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Antioxidant Activity of Olive Pulp and Olive Oil Phenolic Compounds of the Arbequina Cultivar

JOSÉ-RAMÓN MORELLÓ,† SATU VUORELA,‡ MARIA-PAZ ROMERO,† MARIA-JOSÉ MOTILVA,*,† AND MARINA HEINONEN‡

Food Technology Department, CeRTA-TPV, Escuela Técnica Superior de Ingeniería Agraria, Universidad de Lleida, Av/Alcalde Rovira Roure 191, 25198 Lleida, Spain, and Department of Applied Chemistry and Microbiology, Division of Food Chemistry, University of Helsinki, P.O. Box 27, 00014 University of Helsinki, Finland

The aim of this study was to characterize antioxidant activities of phenolic compounds that appear in olive pulp and olive oils using both radical scavenging and antioxidant activity tests. Antiradical and antioxidant activities of olive pulp and olive oil phenolic compounds were due mainly to the presence of a 3,4-dihydroxy moiety linked to an aromatic ring, and the effect depended on the polarity of the phenolic compound. Glucosides and more complex phenolics exhibited higher antioxidant activities toward oxidation of liposomes, whereas in bulk lipids aglycons were more potent antioxidants with the exception of oleuropein. Lignans acted as antioxidants only in liposomes, which could partly be due to their chelating activity, because liposome oxidation was initiated by cupric acetate. The antioxidant activity of virgin olive oil is principally due to the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), a secoiridoid derivative (peak RT 36, structure unidentified), and luteolin.

KEYWORDS: Olive oil; phenolic compounds; secoiridoid derivatives; antioxidant activity

INTRODUCTION

The traditional Mediterranean diet is characterized by an abundance of plant foods (fruits, vegetables, cereals, legumes) in which olive oil is the principal source of fat. Olive oil may have a role in the prevention of coronary heart disease and certain cancers because of its high levels of monosaturated fatty acids and phenolic compounds (*1*). Olives (*Olea europaea* L.) and virgin olive oils provide a rich source of natural antioxidants. These include carotenoids, tocopherols, and phenolic compounds, which may act, by different mechanisms, as an effective defense against reactive species. The content of phenolic compounds is an important parameter in the evaluation of virgin olive oil quality because phenols largely contribute to oil flavor and aroma and protect it from oxidation (*2*, *3*).

Phenolic compounds of the Oleaceae family can be characterized by the presence of a number of coumarin-like compounds known as secoiridoids. These compounds are related to the iridoids, which are produced via secondary metabolism of monoterpenes as precursors of various indole alkaloids. In olive, oleuropein, demethyloleuropein, ligstroside, and oleoside represent the predominant phenolic oleosides (*4*), whereas verbascoside (*5*) is the main hydroxycinnamic derivative of olive fruit (*6*). Oleuropein is generally the most prominent phenolic compound in olive cultivars and may reach concentrations of up to 140 mg g^{-1} on a dry matter basis in young olives (*7*).

During olive oil extraction various secoiridoid derivatives are formed, and they have been identified both in olive oil and in vegetation waters (*7*, *8*). The apparent reduction in glycosidic and flavonoid compounds in olive oil compared to olive pulp maybe attributed to glycosidic modification or degradation as a result of olive oil extraction, which may arise due to the addition of water to the olive paste. The amount of phenolic compounds in olive oil varies from 150 to 700 mg/kg, depending on several factors such as cultivar, degree of maturation, possible infestation by the olive fly *Bactrocera oleae*, climate, and production steps (*9*). In olive oil the phenolic content serves as an important qualitative parameter due to its correlation with peroxide number, acidity, and sensorial quality (*10*, *11*). Virgin olive oil contains a large number of phenolic compounds including phenyl alcohols, namely, 3,4-dihydroxyphenylethanol (3,4-DHPEA, or hydroxytyrosol) and *p*-4-hydroxyphenylethanol (*p*-HPEA, or tyrosol) as well as phenyl acids. Derivatives 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 (*8*, *12*, *13*).

Due to their chemical properties, the phenolic compounds inhibit lipid oxidation (*14*) and exhibit physiological activities

* Corresponding author (telephone + 34 973 702817; fax + 34 973 702596; e-mail motilva@tecal.udl.es).
† Universidad de Lleida.
‡ University of Helsinki.

