THE Society for Food Science & Technology
Journal of Food Science
A Publication of the Institute of Food Technologists

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

A. BAIANO, G. GAMBACORTA, C. TERRACONE, M.A. PREVITALI, C. LAMACCHIA, AND E. LA NOTTE

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; Warhrburg 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), depending on cultivars, place of origin, agronomic techniques, olive ripening, possible infestation by the olives fly *Bactrocera oleae* (Gómez-Caravaca and others 2008), extraction methods, and storage conditions but results obtained by different researchers are hardly comparable because of the variety of methods proposed for their determination. In fact, the widely employed Folin–Ciocalteau reagent is not specific for phenols and the HPLC analysis is limited by the complexity of the phenolic fraction.

The polar fraction of virgin olive oil is rich in simple and complex phenolic antioxidants with the latter being the most abundant (Nissiotis and Tasioula-Margari 2002). Phenolic compounds identified in virgin olive oil include 3,4-dihydroxyphenylethanol (3,4-DHPEA, or hydroxytyrosol) and *p*-4-hydroxyphenylethanol (p-HPEA, or tyrosol) (Montedoro and others 1992b, 1993; Angerosa and others 1995), gallic, caffeic, vanillic, *p*-coumaric, syringic, ferulic, homovanillic, *p*hydroxybenzoic, protocatecuic 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 dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), an isomer of oleuropein aglycon (3,4-DHPEA-EA), and the dialdehydic form of elenolic 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-Pancorbo 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 founding 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.

MS 20080668 Submitted 9/3/2008, Accepted 11/19/2008. *Authors are with the Dept. of Food Science, Univ. of Foggia, Via Napoli, 25-71100 Foggia, Italia. Authors Baiano, Gambacorta, Lamacchia, and La Notte are also with Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità, Via Napoli, 25-71100 Foggia, Italia. Direct inquiries to author Baiano (E-mail: a.baiano@unifg.it).*

© 2009 Institute of Food Technologists®
doi: 10.1111/j.1750-3841.2009.01072.x
Further reproduction without permission is prohibited

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 *p*-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 Tsimidou 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 *o*-diphenols can be related to hydrogen 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 $>$ oleuropein $>$ tyrosol (Chimi and others 1991). A synergistic effect between oleuropein, 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 oleuropein aglycon, and oleuropein 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 (Antolovich 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 countrysides near Cerignola and Torremaggiore (Apulia, Italy), respectively. In particular, samples of *Cellina di Nardò*, *FS17*, *Grignano*, *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 (K_{232}, K_{270}), 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, Advantec, Toyo Roshi 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 Na_2CO_3 (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

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 the 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 2008).

Quantification 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 $\cdot^+$ radical cation in comparison with that of a standard antioxidant (Trolox).

The β-carotene-linoleate test is based on the thermal (50 °C) autoxidization 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.

	Total phenolic content at production (μg/g gall. ac. eq)	Total phenolic content at 6 mo of storage (μg/g gall. ac. eq)	Total phenolic content at 12 mo of storage (μg/g gall. ac. eq)
***Cellina di Nardò* - C**	205.11 ± 5.25 **E**	165.70 ± 3.82 **E**	46.18 ± 1.85 **C**
***FS17* – C**	140.45 ± 3.96 **B**	114.37 ± 3.15 **A**	36.15 ± 3.54 **B**
***Grignano* – C**	143.59 ± 5,89 **B**	125.53 ± 4.80 **B**	26.53 ± 2.94 **A**
***Moraiolo* – C**	179.88 ± 5.76 **C**	144.15 ± 7.44 **C**	37.72 ± 1.72 **B**
***Nociara* – C**	185.45 ± 8.12 **D**	147.68 ± 4.97 **C**	42.15 ± 3.32 **B C**
***Ogliarola Barese* – C**	133.68 ± 6.16 **A**	123.92 ± 4.77 **B**	30.48 ± 1.36 **A B**
***Peranzana* – C**	190.10 ± 6.75 **D**	159.07 ± 3.28 **D**	59.48 ± 16.63 **D**
***Picholine* – C**	202.30 ± 7.32 **E**	192.01 ± 9.35 **F**	42.26 ± 4.92 **B C**
***Cima di Melfi* – T**	225.09 ± 5.90 **b**	174.77 ± 4.28 **b**	115.65 ± 4.67 **c**
***Coratina* – T**	322.18 ± 9.73 **d**	284.67 ± 10.47 **e**	55.36 ± 3.20 **b**
***Frantoio* – T**	178.34 ± 3.63 **a**	179.67 ± 7.58 **b**	39.11 ± 9.94 **a**
***Leccino* – T**	228.58 ± 6.91 **b**	171.91 ± 4.88 **b**	147.32 ± 4.71 **d**
***Moraiolo* – T**	179.93 ± 7.61 **a**	132.39 ± 8.58 **a**	37.59 ± 3.68 **a**
***Nociara* – T**	256.85 ± 9.28 **c**	205.55 ± 7.42 **c**	62.98 ± 5.01 **b**
***Peranzana* – T**	253.36 ± 9.63 **c**	227.52 ± 8.79 **d**	60.66 ± 8.95 **b**

