Changes in Phenolic Content and Antioxidant Activity of Italian Extra-Virgin Olive Oils during Storage

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ABSTRACT: Phenolic composition and antioxidant activity of extra-virgin olive oils extracted from several Italian varieties were studied at production and during storage. The antioxidant activity was measured according to the following tests: in the aqueous phase, by radical scavenging of the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation; and in the lipid phase, using the β-carotene bleaching method. The phenolic content was not correlated to the oxidation indices (peroxide value and spectrophotometric constants). The phenolic contents and profiles of the various cultivars showed remarkable differences. The phenolic content was strongly correlated with the antioxidant activity measured according to the β-carotene test and weakly correlated with the radical scavenging ability.

Keywords: ABTS, antioxidant, β-carotene, phenolic, virgin olive oil

Introduction

The Mediterranean diet, which is largely vegetarian in nature, includes the consumption of noticeable amounts of extra-virgin olive oil. To be an extra-virgin olive oil, it must be obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil and without any treatment other than washing, decantation, centrifugation, or filtration (EC Reg. 1513/2001).

The health-promoting properties of extra-virgin olive oil concern the ability to prevent diseases that may be related to oxidative damages such as coronary heart diseases, stroke, and certain types of cancers (Fito and others 2000; Covas and others 2001; Aguilera and others 2002; Warburg and others 2002; Bendini and others 2007). The protective role of virgin olive oil is the result of its specific composition including high proportion of monounsaturated fatty acids (oleic acid), a balanced presence of polyunsaturated fatty acids, and minor components such as phenolic compounds (Owen and others 2000) tocopherols, and carotenoids, known to act as antioxidants against reactive species (Boskou 1996) at different levels in the oxidative sequence involving lipid molecules.

A virgin olive oil contains at least 30 phenolic compounds (Bendini and others 2007). Phenolic total amount and composition of olive oil varies from 50 to 1000 mg/kg (Montedoro and others 1992a,b), depending on cultivars, place of origin, agronomic techniques, olive ripening, possible infestation by the olives fly (Bactrocera oleae) (Owen and others 1995), gallic, caffeic, vanillic, p-coumaric, syringic, ferulic, homovanillic, phodroxycbenzoic, protocateucic acids (Montedoro and others 1992b; Mannino and others 1993), and lignans (Brenes and others 1999; Owen and others 2000). Derivates of 3,4-DHPEA, in particular the dialdehyde form of phenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), an isomer of oleuropein aglycon (3,4-DHPEA-EA), and the dialdehydic form of phenolic acid linked to p-HPEA (p-HPEA-EDA) have been identified as the major secoiridoid compounds of virgin olive oil (Montedoro and others 1993; Baldioli and others 1996; Brenes and others 1999). More than 45 phenolic compounds were characterized in olive oil through the application of capillary electrophoresis and HPLC-TOF-MS (Carrasco-Panconero and others 2007). The type as well as the level of these compounds is an important parameter in evaluating the quality and nutritive value of virgin olive oil.

Lavelli and others (2006) studied the extent of degradation of phenolic derived from olive secoiridoids and the antioxidant activity of several monovarietal extra-virgin olive oils during storage. They found that degradation occurred in a similar pattern in all the considered oils and that oleuropein derivatives were less stable than the corresponding ligstroside derivatives. Based on their results, despite the antioxidant depletion, the oils with high phenolic content maintained their beneficial properties during all the commercial life. Gómez-Alonso and others (2007) investigated the evolution of phenolics during 21 mo storage at room temperature finding that the reduction of total phenolic compounds ranged from 43% to 73%, and was higher in samples whose initial phenol contents were greater. Hydroxytyrosol increased linearly in most samples, whereas its complex forms decreased considerably.
Storage affects the phenolic profile through the oxidative stress and the consequent formation of oxidized phenols (Armaforte and others 2007). In particular, 3,4-DHPEA and β-HEA increased during storage. The authors concluded that it may be feasible to use the ratio fresh phenols/oxidized phenols as an interesting means of determining the freshness/aging ratio of the oil.