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(*15*, *16*). Total hydrophilic phenolic compounds and the oleosidic forms of 3,4-DHPEA were correlated ($r = 0.97$) with the oxidative stability of virgin olive oil (*12*, *17*). When tested in oil, 3,4-DHPEA and its derivatives have shown much stronger antioxidant activities than that of α-tocopherol (*12*, *17*). The antioxidant capacities of oleuropein and its aglycons and minor phenols have been studied using different methods such as the DPPH test (*17*), the ABTS test and soybean liposome system (*18*), the *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) method, and copper-chelating capacity (*19*), but very few studies have been made on the antioxidant activities of the phenols present in olive pulp and olive oil using the radical scavenging activity test with 1,1-diphenyl-2-picrylhydrazyl (DPPH), liposome oxidation model, and methyl linoleate oxidation.

The aim of this study was to evaluate antioxidant activities of phenolic compounds present in olive pulp and virgin olive oil from the Arbequina cultivar. Scavenging of free radicals was measured using the DPPH test, and the antioxidant activity was measured during oxidation of both liposomes (polar lipid model) and methyl linoleate (nonpolar lipid model).

MATERIALS AND METHODS

Chemicals. Apigenin, apigenin-7-glucoside, luteolin, luteolin-7-*O*-glucoside, oleuropein, rutin, tyrosol, vanillin, and verbascoside were purchased from Extrasynthése (Genay, France); *p*-coumaric and vanillic acid were obtained from Fluka Co. (Buchs, Switzerland), and copper (II) acetate was obtained from Merck (Darmstadt, Germany). DPPH, pyrogallol, and l-α-phosphatidylcholine (lecithin from soybean) with a phosphatidylcholine (PC) content of 40% were obtained from Sigma Chemical Co. (St. Louis, MO). Methanol (HPLC grade) was from Rathburn Chemicals Ltd. (Walkerburn, Scotland), and water was of Milli-Q quality (Millipore Corp., Bedford, MA).

Oil and Phenolic Compounds Extraction. Olive drupes (*Olea europaea* L.) were picked in olive groves of the Arbequina cultivar located in the area of Les Garrigues (Catalonia, Spain), put into 3 kg boxes, and taken directly to the pilot plant, where they were processed. An Abencor analyzer (MC2 Ingenierias y Sistemas, Sevilla, Spain) was used to process the olives in the pilot extraction plant. The unit consisted of three essential elements: the mill, the thermobeater, and the pulp centrifuge. After being processed in the mill, the oil was separated by decanting, transferred into dark glass bottles, and stored in the dark at 4 °C.

Olive fruit phenolic compounds were extracted as described by Tovar et al. (*20*). Approximately 1 g of ground olive pulp from 20 olives was mixed in duplicate with 40 mL of hexane and agitated for 4 min; the upper phase was recovered, and the extraction was repeated twice successively with the lower phase to allow removal of pigments and most of the lipids. Phenolic compounds were extracted with 80 mL of 80% (v/v) methanol containing 400 mg/L sodium metabisulfite. The mixture was homogenized for 30 s using a Polytron homogenizer to separate the hydromethanolic phase. This procedure was repeated twice. The hydromethanolic phases were combined and filtered.

Phenolic compounds were extracted from virgin olive oil following the procedure reported in Romero et al. (*21*); 2 × 20 mL of methanol/water (80:20 v/v) was added to 45 g of virgin olive oil and homogenized for 2 min with a Polytron. The two phases were separated by centrifugation at 3000 rpm for 10 min. Aqueous alcoholic extracts were then combined and concentrated in a vacuum at <35 °C until a syrupy consistency was reached. Five milliliters of acetonitrile was added to the extract, and it was washed with 3 × 20 mL of hexane. The nonpolar phases were also purified with 5 mL of acetonitrile. Finally, an aliquot of 2 mL was evaporated under a stream of nitrogen.

Analysis and Fraction Collection of Phenolic Compounds by HPLC and ESI-MS. The phenolic fractions (olive pulp and olive oil) extracted were dissolved in 1 mL of methanol and analyzed by HPLC according to the method of Morelló et al. (*22*). Chromatograms were obtained at 278 and 339 nm.

Phenolic isolates were obtained from olive oil using a semipreparative HPLC column Spherisorb ODS-2 (5 μm, 25 cm × 10 mm i.d., Technokroma, Barcelona, Spain) and a flow rate of 4 mL/min (*23*).