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.

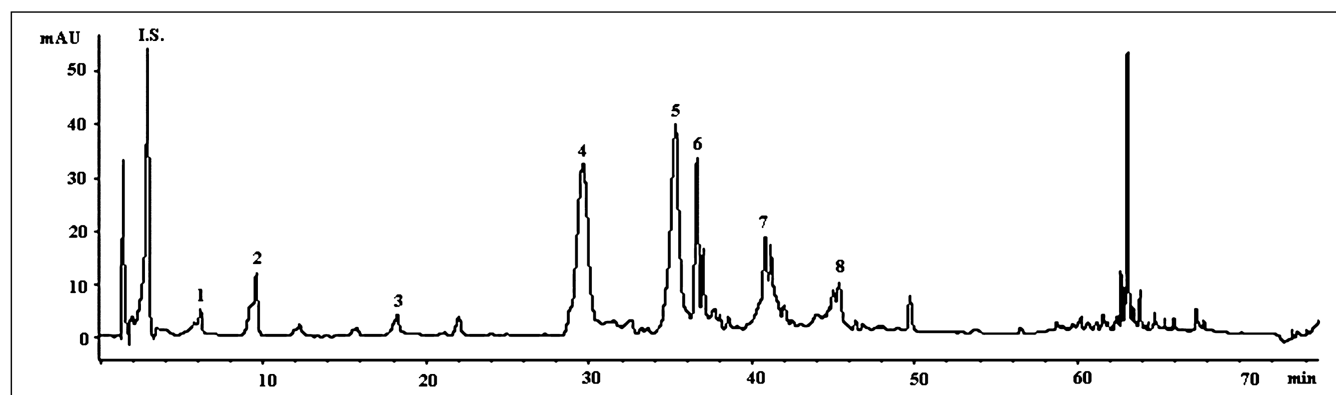

Figure 1 — Phenolic profile of Picholine oils obtained by olives picked in the Cerignola countryside. (A) At production (B) after 6 mo of storage. I.S., gallic acid, (1) hydroxytyrosol, (2) tyrosol, (3) vanillin, (4) 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol), (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).

synthetic β-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 μL of phenolic extracts immediately put into a water bath at 50 °C. The absorbance at 470 nm of samples and of a control containing an aqueous solution of methanol (70%, v/v) was monitored at regular intervals (15 min) on a Varian Cary 50 Scan UV-Visible spectrophotometer (Palo Alto, Calif., U.S.A.) until the complete β-carotene bleaching (after about 2 h). 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 $ABTS^{\bullet+}$ is the oxidizable substrate. Metamyoglobin (MetMb) supplies the ferric ions needed for the formation of hydroxyl radicals (OH·) following the Fenton reaction (Winterbourn 1995). H_2O_2 is the reaction starter. A solution of ABTS (246 μL, 610 μM), saline phosphate buffer (PBS, 524 μL, pH 7.4), and purified MetMb in PBS (410 μL, 6.1 μM) were prepared. Fresh ABTS solutions were prepared each day. Three hundred microliters of hydrogen peroxide were added to generate the $ABTS^{\bullet+}$. Twenty microliters of the phenolic extracts were added to the mixture to evaluate their antioxidant properties. Trolox 2.5 mM (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich, Steinhein, 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 $ABTS^{\bullet+}$, 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

Table 2—Composition of phenolic extracts from Cerignola and Torremaggiore oils at production (μg/g).