Due to their chemical properties, the phenolic compounds inhibit lipid oxidation (Teissedre and others 1996). The contribution of phenols to the virgin olive oil stability and antioxidant activity was estimated to be higher than that of other compounds (Aparicio and others 1999; Gutierrez and others 2001; Pellegrini and others 2001). Various researchers have demonstrated a positive linear relationship between oil stability and total phenolic content (Gutfinger 1981; Baldioli and others 1996). Baldioli and others (1996) and Gordon and others (2001) found that the total hydrophilic phenolic compounds and the oleosidic forms of 3,4-DHPEA are to be correlated with the oxidative stability of virgin olive oil. According to Papadopoulos and Boskou (1991) and Tsimi-dou and others (1992), hydroxytyrosol (but not tyrosol) concentration is closely correlated with the stability of the oil.

Natural antioxidants exert their antioxidant activity through various mechanisms: preventing first chain initiation by scavenging initiating radicals, metal chelating, decreasing localized oxygen concentration, and decomposing peroxides (Aruoma 1996). The antioxidant properties of α-diphenols can be related to hydroxyl donation, that is, their ability to improve radical stability by forming an intramolecular hydrogen bond between the free hydrogens of their hydroxyl group and their phenoxyl radicals. Although investigations on the structure–activity relationship of olive oil phenols are yet to be carried out, similar studies have been performed on flavonoids and have indicated that, in general, the degree of antioxidant activity is correlated with the number of hydroxyl substitutions (Rice-Evans and others 1996; Cao and others 1997).

According to Chimi and others (1991), among the phenolic compounds contained in olive oil, the antioxidant effect was, in a decreasing order: hydroxytyrosol > oleanuropein > tyrosol (Chimi and others 1991). A synergistic effect between oleanuropein, which is a derivative of hydroxytyrosol, and α-tocopherol was reported (Baldioli and others 1996). A more recent study by Carrasco-Pancorbo and others (2005) classified hydroxytyrosol, deacetoxy oleanuropein aglycon, and oleanuropein aglycon as the strongest antioxidant in virgin olive oils.

An important aspect of the study of phenolic compounds is the assessment of their antioxidant activity. Various methods have been introduced to test the antioxidant activity of olive oil. They can be divided into 2 groups: (1) assay of the radical scavenging ability and (2) assay of the ability to inhibit the oxidation of a lipidic substrate (Schwarz and others 2001). The radical scavenging tests measure either the reduction of stable radicals or radicals generated by radiolysis, photolysis, or other reactions. The elements involved in an oxidation reaction are a substrate, an oxidant, an initiator, intermediates, and final products and the measurement of one of these can be used to assess antioxidant activity (Antolvich and others 2002). An important limitation of these tests is that the reducing capacity does not necessarily reflect antioxidant activity (Katalinic and others 2006; Wong and others 2006).

In this study, methanolic extracts of extra-virgin olive oil from 15 Italian cultivars grown in the Daunia district were analyzed for their phenolic content and antioxidant activity and the change of these indices during storage was also considered. The antioxidant activity was measured according to the following tests: in the aqueous phase, by radical scavenging of the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation; and in the lipid phase, using the β-carotene bleaching method.

### Materials and Methods

#### Oil samples

Extra-virgin olive oil samples were obtained from healthy olive fruits (Olea europaea L.) manually picked in 2 olive groves located in the countryside near Cerignola and TorreMaggiore (Apulia, Italy), respectively. In particular, samples of Cellina di Nardo, FS17, Gignarino, Moraiolo, Nociara, Ogliarola barese, Peranzana, and Picholine were withdrawn in the 1st area, whereas samples of Cima di Melfi, Coratina, Frantoio, Leccino, Moraiolo, Nociara, and Peranzana were collected in the 2nd one. The names of oils correspond to different Italian varieties from South of Italy or of new introduction in those areas.

The same agronomic techniques were applied for all cultivars. Olive fruits were harvested in the year 2005 and the relative oils were obtained within 24 h by crushing the olives by traditional processing technique in the same Italian oil factory. The obtained extra-virgin oils were stored in 1-L dark glass bottles at room temperature and withdrawn at production and at regular intervals of time during 12 mo of storage.

#### Quality indices

Acidity, peroxide value, spectrophotometric indices (K₂₃₂, K₂₇₀), and sensory score were determined following the analytical methods described by the EEC Regulation 2568/91.