Fractions were collected peak by peak. The phenolic fraction was removed by rotatory evaporation at reduced pressure, lyophilized, and stored at −18 °C under N_2 atmosphere until being analyzed. Phenolic isolates were analyzed using a ZMD mass spectrometer (Waters Inc.) equipped with an electrospray ionization ion source (ESI). The ion spray mass spectra in the negative-ion mode were obtained under the following conditions: capillary voltage, 2.5 kV; cone voltage, 10 V; desolvation temperature, 400 °C; and source temperature, 120 °C.

Scheme 1 shows the chemical structures of the phenolic compounds studied. 3,4-DHPEA-EDA, *p*-HPEA-EDA, lignans, *p*-HPEA-EA, 3,4-DHPEA-EA, secoiridoid derivatives peaks RT 36, RT 38, and RT 39, and peak RT 42 were isolated from olive oil, whereas oleuropein, rutin, verbascoside, apigenin, apigenin-7-glucoside, luteolin, and luteolin-7-*O*-glucoside were commercial standards.

Determination of the Radical Scavenging Activity (RSA). RSA was tested by measuring the decrease in the absorption at 517 nm of DPPH solution after the addition of the antioxidant solution. In a cuvette, 2950 μL of 0.1 mM methanolic DPPH solution was mixed with 50 μL of a 1 mg/mL antioxidant solution, and the absorption was monitored at intervals of 15 s for 5 min. The resulting difference is expressed as the percentage (percentual reduction) of radicals scavenged after 4 min. Pyrogallol was used as positive control to test DPPH solution.

Antioxidant Activity in Liposome Model. Lecithin-liposomes were prepared as previously described by Huang et al. (*24*) using a sonicator (Ikasonic U50 Control, Staufen, Germany). For the assay methanolic solutions of standards and olive oil phenolics were added into screw-capped 100 mL Erlenmeyer flasks to reach final concentrations of 10 and 25 μM for pure compounds or 4.2 and 8.3 μg/mL for phenolic isolates. Methanol was evaporated by flushing with nitrogen. Liposome samples were then weighed into the flasks and diluted with Milli-Q water to a final lecithin concentration of 0.8% (by weight). The samples were oxidized by adding cupric acetate (3 μM) and shaking at 37 °C in the dark. Aliquot samples were taken for both hexanal (500 μL) and conjugated diene (100 μL) analyses after 0 and 72 h. Hexanal was measured by static headspace gas chromatography (GC) as described previously by Frankel et al. (*25*). Conjugated dienes were measured by UV spectrophotometry at 234 nm (Perkin-Elmer, Lambda 11/Bio UV−vis spectrophotometer, Überlingen, Germany). The antioxidant activity of phenolic compounds was calculated as a percent of inhibition of conjugated diene and hexanal production and expressed as $(C - S)/C) \times 100$, where C is the amount of hexanal or conjugated diene hydroperoxides formed in the control sample and S is the amount of hexanal or conjugated dienes formed in the sample containing phenolic compound. The antioxidant activity is expressed as percentual inhibition of hexanal and hydroperoxides after 72 h of incubation (*26*).