	From Cerignola								From Torremaggiore						
	Cellina di Nardò	FS 17	Grignano	Moraiolo	Nociara	Ogliarola barese	Peranz.	Picholine	Cima di Melfi	Coratina	Frantoio	Leccino	Moraiolo	Nociara	Peranzana
Hydroxytyrosol	1.64 ± 0.09	1.26 ± 0.02	1.72 ± 0.03	0.36 ± 0.03	1.85 ± 0.02	2.41 ± 0.09	2.85 ± 0.87	1.42 ± 0.02	0.45 ± 0.09	2.26 ± 0.08	0.54 ± 0.00	0.46 ± 0.01	0.36 ± 0.01	1.03 ± 0.03	1.52 ± 0.00
Tyrosol	1.64 ± 0.06	3.37 ± 0.09	5.74 ± 0.01	3.60 ± 0.02	11.13 ± 0.97	7.35 ± 0.31	2.85 ± 0.25	6.47 ± 0.05	3.60 ± 0.53	9.34 ± 1.35	3.92 ± 0.06	2.51 ± 0.46	3.42 ± 0.04	3.34 ± 0.24	3.80 ± 0.05
Vanillin	2.67 ± 0.10	1.97 ± 0.02	3.45 ± 0.01	0.90 ± 0.00	1.48 ± 0.36	1.60 ± 0.01	1.90 ± 0.36	1.42 ± 0.33	1.58 ± 0.03	1.29 ± 0.07	1.96 ± 0.04	1.60 ± 0.04	1.44 ± 0.14	1.28 ± 0.02	1.27 ± 0.03
3,4-DHPEA-EDA	57.64 ± 0.10	25.42 ± 3.25	21.11 ± 0.47	57.92 ± 3.12	42.84 ± 1.96	35.83 ± 2.38	46.76 ± 0.25	34.39 ± 1.39	54.47 ± 0.69	69.91 ± 2.33	34.60 ± 0.28	76.80 ± 1.35	24.83 ± 2.08	60.36 ± 2.01	75.75 ± 2.12
p-HPEA-EDA	33.02 ± 0.16	19.24 ± 2.31	20.10 ± 0.00	46.23 ± 4.86	47.29 ± 2.78	24.60 ± 1.48	25.66 ± 0.92	32.97 ± 1.34	51.32 ± 0.85	61.86 ± 2.17	24.43 ± 0.78	44.80 ± 0.13	26.63 ± 3.31	62.93 ± 0.42	40.79 ± 2.01
Pinoresinol	9.85 ± 0.02	7.44 ± 0.32	7.61 ± 0.75	23.20 ± 2.39	11.68 ± 0.31	17.65 ± 0.95	5.13 ± 0.00	9.51 ± 0.23	32.41 ± 0.28	12.89 ± 0.19	25.32 ± 0.46	8.91 ± 0.32	33.47 ± 3.46	27.74 ± 0.36	7.85 ± 0.33
3,4-DHPEA-EA	7.79 ± 0.68	6.18 ± 0.52	5.60 ± 0.65	18.17 ± 1.72	10.20 ± 0.66	15.11 ± 0.91	9.70 ± 0.11	8.09 ± 0.38	11.93 ± 0.45	7.09 ± 0.07	14.98 ± 0.57	13.71 ± 1.97	14.57 ± 1.31	13.87 ± 1.20	8.61 ± 0.00
p-HPEA-EA	3.28 ± 1.52	0.70 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	1.30 ± 0.78	0.00 ± 0.00	1.90 ± 0.02	3.03 ± 0.04	0.00 ± 0.00	4.19 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

(*Peranzana*) to 0.65 ± 0.04 g/100 g (*Grignano*) whereas those produced around Torremaggiore showed lower values included within 0.30 ± 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 ± 0.2.0 meqO_2/kg of *Ogliarola barese* whereas, oils from Torremaggiore were included in the range 4.8 ± 0.1 (*Coratina*) to 12.3 ± 0.1.3 meqO_2/kg (*Moraiolo*).

