinoresinol; 6, (+)-1-acetoxypinoresinol; IS, internal standard (3,4-dihydroxyphenylacetic acid) added for graphical clarity. Other analytical conditions are given under Materials and Methods.

the 230/280 nm ratio was higher than that of hydroxytyrosol (**Figure 3H**). On the other hand, the CZE shape of the UV spectrum of (+)-pinoresinol should be similar to that of hydroxytyrosol, where the 230/280 nm ratio was lower than that of (+)-1-acetoxypinoresinol and tyrosol, as recorded by HPLC-UV. Anyway, CE behavior of (+)-pinoresinol needs to be thoroughly investigated in further studies.

Quantification of Phenolic Compounds by HPLC and CZE in the Cv. Nostrana di Brisighella Extracts at Different Ripening Stages. Figure 4 reports the HPLC (**A**) and the CZE (**B**) separations of the Nostrana di Brisighella samples, obtained by processing olive fruits at different ripeness stages (RS_{I-IV}). To evaluate the amount of the phenolic compounds (tyrosol, hydroxytyrosol, and vanillic acid as simple phenols, DAOA as secoiridoid derivative, and (+)-1-acetoxypinoresinol as lignan), a quantification based on peak areas was arranged. Unfortunately, a direct quantification by means of calibration curves was not possible because most of the compounds identified were not commercially available.

Table 2 shows the trend of total phenols (TPs, expressed as peak areas) of the Nostrana di Brisighella samples, by both capillary electrophoresis and liquid chromatography.

The highest amount of simple phenols (sum of tyrosol, hydroxytyrosol, and vanillic acid peak areas) was found at the second harvest time sample (*23*). This result might be explained by the increase of hydrolytic enzymatic activity during maturity of olives. A positive Pearson's correlation was found between CZE and HPLC determination of simple phenols ($r^2_{AREA} = 0.920$, $p < 0.0001$). The amount of secoiridoids (expressed as deacetoxy oleuropein aglycon peak area) decreased with ripening. A positive Pearson's correlation was found between CZE and HPLC determination of the DAOA ($r^2_{AREA} = 0.783$, $p < 0.003$). The contents of lignans [expressed as (+)-1-acetoxypinoresinol peak area] in the oils at the four ripeness stages were not significantly different ($p < 0.05$), confirming their stability during the ripening time, reported by other authors (*7*, *10*). As **Table 2** shows, the amount of total phenols (TPs, expressed as the sum of these three classes of compounds) decreased with the maturity of olives, as reported in the literature (*15*−*18*, *26*). The positive Pearson's correlation found between the TPs-CZE and TPs-HPLC ($r^2_{AREA} = 0.845$, $p < 0.001$) confirmed the good agreement in the quantification of the main phenolic compounds of olive oil achieved by the two techniques.

Thus, both HPLC and CZE analyses of olive oil samples showed that the amount of secoiridoids (DAOA) decreased with the olive's ripening degree (see **Figure 4A**,**B**), whereas the concentration of simple phenols (phenylethyl alcohols and phenolic acids) and lignans [(+)-1-acetoxypinoresinol] remained quite similar during the ripening period considered, similar to that previously reported by Rotondi et al. (*15*).

Statistical Correlation among Quantification by HPLC and CZE, Spectrophotometric Assays, and Antioxidant Activity of the Nostrana di Brisighella Extracts. Table 2 shows the spectrophotometric indices and the antioxidant activity results of the Nostrana di Brisighella samples at four ripening stages.

Table 2. Spectrophotometric Indices, Antioxidant Activity, Oxidative Stability, and High-Performance Analysis Quantification (Based on Peak Area, CZE at 200 nm, and HPLC at 280 nm) of the Nostrana di Brisighella Samples at Four Ripening Stages (RS_{I-IV})[a]

	RS_I	RS_{II}	RS_{III}	RS_{IV}
harvest date	Oct 22, 2002	Nov 7, 2002	Nov 19, 2002	Dec 3, 2002
OSI time	47.1 ± 0.3a	43.1 ± 0.5b	38.4 ± 0.8c	35.3 ± 0.1d
TPCs	441.4 ± 29.1a	379.5 ± 18.3b	277.4 ± 14.6c	209.6 ± 22.4d
o-diphenols	212.2 ± 14.1a	228.1 ± 8.5a	153.5 ± 10.2b	127.5 ± 11.9c
ARP	4.02	3.99	2.44	2.05
TPs-HPLC	6364.1 ± 739.0a	6322.7 ± 726.0a	4976.4 ± 268.5ab	4263.1 ± 30.6b
TPs-CZE	77379.3 ± 4588.2a	76671.7 ± 7285.0ab	62314.3 ± 6423.8bc	53000.3 ± 3019.3c