#### Extraction of phenolic compounds

Phenols were recovered from extra-virgin olive oils by liquid–liquid extraction using methanol as solvent and following the procedure reported in Montedoro and others (1992a), opportunely modified. Two milliliters of methanol/water (70:30, v/v) and 2 mL of hexane were added to 5 g of virgin olive oil and mixed with a Vortex for 10 min. The hydroalcoholic phase containing phenolics was separated from the oily phase by centrifugation (6000 rpm, 4 °C, 10 min). Hydroalcoholic phases were collected and submitted to another centrifugation (13000 rpm, at room temperature, 4 min). Finally, hydroalcoholic extracts were recovered with a syringe and then filtered though a 0.45-μm nylon filter (DISMIC-13NP, Advan-tec, Toshi Roshai Kaisha, Tokyo, Japan) before analysis.

The extracts prepared for HPLC analysis were obtained according to the same protocol, but with the addition of 0.5 mL of a solution of gallic acid (internal standard) at the concentration of 100 ppm, prepared in methanol/water (70:30, v/v).

#### Total phenolic content

The determination of the total phenolic compounds included the use of the Folin–Ciocalteau reagent and the method was adapted from Di Stefano and others (1989). In a test tube, 100 μL of phenolic extract or phenolic standard were mixed with the Folin–Ciocalteau reagent (100 μL, 2N) and, after 4 min, with an aqueous solution of Na₂CO₃ (800 μL, 5%). The mixture was heated in a 40 °C water bath for 20 min and the total phenol content was determined colorimetrically at 750 nm. The standard curve was prepared using diluted solutions of gallic acid in a methanol/water solution (70:30, v/v). The total phenolic content was expressed as milligrams of gallic acid equivalents per kilogram of oil.

#### HPLC phenolic profile

The HPLC analysis of the phenolic extracts was carried out according to Gambacorta and others (2006), using a HPLC binary
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system (Agilent, model G1311A, Santa Clara, Calif., U.S.A.) equipped with a 7725 Rheodyne injector, a 20-µL sample loop, a diode array detector (Agilent, model G1315Bm), and a ChemStation integrator (Agilent) for data acquisition. The stationary phase was a Nova-Pack C18 analytical column (150 × 3.9 mm i.d.) with a particle size of 4 µm (Waters, Milford, Mass., U.S.A.). The mobile phases for chromatographic analysis were (A) water:acetic acid (98:2, v/v) and (B) methanol:acetonitrile (1:1, v/v) at constant flow rate of 1 mL/min. The gradient program of solvent was as follows: 0 to 30 min 100% A; 30 to 45 min 70% A; 45 to 55 min 50% A; 55 to 65 min 40% A; 65 to 75 min 0% A.

The identification of some phenolic components was carried out comparing the peak retention times with those obtained by injection of pure standards (hydroxytyrosol and tyrosol, purchased at Extrasynthese, Genay Cedex, France; vanillin, purchased at Sigma-Aldrich, Milan, Italy) analyzing the obtained spectra and by LC-MS analysis. The identification of some phenolic components was carried out comparing the peak retention times with those obtained by injection of pure standards (hydroxytyrosol and tyrosol, purchased at Extrasynthese, Genay Cedex, France; vanillin, purchased at Sigma-Aldrich, Milan, Italy) analyzing the obtained spectra and by LC-MS analysis. The identification of other phenolics (3,4-DHPEA-EDA, p-HPEA-EDA, pinoresinol, 3,4-DHPEA-EA, p-HPEA-EA) was made on the basis of studies found in the literature (Brenes and others 2000; Gómez-Alonso and others 2002, 2007; Morello and others 2004; Gómez-Rico and others 2006).

Quantity of phenolic compounds was performed according to the method of the internal standard (gallic acid, Extrasynthese) and on the basis of the response factors. The response factors were determined taking into account the recovery percentages of the phenolic compounds and the internal standard. The recovery percentages were the following: p-HPEA-EDA 76.85%, pinoresinol 76.98%, 3,4-DHPEA-EDA 77.35%, hydroxytyrosol 77.71%, tyrosol 79.19%, and gallic acid 81.24%.

Evaluation of the antioxidant activity
The antioxidant activity of the oil phenolic extracts was evaluated according to 2 tests: the β-carotene-linoleate model system (Lee and others 1995), in which the antioxidant activity was measured by the ability of a compound to minimize the loss of β-carotene in an emulsified aqueous system in the presence of oxygen at high temperature (50 °C), and the ABTS/MetMb method (Miller and others 1993), based on the abilities of the antioxidants present into the extracts to scavenge the ABTS·+ radical cation in comparison with that of a standard antioxidant (Trolox).