Among oils from Cerignola, K_{232} 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, K_{270} 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.

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-EA, it was absent from many samples with the exception of *Cellina di Nardò*, *FS17*, *Nociara*, *Peranzana*, *Picholine* from Cerignola and *Coratina* 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.

During oils storage (data not shown), a decrease in vanillin, 3,4-DHPEA-EDA and p-HPEA-EDA was detected whereas, during the first 6 mo of storage, an increase of tyrosol and hydroxytyrosol was measured as a consequence of degradation of ligstroside and oleuropein.

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 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.

	At production		After 6 mo of storage		After 12 mo
	AAC	% Antioxidant activity oils	AAC at 6 mo of storage	% Antioxidant activity oils at 6 mo of storage	of storage AAC
***Cima di Melfi* – T**	79.33 ± 1.06 **a b**	54.23 ± 2.98 **b**	53.52 ± 7.97 **b**	69.58 ± 8.10 **c**	44.86 ± 6.86 **d**
***Coratina* - T**	87.56 ± 1.62 **c**	65.07 ± 1.23 **d**	65.32 ± 5.85 **b**	94.48 ± 0.74 **d**	30.52 ± 1.18 **c**
***Frantoio* - T**	78.20 ± 3.28 **a b**	40.85 ± 1.30 **a**	50.83 ± 7.94 **b**	58.54 ± 5.98 **b**	22.19 ± 0.63 **b**
***Leccino* - T**	73.95 ± 2.63 **a**	51.16 ± 5.16 **b**	56.58 ± 9.82 **b**	56.82 ± 1.81 **b**	42.62 ± 3.03 **d**
***Moraiolo* - T**	80.45 ± 3.48 **b**	38.49 ± 3.10 **a**	49.19 ± 7.28 **a b**	55.21 ± 2.81 **b**	20.01 ± 3.59 **a**
***Nociara* - T**	76.96 ± 3.26 **a b**	51.80 ± 3.08 **b**	54.62 ± 7.43 **b**	11.56 ± 1.54 **a**	24.10 ± 2.62 **a b**
***Peranzana* - T**	80.12 ± 3.88 **a b**	59.46 ± 2.44 **c**	54.87 ± 5.42 **b**	76.20 ± 5.64 **c**	27.62 ± 2.23 **b c**
***Cellina di Nardò* - C**	74.12 ± 1.28 **A**	56.94 ± 1.99 **E**	74.59 ± 11.93 **B**	58.44 ± 1.88 **D**	19.46 ± 1.33 **A B**
***FS17* – C**	70.67 ± 4.02 **A**	33.22 ± 2.51 **B**	53.14 ± 8.48 **A**	42.14 ± 1.73 **A**	16.25 ± 1.10 **A**
***Grignano* - C**	73.13 ± 2.14 **A**	34.19 ± 1.29 **B**	49.75 ± 4.90 **A**	42.29 ± 3.32 **A**	18.06 ± 1.87 **A B**
***Moraiolo* - C**	74.11 ± 2.06 **A**	40.41 ± 2.06 **C**	57.25 ± 3.46 **A B**	50.10 ± 1.93 **B**	21.70 ± 1.04 **A B**
***Nociara* - C**	70.00 ± 2.23 **A**	39.78 ± 2.92 **C**	53.46 ± 9.48 **A**	50.83 ± 1.48 **B C**	19.15 ± 1.35 **A B**
***Ogliarola Barese* - C**	72.20 ± 3.58 **A**	29.23 ± 2.77 **A**	55.80 ± 12.23 **A B**	42.03 ± 1.89 **A**	16.13 ± 1.70 **A**
***Peranzana* - C**	72.57 ± 1.17 **A**	47.03 ± 3.34 **D**	70.11 ± 11.87 **B**	54.32 ± 1.40 **C**	24.72 ± 5.27 **B**
***Picholine* - C**	87.82 ± 1.37 **B**	44.24 ± 4.17 **D**	57.78 ± 8.54 **A B**	54.69 ± 2.89 **C D**	19.73 ± 4.56 **A B**

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.