[a] OSI time in hours; TPCs (total phenolic compounds using the Folin–Ciocalteu reagent) in mg of gallic acid/kg of oil; *o*-diphenols in mg of gallic acid/kg of oil; ARP expressed as $1/EC_{50}$ (*15*); TPs-HPLC (total phenols by HPLC quantification) in mAU (peak area); TPs-CZE (total phenols by CZE quantification) in AU (peak area). Different letters in the same row indicate significant differences ($p < 0.05$).

Table 3. Factorial Coordinates of the Variables Based on the Statistical Correlations[a]

	PC 1	PC 2
OSI time	–0.958	0.278
TPCs	–0.969	0.185
o-diphenols	–0.945	–0.185
ARP	–0.989	–0.003
TPs-HPLC	–0.933	–0.231
TPs-CZE	–0.926	–0.057

[a] PC, principal component; OSI time value; TPCs, total phenolic compounds using the Folin–Ciocalteu reagent; *o*-diphenols using spectrophotometric method; ARP, antiradical power; TPs-HPLC, total phenols by HPLC quantification; TPs-CZE, total phenols by CZE quantification.

Similarly to that reported by the HPLC and the CZE quantifications of total phenols, the spectrophotometric indices (TPCs by the Folin−Ciocalteu reagent and *o*-diphenols), the radical scavenging activity (measured by the DPPH test), and the oxidative stability (OSI) decreased with the ripeness stage of olives, as reported by several authors (*15*−*18*, *26*).

Interesting positive Pearson's correlation between TPCs and TPs-HPLC ($r^2_{AREA} = 0.888$, $p < 0.0001$), between *o*-diphenols and TPs-HPLC ($r^2_{AREA} = 0.872$, $p < 0.0001$), between OSI and TPs-HPLC ($r^2_{AREA} = 0.834$, $p < 0.001$), and between ARP and TPs-HPLC ($r^2_{AREA} = 0.901$, $p < 0.0001$) were found. Similarly, positive Pearson's correlation between TPCs and TPs-CZE ($r^2_{AREA} = 0.846$, $p < 0.001$), between *o*-diphenols and TPs-CZE ($r^2_{AREA} = 0.841$, $p < 0.001$), between OSI and TPs-CZE ($r^2_{AREA} = 0.871$, $p < 0.0001$), and between ARP and TPs-CZE ($r^2_{AREA} = 0.900$, $p < 0.0001$) were also recorded, indicating that both high-performance analytical techniques can satisfactorily explain the tendency of phenolic compounds of olive oil during the ripeness observed by the spectrophotometric indices and the evaluation of antioxidant activity.

Positive Pearson's correlation between spectrophotometric indices and evaluation of antioxidant activity were also found: TPCs and *o*-diphenols ($r^2 = 0.879$, $p < 0.0001$), TPCs and OSI ($r^2 = 0.967$, $p < 0.0001$), TPCs and ARP ($r^2 = 0.940$, $p < 0.0001$), *o*-diphenols and OSI ($r^2 = 0.853$, $p < 0.0001$), *o*-diphenols and ARP ($r^2 = 0.962$, $p < 0.0001$), and OSI and ARP ($r^2 = 0.944$, $p < 0.0001$).

The correlations recorded could be explained by the contribution of the phenolic compounds to the antioxidant activity and the oxidative stability of the extra virgin olive oil (*15*, *27*).

Principal component analysis (PCA) revealed that the different analytical indices (variables) provide similar information because the individual vectors have the same direction and similar intensity with respect to principal component 1 (PC 1) as **Table 3** shows. Noticeably, the first two discriminant functions of the statistical analysis explain 94.3% of the total variance and, particularly, PC 1 alone describes 90.9% of the total variance. This evidence is due to the fact that all of the analytical assays have the same tendency and, consequently, they have an effect on the same principal component (PC 1).

As **Figure 5** and **Table 3** show, the TPs-CZE and TPs-HPLC vectors demonstrate that these two analytical techniques provide the same results and tendency, independent of the measure units.