The β-carotene-linoleate test is based on the thermal (50 °C) autoxidation of linoleic acid and the consequent formation of the peroxidic radical that is able to scavenge hydrogen atoms from the β-carotene molecule, determining its bleaching. The β-carotene bleaching is detectable through the absorbance decrease, which is greater when the antioxidant content is low. Five milligrams of

Table 1 — Total phenolic content of oils at production, 6 and 12 mo of storage.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Total phenolic content at production (µg/g gall. ac. eq)</th>
<th>Total phenolic content at 6 mo of storage (µg/g gall. ac. eq)</th>
<th>Total phenolic content at 12 mo of storage (µg/g gall. ac. eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellina di Nardò – C</td>
<td>205.11 ± 5.25 E</td>
<td>165.70 ± 3.82 E</td>
<td>46.18 ± 1.85 C</td>
</tr>
<tr>
<td>FS17 – C</td>
<td>140.45 ± 3.96 B</td>
<td>114.37 ± 3.15 A</td>
<td>36.15 ± 3.54 B</td>
</tr>
<tr>
<td>Grignano – C</td>
<td>143.59 ± 5.89 B</td>
<td>125.53 ± 4.80 B</td>
<td>26.53 ± 2.94 A</td>
</tr>
<tr>
<td>Moraiolo – C</td>
<td>179.88 ± 5.76 C</td>
<td>144.15 ± 7.44 C</td>
<td>37.72 ± 1.72 C</td>
</tr>
<tr>
<td>Nociara – C</td>
<td>185.45 ± 8.12 D</td>
<td>147.68 ± 4.97 C</td>
<td>42.15 ± 3.32 B</td>
</tr>
<tr>
<td>Ogliarola Barese – C</td>
<td>133.68 ± 6.16 A</td>
<td>123.92 ± 4.77 B</td>
<td>30.48 ± 1.36 A</td>
</tr>
<tr>
<td>Peranzana – C</td>
<td>190.10 ± 6.75 D</td>
<td>159.07 ± 3.28 D</td>
<td>59.48 ± 16.63 D</td>
</tr>
<tr>
<td>Picholine – C</td>
<td>202.30 ± 7.32 E</td>
<td>192.01 ± 9.35 F</td>
<td>42.26 ± 4.92 B</td>
</tr>
<tr>
<td>Cima di Melfi – T</td>
<td>225.09 ± 5.90 b</td>
<td>174.77 ± 4.28 b</td>
<td>115.65 ± 4.67 c</td>
</tr>
<tr>
<td>Coratina – T</td>
<td>322.18 ± 9.73 d</td>
<td>284.67 ± 10.47 e</td>
<td>55.36 ± 3.20 b</td>
</tr>
<tr>
<td>Frantoio – T</td>
<td>178.34 ± 3.63 a</td>
<td>179.67 ± 7.58 b</td>
<td>39.11 ± 9.94 a</td>
</tr>
<tr>
<td>Leccino – T</td>
<td>228.58 ± 6.91 b</td>
<td>171.91 ± 4.88 b</td>
<td>147.32 ± 4.71 d</td>
</tr>
<tr>
<td>Moraiolo – T</td>
<td>179.93 ± 7.61 a</td>
<td>132.59 ± 8.58 a</td>
<td>37.59 ± 3.68 a</td>
</tr>
<tr>
<td>Nociara – T</td>
<td>256.85 ± 9.28 c</td>
<td>205.55 ± 7.42 c</td>
<td>62.98 ± 5.01 b</td>
</tr>
<tr>
<td>Peranzana – T</td>
<td>253.36 ± 9.63 c</td>
<td>227.52 ± 8.79 d</td>
<td>60.66 ± 8.95 b</td>
</tr>
</tbody>
</table>

In columns, different letters indicate significant differences among cultivars (P < 0.05): capital letters represent comparison of oils from Cerignola; lowercase letters represent comparison of oils from Torremaggiore.

Data are expressed as µg/g gallic acid equivalents since quantification has been done on the basis of the calibration curve of gallic acid and taking into account its molecular weight.

C = Cerignola; T = Torremaggiore.