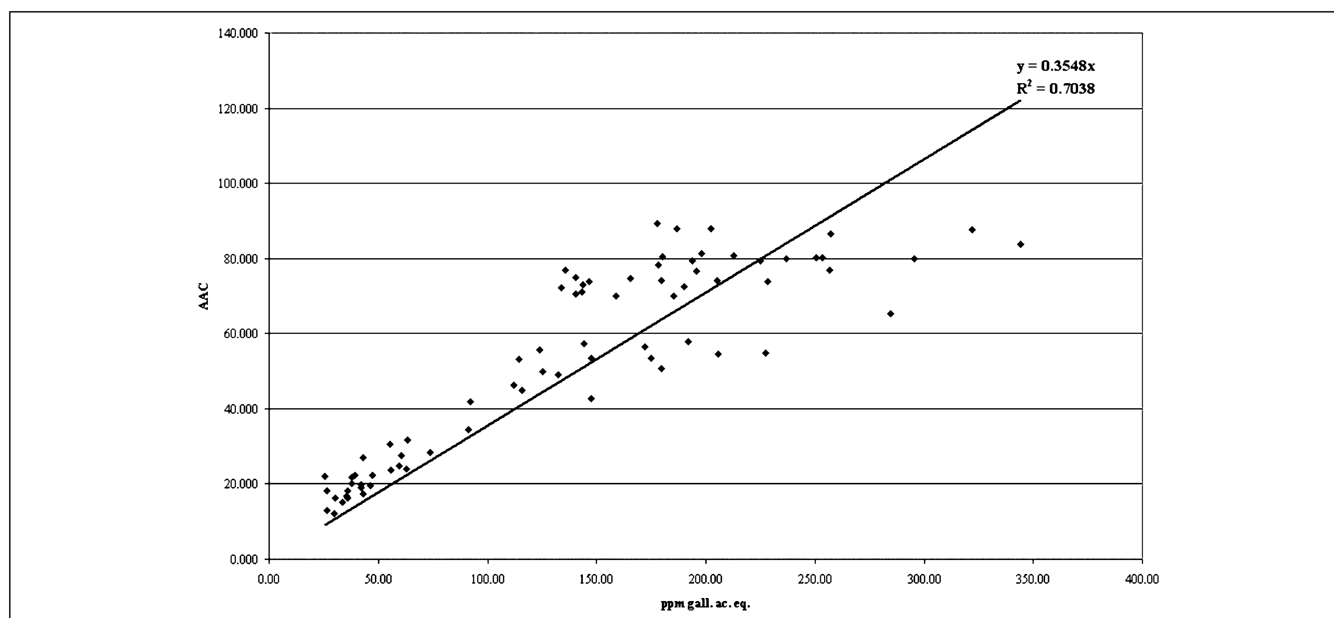

Figure 2—Linear correlation among phenolic content and antioxidant activity of oils measured according to the β-carotene test.

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* > *Frantoio*, *Leccino*, *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

Aguilera CM, Ramirez-Tortosa MC, Mesa MD, Ramirez-Tortosa CL, Gil A. 2002. Sunflower, virgin-olive and fish oils differentially affect the progression of aortic lesions in rabbits with experimental atherosclerosis. Atheroscl 162:335–44.

Angerosa F, d'Alessandro N, Konstantinou P, Di Giacinto L. 1995. GC–MS evaluation of phenolic compounds in virgin olive oil. J Agric Food Chem 43:1802–7.

Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. 2002. Methods for testing antioxidant activity. Analyst 127:183–98.

Aparicio R, Roda L, Albi MA, Gutierrez F. 1999. Effect of various compounds on virgin olive oil stability measured by Rancimat. J Agric Food Chem 47:4150–5.