In conclusion, this work reports a qualitative and semiquantitative comparison between HPLC and CZE separations of extra virgin olive oil phenolic compounds. The main advantage of the CE separation here reported is the short analysis time, compared to the HPLC method. Actually, tyrosol, hydroxytyrosol, vanillic acid, (+)-1-acetoxypinoresinol, and deacetoxy oleuropein aglycon are analyzed within 10 min by CZE, whereas HPLC separation requires up to 75 min. However, (+)-pinoresinol, an important phenolic compound belonging to the lignan class, is not detected with satisfactory sensitivity by CZE; therefore, the CE behavior of (+)-pinoresinol needs to be more investigated. This preliminary semiquantitative study shows that CZE and HPLC record the same decreasing tendency of phenolic compounds with ripening degree, confirming the correct attribution of CZE peaks. Unfortunately, for this last technique, the lack of a mass spectrometric detection did not allow the sure peak identification or the detection of compounds having inadequate UV-absorbing properties, reducing the potential of this technique.

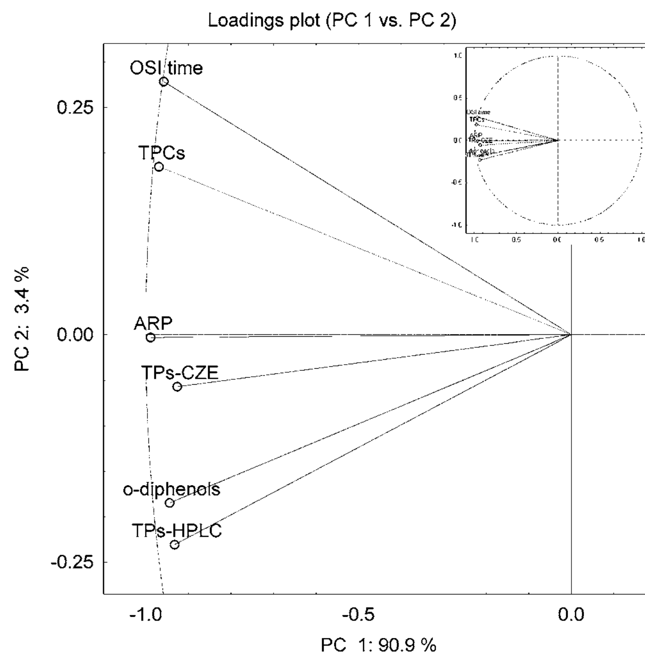

Figure 5. Loading plot of the first two principal components of PCA applied to the analytical indices. The inset reports the complete graphic of the factorial coordinates.

ABBREVIATIONS USED

DAOA, deacetoxy oleuropein aglycon; TPCs, total phenolic compounds; TPs-CZE, total phenols by capillary zone electrophoresis; TPs-HPLC, total phenols by high-performance liquid chromatography; ARP, antiradical power; OSI, oxidative stability instrument (or oxidative stability index).

ACKNOWLEDGMENT

We gratefully thank IBIMET-CNR and Dr. Annalisa Rotondi for the kind collaboration and donation of the Nostrana di Brisighella and Ghiacciolo samples.

LITERATURE CITED

(1) Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, R.; Würtele, G.; Spiegelhalder, B.; Bartsch, H. Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncol.* **2000**, *1*, 107−112.

(2) Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, V.; Spiegelhalder, B.; Bartsch, H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *Eur. J. Cancer* **2000**, *36*, 1235−1247.

(3) Owen, R. W.; Mier, W.; Giacosa, A.; Hull, W. E.; Spiegelhalder, B.; Bartsch, H. 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.

(4) Owen, R. W.; Mier, W.; Giacosa, A.; Hull, W. E.; Spiegelhalder, B.; Bartsch, H. Identification of lignans as major components in the phenolic fraction of olive oil. *Clin. Chem.* **2000**, *46*, 976−988.

(5) Angerosa, F.; D'Alessandro, N.; Corana, F.; Mellerio, G. Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography−chemical ionization mass spectrometry *J. Chromatogr. A* **1996**, *736*, 195−203.

(6) Mateos, R.; Espartero, J. L.; Trujillo, M.; Rìos, J. J.; Leòn-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185−2192.

(7) Brenes, M.; Hidalgo, F. J.; García, A.; Rìos, J. J.; García, P.; Zamora, R.; Garrido, A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *J. Am. Oil Chem. Soc.* **2000**, *77*, 715−720.

(8) Brenes, M.; García, A.; García, P.; Rios, J. J.; Garrido, A. Phenolic compounds in spanish olive oils. *J. Agric. Food Chem.* **1999**, *47*, 3535−3540.