Figure 1 — Phenolic profile of Picholine oils obtained by olives picked in the Cerignola countryside. (A) At production (B) after 6 mo of storage. I.S., gallic acid, (1) hydroxytyrosol, (2) tyrosol, (3) vanillin, (4) 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol), (5) p-HPEA-EDA (dialdehydic form of elenolic acid linked to p-hydroxyphenylethanol), (6) pinoresinol, (7) 3,4-DHPEA-EA (aglycon isomer of oleuropein), (8) p-HPEA-EA (aglycon isomer of ligstroside).
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Synthetic \( \beta \)-carotene type II (Sigma, St. Louis, Mo., U.S.A.) were dissolved in 50 mL of chloroform (J.T. Baker, Mallinckrodt Baker, Milano, Italia). Three milliliters of this solution were pipetted into a round-bottom flask containing 40 mg of linoleic acid (Sigma) and 400 mg of Tween 40 (Sigma). After evaporation of chloroform to dryness under vacuum at 40 °C, 100 mL of distilled water enriched with oxygen were added to the flask and the mixture was shaken to form a liposome solution. Aliquots of 1.5 mL of this solution were pipetted into test tubes containing 20 \( \mu \)L of phenolic extracts immediately put into a water bath at 50 °C. The absorbance decreased rapidly in the absence of antioxidants and slowly in sample extracts. The antioxidant activity was expressed as the antioxidant coefficient AAC:

\[
AAC = \left( 100 - \frac{\text{Abs of extract}_{0 \text{min}} - \text{Abs of extract}_{240 \text{min}}}{\text{Abs of control}_{0 \text{min}} - \text{Abs of control}_{240 \text{min}}} \right) \times 100
\]

Analyses were carefully performed to avoid heavy metal contamination that acts as prooxidant.

In the ABTS assay, the antioxidant activity was measured through the ability of antioxidants to scavenge the ABTS radical cation (a blue/green chromophore) inhibiting its absorption at 734 nm. The ABTS\(+\) is the oxidizable substrate. Metmyoglobin (MetMb) supplies the ferric ions needed for the formation of hydroxyl radicals (OH\(-\)) following the Fenton reaction (Winterbourn 1995). \( \text{H}_2\text{O}_2 \) is the reaction starter. A solution of ABTS (246 \( \mu \)L, 610 \( \mu \)M), salmine phosphate buffer (PBS, 524 \( \mu \)L, pH 7.4), and purified MetMb in PBS (410 \( \mu \)L, 6.1 \( \mu \)M) were prepared each day. Three hundred microliters of hydrogen peroxide were added to generate the ABTS\(+\). Twenty microliters of the phenolic extracts were added to the mixture to evaluate their antioxidant activities. Trolox \( 2.5 \) mM (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich, Steinheim, Germany) was prepared in a methanolic aqueous solution (70%, v/v) as a stock standard. The antioxidant activity of this solution and of a methanolic aqueous solution (70%, v/v) was also measured. The antioxidant activities of Trolox and the methanolic solution were considered as equal to 100% and 0% of inhibition of the formation of the radical ABTS\(+\), respectively. Obviously, the phenolic extracts showed intermediate values.

### Statistical analysis

Mean values and standard deviations were determined. The discussion of the results was based on the one-way analysis of variance (ANOVA) and the Holm test at a confidence level of 95% performed by means of the Kaleidagraph statistical software (version 3.6.2; Synergy Software, Reading, Pa., U.S.A.).

### Results and Discussion

#### Quality indices

According to EU Regulation 1989 (2003), an extra-virgin olive oil is a liquid fat that conforms to a series of chemical parameters (free fatty acid percentage \( \leq 0.8 \) g oleic acid/kg oil, peroxide value \( \leq 20 \) meqO\(_2\)/kg, \( K_{232} \leq 2.50, K_{270} \leq 0.22, \) median of defects = 0, median of fruity \( > 0 \), is free of defects.

The chemical parameters measured on the oils at production were included in the values mentioned previously. Concerning acidity, oils from Cerignola showed values ranging from 0.50 ± 0.00
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(Peranzana) to 0.65 ± 0.04 g/100 g (Grignano) whereas those produced around Torremaggiore showed lower values included within 0.39 ± 0.03 (Cima di Melfi) and 0.59 ± 0.03 g/100 g (Leccino). Peroxide value of oils from Cerignola ranged from 7.2 ± 0.5 of Cellina di Nardò to 12.1 ± 0.2 meqO₂/kg of Ogliarola barese whereas, oils from Torremaggiore were included in the range 4.8 ± 0.1 (Coratina) to 12.3 ± 0.13 meqO₂/kg (Moraiolo).