Armaforte E, Mancebo-Campos V, Bendini A, Desamparados Salvador M, Fregapane G, Cerretani L. 2007. Retention effects of oxidized polyphenols during analytical extraction of phenolic compounds of virgin olive oil. J Separation Sci 30:2401–6.

Aruoma O. 1996. Assessment of potential pro-oxidant and antioxidant actions. J Am Oil Chem Soc 73:1617–25.

Baldioli M, Servili M, Perretti G, Montedoro GF. 1996. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. J Am Oil Chem Soc 73:1589–93.

Bendini A, Cerretani L, Carrasco-Pancorbo A, Gómez-Caravaca AM, Segua-Carretero A, Fernández-Gutiérrez A, Lercker G. 2007. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules 12:1679–719.

Boskou D. 1996. Olive oil composition. In: Boskou D, editor. Olive oil: chemistry and technology. Champaign, Ill.: AOCS Press. p 52–83.

Brenes M, Garcia A, Garcia P, Rios JJ, Garrido A. 1999. Phenolic compounds in Spanish olive oils. J Agric Food Chem 47:3535–40.

Brenes M, Garcia A, Garcia P, Garrido A. 2000. Rapid and complete extraction of phenols from olive oil and determination by means of a coulometric electrode array system. J Agric Food Chem 48:5178–83.
Cao G, Sofic E, Prior RL. 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radical Biol Med 22:749–60.
Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Del Carlo M, Gallina-Toschi T, Lercker G, Compagnone D, Fernández-Gutiérrez A. 2005. Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. J Agric Food Chem 53:8918–25.
Carrasco-Pancorbo A, Neusüß C, Pelzing M, Segura-Carretero A, Fernández-Gutiérrez A. 2007. CE- and HPLC-TOF-MS for the characterization of phenolic compounds in olive oil. Electrophoresis 28:806–21.
Chimi H, Cillard J, Cillard P, Rahmani M. 1991. Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. J Am Oil Chem Soc 68:307–12.
Covas MI, Fito M, Marrugat J, Miro E, Farre M, de la Torre R, Gimeno E, Lopez-Sabater MC, Lamuela-Raventos R, de la Torre-Boronat MC. 2001. Coronary heart disease protective factors: antioxidant effect of olive oil. Therapie 56:607–11.
Di Stefano R, Cravero MC, Genilizzi N. 1989. Metodi per lo studio dei polifenoli nei vini. L'Enotecnico 5:83–9.
EC Regulation 1513. 2001. Off J Eur Com, L 201, 26th Jul 2003.
EU Regulation 1989. 2003. Off J Eur Com, L 295, 13th Nov 2003.
Fito M, Covas MI, Lamuela-Raventos RM, Vila J, Torrents J, de la Torre C, Marrugat J. 2000. Protective effect of olive oil and its phenolic compounds against low density lipoprotein oxidation. Lipids 35:633–8.
Gambacorta G, Previtali MA, Pati S, Baiano A, La Notte E. 2006. Characterization of the phenolic profiles of some monovarietal extravirgin olive oils of Southern Italy. XXIII Intl. Conference on Polyphenols. Winnipeg, Manitoba, Canada 22–25 Aug 2006.
Gómez-Alonso S, Salvador MD, Fregatane G. 2002. Phenolic compounds profile of Cornicabra virgin olive oil. J Agric Food Chem 50:6812–7.
Gómez-Alonso S, Mancebo-Campos V, Desamparados Salvador M, Fregatane G. 2007. Evolution of major and minor componente and oxidation indices of virgin olive oil during 21 months storage at room temperature. Food Chem 100:36–42.
Gómez-Caravaca AM, Cerretani L, Bendini A, Segua-Carretero A, Fernández-Gutiérrez A, Del Carlo M, Compagnone D, Cichelli A. 2008. Effect of fly attack (Bactocera oleae) on phenolic profile and selected chemical parameters of olive oil. J Agric Food Chem 56:4577–83.
Gómez-Rico A, Fregapane G, Desamparados Salvador M. 2008. Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. Food Res Int 41:433–40.
Gordon MH, Paiva-Martins F, Almeida M. 2001. Antioxidant activity of hydroxytyrosol acetate compared with other olive oil polyphenols. J Agric Food Chem 49:2480–5.