(9) Bendini, A.; Bonoli, M.; Cerretani, L.; Biguzzi, B.; Lercker, G.; Gallina Toschi, T. Liquid−liquid and solid-phase extractions of phenols from virgin olive oil and their separation by chromatographic and electrophoretic methods. *J. Chromatogr. A* **2003**, *985*, 425−433.

(10) Montedoro, G.; Servili, M.; Baldioli, R.; Miniati, E. Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *J. Agric. Food Chem.* **1992**, *40*, 1571−1576.

(11) Montedoro, G.; Servili, M.; Baldioli, R.; Miniati, E. Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. *J. Agric. Food Chem.* **1992**, *40*, 1577−1580.

(12) Montedoro, G.; Servili, M.; Baldioli, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

(13) Pirisi, F. M.; Angioni, A.; Cabras, P.; Garau, V. L.; Sanjust Di Teulada, M. T.; Dos Santos, M. K.; Bandino, G. 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.

(14) Bonoli, M.; Montanucci, M.; Gallina Toschi, T.; Lercker, G. Fast separation and determination of tyrosol, hydroxytyrosol and other phenolic compounds in extra-virgin olive oil by capillary zone electrophoresis with ultraviolet-diode array detection *J. Chromatogr. A* **2003**, *1011*, 163−172.

(15) Rotondi, A.; Bendini, A.; Cerretani, L.; Mari, M.; Lercker, G.; Gallina Toschi, T. 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.

(16) Cerretani, L.; Bendini, A.; Rotondi, A.; Mari, M.; Lercker, G.; Gallina Toschi, T. Evaluation of the oxidative stability and organoleptic properties of extra-virgin olive oils in relation to olive ripening degree. *Prog. Nutr.* **2004**, *6*, 50−56.

(17) Tsimidou, M. Polyphenols and quality of virgin olive oil in retrospect. *It. J. Food Sci.* **1998**, *10*, 99−116.

(18) Monteleone, E.; Caporale, G.; Lencioni, L.; Favati, F.; Bertuccioli, M. Optimization of virgin olive oil quality in relation to fruit ripening and storage. In *Food Flavors: Generation, Analysis and Process Influence*; Charalambous, G., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1995; pp 397−418.

(19) Jebe, T. A.; Matlock, M. G.; Sleeter, R. T. Collaborative study of the oil stability index analysis. *J. Am. Oil Chem. Soc.* **1993**, *70*, 1055−1057.

(20) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(21) Parejo, I.; Codina, C.; Petrakis, C.; Kefalas, P.; Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminal chemiluminescence and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. *J. Pharmacol. Toxicol.* **2000**, *44*, 507−512.

(22) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. -Technol.* **1995**, *28*, 25−30.

(23) Bonoli, M.; Bendini, A.; Cerretani, L.; Gallina Toschi, T. Identification of (+)-pinoresinol and (+)-1-acetoxypinoresinol in virgin olive oil by capillary zone electrophoresis (CZE) and high performance liquid chromatography−mass spectrometry detection (HPLC-MSD). In *Proceedings of the 27th International Symposium on Capillary Chromatography*, Riva del Garda, Italy, May 31−June 4 2004; Research Institute for Chromatography: Kortrijk, Belgium, 2004; P20, pp 1−12.

(24) Tsukamoto, H.; Hisada, S.; Nishibe, S. Lignans from bark of the *Olea* plants. I. *Chem. Pharm. Bull.* **1984**, *32*, 2730−2735.

(25) Tsukamoto, H.; Hisada, S.; Nishibe, S. Lignans from bark of the *Olea* plants. II. *Chem. Pharm. Bull.* **1985**, *33*, 1232−1241.

(26) Cerretani, L.; Bendini, A.; Biguzzi, B.; Lercker, G.; Gallina Toschi, T. Evaluation of the oxidative stability of extra-virgin olive oils, obtained by different technological plants, with respect to some qualitative parameters. *Ind. Aliment.* **2003**, *427*, 706−711.

(27) Del Carlo, M.; Sacchetti, G.; Di Mattia, C.; Compagnone, D.; Mastrocola, D.; Liberatore, L.; Cicchelli, A. Contribution of the phenolic fraction to the antioxidant activity and oxidative stability of olive oil. *J. Agric. Food Chem.* **2004**, *52*, 4072−4079.

Received for review July 9, 2004. Revised manuscript received August 20, 2004. Accepted September 5, 2004.

JF048868M