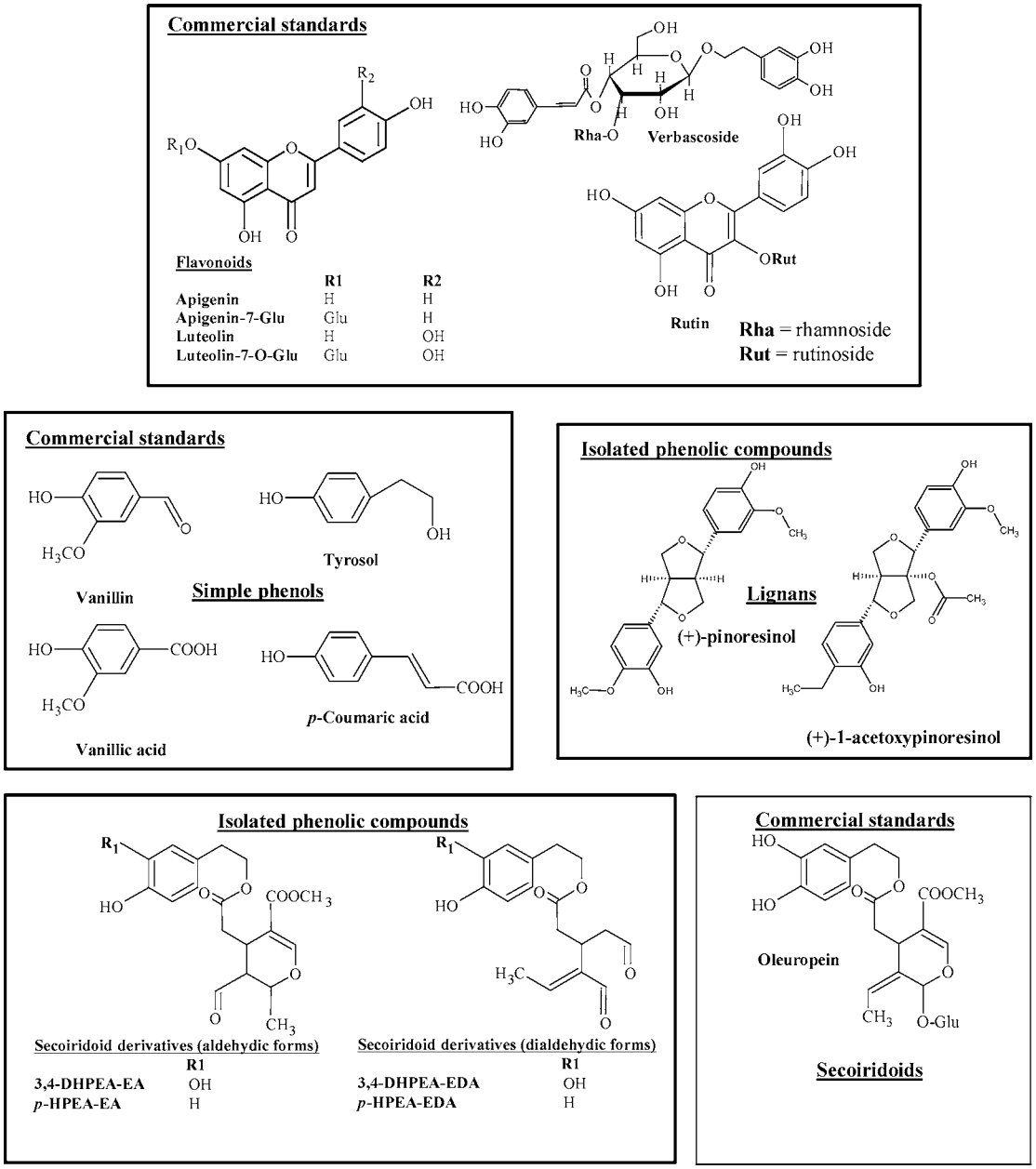
Antioxidant Activity in Methyl Linoleate (MeLo) Model. MeLo samples (0.2 g) were oxidized in the presence of antioxidants at levels of 10 and 25 μM for standards and 4.2 and 8.3 μg/mL for phenolic fractions. MeLo without antioxidant addition served as a control. Antioxidants were added into MeLo as freshly prepared ethanol solutions (50−200 μL), and the solvent was purged by nitrogen flow. Oxidation of MeLo was carried out in the dark at 40 °C for 72 h of incubation. Conjugated diene (CD) analysis was used to follow the degree of oxidation by dissolving 10 mg of sample into isooctane for spectrophotometric measurement.

Statistical Analysis. Each oxidation experiment and DPPH test was performed in duplicate. The data were subjected to an analysis of variance using the SAS version 8.02 (SAS Institute Inc., Cary, NC). Separation of the means was obtained using the least-squares means test, where the significant difference was defined at $p \leq 0.05$.

RESULTS

HPLC profiles of olive pulp and olive oil from the Arbequina cultivar are shown in **Figure 1**. The phenolic compounds appearing in higher amounts in olive pulp (**Figure 1a**) were oleuropein followed by verbascoside and flavonoids such as

Scheme 1. Structures of Phenolic Compounds Used in the Study[a]



[a] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon.