Gorinstein S, Martin-Belloso O, Katrich E, Lojek A, Číž M, Gligelmo-Miguel N, Haruenkit R, Park Y-S, Jung S-T, Trakhtenberg S. 2003. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. J Nutr Biochem 14:154–9.
Gutfinger T. 1981. Phenols in olive oil. J Am Oil Chem Soc 68:966–8.
Gutierrez F, Arnaud T, Garrido A. 2001. Contribution of polyphenols to the oxidative stability of virgin olive oil. J Sci Food Agric 81:1463–70.
Katalinic V, Milos M, Jukic M. 2006. Screening of 70 medicinal plants extracts for antioxidant capacity and total phenols. Food Chem 94:550–7.
Lavelli V, Fregapane G, Desamparados SM. 2006. Effect of storage on secoiridoid and tocopherol contents and antioxidant activity of monovarietal extra virgin olive oils. J Agri Food Chem 54:3002–7.
Lee M, Yang ZY, Li H, Chen L, Sun Y, Gobbo S, Balentine DA, Yang CS. 1995. Analysis f human plasma and urinary tea poliphenols in human subjects. Cancer Epid Biomarkers Prev 4:333–93.
Mannino S, Cosio MS, Bertuccioli M. 1993. High performance liquid chromatography of phenolic compounds in virgin olive oils using amperometric detection. Ital J Food Sci 4:363–70.
Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci 84:407–12.
Montedoro G, Servili M, Baldioli M. 1992a. Simple and hydrolyzable phenolic compounds in virgin olive oil. I Their extraction, separation, and quantitative and semi-quantitative evaluation by HPLC. J Agric Food Chem 40:1571–6.
Montedoro G, Servili M, Baldioli M, Selvaggini R, Miniati E, Maccioni A. 1992b. Simple and hydrolyzable compounds in virgin olive oil. II. Initial characterization of hydrolyzable fraction. J Agric Food Chem 40:1577–80.
Montedoro G, Servili M, Baldioli M, Selvaggini R, Miniati E, Maccioni A. 1993. Simple and hydrolizable compounds in virgin olive oil. III. Spectroscopic characterizations of the secoiridoids derivatives. J Agric Food Chem 41:2228–34.
Morello JR, Motiva MJ, Tovar MJ, Romero MP. 2004. Changes in commercial virgin olive oil (cv. Arbequina) durino storage, with special emphasis on the phenolic fraction. Food Chem 85:357–64.
Nissiotis M, Tasioula-Margari M. 2002. Changes in antioxidant concentration of virgin olive oil during thermal oxidation. Food Chem 77:371–6.
Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, Bartsch B. 2000. Olive oil consumption and health: the possible role of antioxidants. Lancet Oncol 1:107–12.
Papadopoulos G, Boskou D. 1991. Antioxidant effect of natural phenols on olive oil. J Am Oil Chem Soc 68:669–71.
Pellegrini N, Visioli F, Buratti S, Brighenti F. 2001. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. J Agric Food Chem 49:2532–8.
Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure antioxidant activity relationship of flavonoids and phenolic acids. Free Rad Biol Med 20:933–56.
Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Ba TH, Lambelet P, McPhail D, Skibsted LH, Tijburg L. 2001. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Eur Food Res Technol 212:319–28.
Teissedre PL, Frankel EN, Waterhouse AL, Peleg H, German JB. 1996. Inhibition of *In Vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. J Sci Food Agric 70:55–61.
Tsimidou M, Papadopoulos G, Boskou D. 1992. Phenolic compounds and stability of virgin olive oil—Part 1. Food Chem 45:141–4.
Warhrburg U, Kratz M, Cullen P. 2002. Mediterranean diet, olive oil and health. Eur J Lipid Sci Technol 104:675–98.
Winterbourn CC. 1995. Toxicity of iron and hydrogen peroxide: the Fenton reaction. Toxicol Lett 82/83:969–74.
Wong CC, Li HB, Cheng KW, Chen F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using ferric reducing antioxidant power assay. Food Chem 97:705–11.