Among oils from Cerignola, K₂⁷⁰ values were included between 1.56 ± 0.01 of Grignano and 2.02 ± 0.82 of FS17 whereas oils from Torremaggiore ranged from 1.39 ± 0.44 of Leccino and 1.75 ± 0.04 of Frantoio.

Among oils from Cerignola, K₂⁷⁰ values ranged from 0.12 ± 0.00 (Grignano) to 0.21 ± 0.02 (Cellina di Nardò) whereas, oils from Torremaggiore showed values included between 0.11 ± 0.00 (Frantoio) and 0.17 ± 0.01 (Cima di Melfi).

Concerning the sensory tests, all the obtained oils had a median of defects equal to zero and a median of fruity greater than zero. In particular, the median of fruity ranged from 2.8 (Cellina di Nardò) to 5.8 (Peranzana) among oils from Cerignola and from 4.0 (Moraiolo) to 6.0 (Coratina) among oils from Torremaggiore.

The percentage of unidentified compounds ranged from 16.4% (Moraiolo) and 54.5% (Grignano) for Cerignola oils and from 30.9% (Cima di Melfi) and 47.6% (Coratina) for Torremaggiore oils.

The results of the analysis highlighted a greater influence of the place of growing. Concerning the sensory tests, all the obtained oils had a median of defects equal to zero and a median of fruity greater than zero. In particular, the median of fruity ranged from 2.8 (Cellina di Nardò) to 5.8 (Peranzana) among oils from Cerignola and from 4.0 (Moraiolo) to 6.0 (Coratina) among oils from Torremaggiore.

**Evolution of the phenolic content**

The phenolic content of the oils is reported in Table 1. Concerning oils at production, very low linear correlations were found between phenolic content and each of the oxidation indices (peroxide value and spectrophotometric constants). A reduction of the phenolic content of the extra-virgin olive oils was registered from production up to 1 y with more remarkable losses in the last 6 mo of storage. The ANOVA highlighted the different time courses of the decrease of the phenolic concentration among samples. The highest phenolic decrease (about 85%) was detected for Coratina (Torremaggiore) and Grignano (Cerignola) whereas Leccino oil from Torremaggiore, although impoverished of about 35% of its initial phenolic content, showed the highest antioxidant concentration (approximately 150 mg/kg) at the end of storage. As highlighted by the results of the ANOVA, the phenolic contents of the considered oils showed remarkable differences. At production, among oils from Cerignola, the highest antioxidant concentration was detected for Cellina di Nardò and Picholine, followed by Peranzana and Nociara whereas the lowest concentration was detected in Ogliarola barese oil. Concerning oils from Torremaggiore, the richest in phenolics was Coratina whereas Frantoio and Moraiolo showed the lowest phenolic content.

To evaluate the effect of cultivar and place of growing, a pair comparison test was applied to the phenolic content of the 3 varieties (Moraiolo, Nociara, and Peranzana) cultivated both in Cerignola and Torremaggiore fields. The results of the analysis highlighted a greater influence of the place of growing.

**Phenolic profiles of oils**

Figure 1 shows the HPLC profile of phenolic extracts from Picholine extra-virgin olive oils at production and after 6 mo of storage whereas Table 2 reports the specific phenolic composition of all the considered oils at production. These data highlighted the different phenolic profile among samples, to confirm the influence of genotype, place of growing, and olive maturity index. The most abundant compounds were represented by 3,4-DHPEA-EDA, p-HPEA-EDA, and pinoresinol whereas tyrosol, hydroxytyrosol, and vanillin were detected in very low amounts. Concerning p-HPEA-EDA, it was absent from many samples with the exception of Cellina di Nardò, FS17, Nociara, Peranzana, Picholine from Cerignola and Coratina from Torremaggiore.