rutin, luteolin-7-*O*-glucoside, and apigenin-7-glucoside. In virgin olive oil the most abundant phenolic compounds (**Figure 1b**) were secoiridoid derivatives such as 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to hydroxytyrosol), 3,4-DHPEA-EA (aldehydic form of elenolic acid linked to hydroxytyrosol), and *p*-HPEA-EDA (dialdehydic form of elenolic acid linked to tyrosol) followed by lignans, simple phenols such as tyrosol, vanillic acid, vanillin, and *p*-coumaric acid and flavonoid aglycons such as apigenin and luteolin. The chemical structures of the unidentified peaks with retention times of 36, 38, and 39 min (peaks RT 36, RT 38, and RT 39) correspond to secoiridoid derivatives, as they showed two absorption maxima at 210 and 278 nm. Peak RT 42 showed a UV spectrum similar to that of *trans*-cinnamic acid with a maximum at 276 nm. Peak RT 36 was a pure compound that showed a mass spectrum of *m*/*z* 433, whereas peaks 38, 39, and 42 were not homogeneous peaks.

Radical Scavenging Activity. Scavenging activities of phenolic compounds present in olive and olive oil are shown in **Figure 2**. In virgin olive oil high radical scavenging activities were exhibited by the secoiridoid derivative, peak RT 36 (92.8 $\pm$ 1), and luteolin (88.3 $\pm$ 1), followed by a moderate effect of 3,4-DHPEA-EDA (65.1 $\pm$ 8). Verbascoside (85.3 $\pm$ 1) and luteolin-7-*O*-glucoside (81.9 $\pm$ 1) were the most potent radical scavenging phenolic compounds in olive pulp. Differences were found in the radical scavenging activities among flavones (apigenin and luteolin) with a glucose moiety linked at position 7 in the A ring. The monoglucoside of apigenin was as inactive as its aglycon, whereas the radical scavenging activity of luteolin-7-glucoside was slightly lower compared to that of its aglycon. Less than moderate antiradical scavenging activities (below 50% RSA) were observed with rutin and oleuropein, both found in olive pulp, as well as with *p*-coumaric acid and 3,4-DHPEA-EA present in virgin olive oil. Among the phenolics

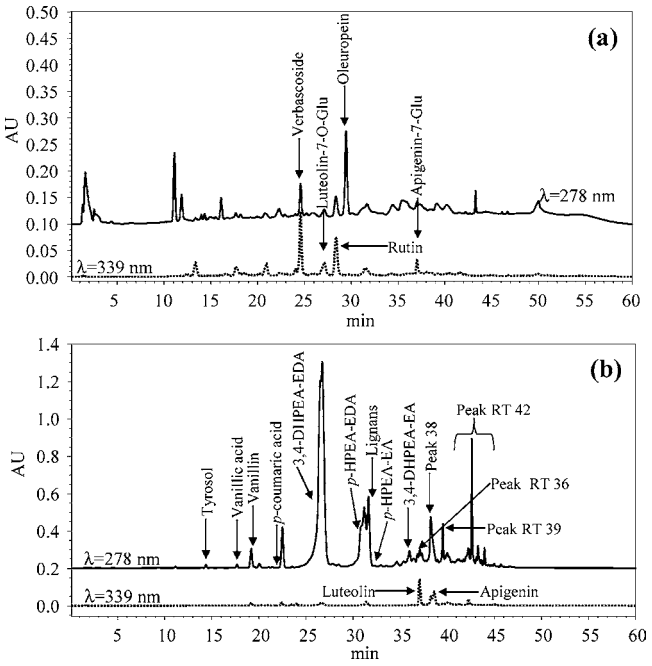


Figure 1. HPLC chromatogram (at 278 and 339 nm) of phenolic profile of Arbequina olive pulp (**a**) and Arbequina olive oil (**b**). 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon. Peaks RT 36, RT 38, and RT 39 are unknown secoiridoid derivatives. Peak RT 42 is an unknown *trans*-cinnamic acid derivative.

isolated from olive oil, lignans (acetoxypinoresinol and pinoresinol), tyrosol, vanillic acid, vanillin, *p*-HPEA-EDA, *p*-HPEA-EA, peak RT 42 and two secoiridoid derivatives, that is, peaks RT 38 and RT 39, exhibited low antiradical activity (<20% RSA).

Liposome Oxidation. At lower concentrations of 10 μM (pure compounds) and 4.2 μg/mL (phenolic isolates), the inhibition of formation of conjugated diene hydroperoxides of olive oil and pulp phenolics decreased in the following order: rutin > luteolin-7-*O*-glucoside > verbascoside = oleuropein > luteolin = lignans (**Table 1**). At higher concentrations of 25 μM (pure compounds) and 8.3 μg/mL (phenolic isolates), the inhibition of the conjugated diene hydroperoxides formation decreased as follows: rutin > lignans > verbascoside = luteolin > oleuropein = luteolin-7-*O*-glucoside.

Luteolin-7-*O*-glucoside, luteolin, and rutin followed by oleuropein, lignans, and verbascoside were the most effective phenolic antioxidants, inhibiting the formation of hexanal during liposome oxidation. The antioxidant effect of olive oil and pulp phenolics was generally higher toward the formation of hexanal than the effect toward conjugated diene hydroperoxide formation. A dose-dependent antioxidant effect was observed with most olive phenolics.

Methyl Linoleate Model. Inhibition of the formation of conjugated diene hydroperoxides decreased in the following order: 3,4-DHPEA-EDA = oleuropein = luteolin > peak RT 36 at both phenolic concentrations. Among olive pulp phenolics the only compounds showing an inhibitory effect toward the formation of conjugated diene hydroperoxides were oleuropein and verbascoside, whereas no antioxidant activity was observed with flavonoids. The effective antioxidants in olive oil toward oxidation of bulk lipids were oleuropein derivatives (3,4-DHPEA-EDA and peak RT 36). Luteolin at concentrations of 10 and 25 μM had an inhibitory effect toward the formation of conjugated diene hydroperoxides (80.8 and 95.0%, respectively), whereas its glucosidic form (luteolin-7-*O*-glucoside) did not act as an antioxidant.

Correlation between Tests. Phenolic compounds in olive showed a statistically significant correlation between the radical scavenging activity test and the inhibition of oxidation of both liposomes and bulk lipids (MeLo). When RSA results were compared to the inhibition of oxidation in MeLo, the correlation was moderate ($r = 0.67$, lower concentration; $r = 0.63$, higher concentration). When the radical scavenging activity results were compared to the antioxidant activities of phenolic compounds in liposome model test, the correlation depended on the type of oxidation products measured. A higher correlation was found between the inhibition of hexanal formation on the liposome model and radical scavenging activity ($r = 0.63$, lower concentration; $r = 0.58$ higher concentration). When radical scavenging activity and inhibition of formation of conjugated diene hydroperoxides in liposomes were compared, correlations of $r = 0.56$ (lower concentration) and $r = 0.52$ (higher concentration) were found.

Phenolic compounds in olive oil showed a significantly higher correlation between the radical scavenging activity and the antioxidant activity toward the oxidation of MeLo (percent inhibition of conjugated diene hydroperoxides) at both lower ($r = 0.79$) and higher ($r = 0.85$) concentrations. With olive pulp, the radical scavenging activities correlated very well with the inhibition of liposome oxidation (CD or hexanal measurement) at the concentration level of 10 μM ($r = 0.91$, lower concentration; $r = 0.88$, higher concentration).

DISCUSSION

Oleuropein obtained in olive pulp and secoiridoid derivatives, 3,4-DHPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA-EDA, and *p*-HPEA-EA, extracted from olive oil are ubiquitous to the Oleacea family (*27*). In this study, 3,4-DHPEA-EDA (280−1278 ppm), an unknown secoiridoid derivative (peak RT 36) (52−195 ppm), and luteolin (0−4 ppm) were found to be the most active virgin olive oil antioxidants, whereas verbascoside and luteolin-7-glucoside were the most effective antioxidants in olive pulp. Higher radical scavenging activities were found with phenolic compounds that bear two hydroxyl groups linked to an aromatic ring on the ortho position (3,4-DHPEA-EDA, oleuropein, and peak RT 36), whereas with ligstroside derivatives such as *p*-HPEA-EDA and *p*-HPEA-EA bearing only one hydroxyl substituent the antiradical activity is close to zero.

The secoiridoid derivatives differ from oleuropein by not having a glucose linked to elenolic acid. The glucose moiety in oleuropein increases antioxidant activity in the MeLo model, conferring a lower solubility of hydrophilic oleuropein compared to the secoiridoid derivatives. This is in accordance with the phenomenon of polar paradox first reported by Porter et al. (*28*), that is, hydrophilic antioxidants being more effective than lipophilic antioxidants in bulk oils compared to lipophilic antioxidants.