Antioxidant activity of oils measured according to the β-carotene test

Data referred to oils at production, 6 and 12 mo of storage are reported in Table 3. At production, the antioxidant activity of the oils from Torremaggiore followed this order: Coratina > Moraiolo > Peranzana, Nociara, Frantoio > Leccino. Among oils from Cerignola, the highest AAC was measured for Picholine. The other oils showed the same antioxidant values. After 3 mo of storage (data not shown) the antioxidant activity remained unchanged whereas a decrease

<table>
<thead>
<tr>
<th>Table 3—Antioxidant activity measured according to the β-carotene test (results expressed as AAC) and the ABTS test (results expressed as % antioxidant activity) of oils at production and after 6 and 12 mo of storage.</th>
</tr>
</thead>
<tbody>
<tr>
<td>At production</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>AAC at 6 mo of storage</td>
</tr>
<tr>
<td>Cima di Melfi – T</td>
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<tr>
<td>Coratina – T</td>
</tr>
<tr>
<td>Frantoio – T</td>
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<tr>
<td>Leccino – T</td>
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<tr>
<td>Moraiolo – T</td>
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<tr>
<td>Nociara – T</td>
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<tr>
<td>Peranzana – T</td>
</tr>
<tr>
<td>Cellina di Nardò – C</td>
</tr>
<tr>
<td>FS17 – C</td>
</tr>
<tr>
<td>Grignano – C</td>
</tr>
<tr>
<td>Moraiolo – C</td>
</tr>
<tr>
<td>Nociara – C</td>
</tr>
<tr>
<td>Ogliarola Barese – C</td>
</tr>
<tr>
<td>Peranzana – C</td>
</tr>
<tr>
<td>Picholine – C</td>
</tr>
</tbody>
</table>

In columns, different letters indicate significant differences (P < 0.05): lowercase letters have been used to compare Torremaggiore oils; capital letters have been used to compare Cerignola oils.

C = Cerignola; T = Torremaggiore.
of about 40% was registered for almost all oils after 6 mo. At 9 (data not shown) and 12 mo of storage, a stronger decrease was detected as a consequence of the loss in phenolic content. At 12 mo the antioxidant activity of the oils from Torremaggiore followed this order: Cima di Melfi, Leccino > Coratina > Peranzana > Nociara > Frantoio > Moraiolo. At the end of storage, among oils from Cerignola, the highest antioxidant activity was reported for Peranzana and the lowest was detected for FS17 and Ogliarola barese.

Figure 2 highlights the strong positive linear correlation existent between phenolic content and antioxidant activity measured through the β-carotene test (R = 0.839), in agreement with data reported in the literature (Gorinstein and others 2003).

As already seen for the phenolic content, the ANOVA highlighted the different time course of the decrease of the antioxidant activity among samples.

To evaluate the effect of cultivar and place of growing on the antioxidant activity measured according to the β-carotene test, a pair comparison test was applied to the 3 varieties (Moraiolo, Nociara, and Peranzana) cultivated in both the locations. The results put in evidence the negligible influence of genotype with respect to the place of growing.

Antioxidant activity of oils measured according to the ABTS test

The radical scavenging ability of phenolic extracts was also tested and the results are reported in Table 3.

At production, the antioxidant capacity of oils from Torremaggiore was, in the order: Coratina > Peranzana > Cima di Melfi, Nociara, Leccino > Frantoio, Moraiolo. After 6 mo, this order changed only a little: Coratina > Peranzana > Cima di Melfi, Nociara, Leccino > Frantoio, Moraiolo > Nociara. At production, among oils from Cerignola, the highest radical scavenging activity was shown by Cellina di Nardò whereas the lowest value was detected for Ogliarola barese. After 6 mo, the cultivars showing the lowest radical scavenging activity included Ogliarola barese, Grignano, and FS17. The radical scavenging ability did not suffer a decrease during oil storage. On the contrary, it significantly increased, probably as a consequence of the changes occurred in the phenolic profile during storage.

Concerning the relationship between phenolic content and radical scavenging ability, the coefficient R was equal to 0.550, indicating a medium contribution of phenolic compounds to the scavenging capacity of these extra-virgin olive oils.

The comparison between the radical scavenging ability of couples of cultivars growing in both the locations put in evidence a certain influence of the genotype for Moraiolo and Peranzana at production. The place of growing showed a stronger effect during storage.

Conclusions

The phenolic content and profiles of the considered Italian cultivars greatly depended on place of growing and olive maturity index. A good correlation was found between total phenolic content and antioxidant activity measured according to the β-carotene method but not between total phenolic content and ABTS data indicating that phenolic compounds supplied a great contribute to the inhibition of the lipidic oxidation but were not able to scavenge free radicals.

References


Figure 2—Linear correlation among phenolic content and antioxidant activity of oils measured according to the β-carotene test.