Depending on the elenolic acid structure, whether it is open (dialdehydic form) or closed (aldehydic form), the antioxidant activity among secoiridoid derivatives varies. 3,4-DHPEA-EDA, which bears a dialdehydic form of elenolic acid linked to hydroxytyrosol, exhibits an antioxidant activity higher than that of DHPEA-EA (closed ring, aldehydic form) toward bulk lipid (MeLo) oxidation.

The highest radical scavenging activity (92.8 ± 1) was found with the peak RT 36 (secoiridoid derivative), suggesting the

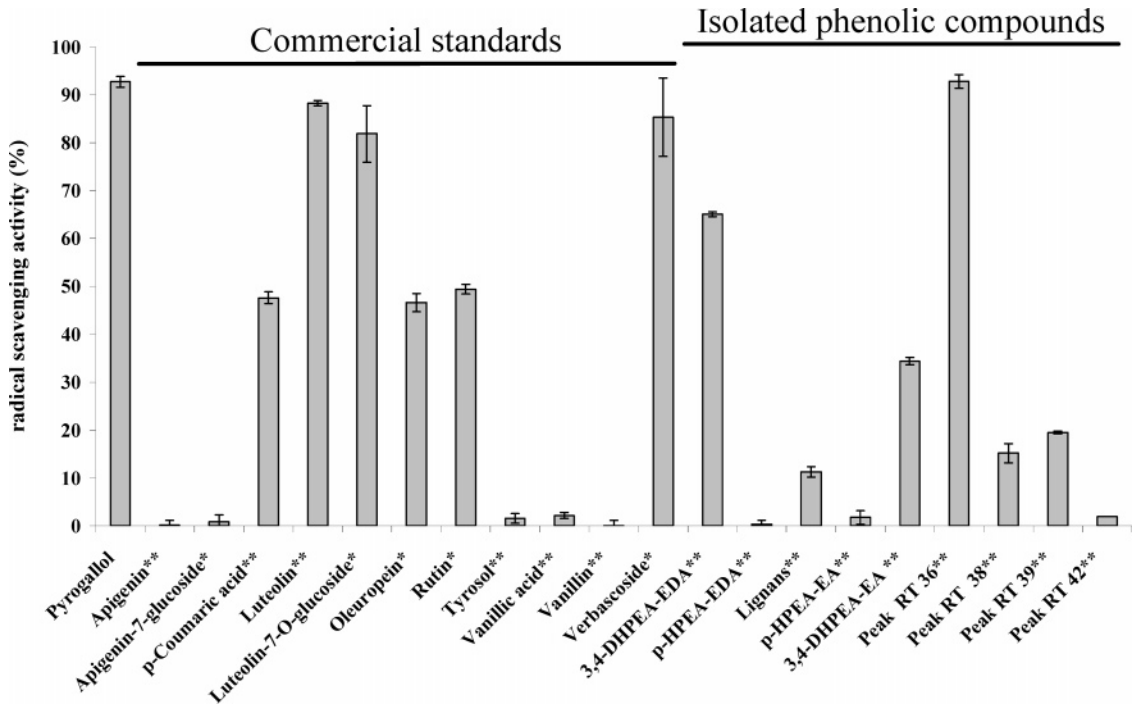


Figure 2. Radical scavenging activities of phenolic compounds (1 mg/mL) present in Arbequina olive and olive oil using DPPH test: *, olive pulp phenols; **, virgin olive oile phenols. See **Scheme 1** and **Figure 1** for detailed description on the phenolic compounds.

Table 1. Antioxidant Activities of Phenolic Compounds Isolated from Arbequina Cultivar Olive Oil and Olive Pulp toward Liposome and Bulk Methyl Linoleate (MeLo) Oxidation (Percent Inhibition, Mean ± SD)[a]

	liposome model, % inhibition of CD[b]		liposome model, % inhibition of hexanal		methyl linoleate, % inhibition of CD	
pure compound	10 μM	25 μM	10 μM	25 μM	10 μM	25 μM
apigenin	12.0 ± 3.2 gh*	25.5 ± 4.4 gh*	21.4 ± 0.9 efg*	40.0 ± 2.4 fgh*	–5.4 ± 3.2 ef	–1.2 ± 1.4 cde
apigenin-7-glucoside	11.4 ± 1.4 gh	8.1 ± 1.8 j	12.5 ± 2.1 gh	28.5± 6.5 hi	1.4 ± 4.4 de	2.7 ± 0.7 cd
p-coumaric acid	19.3 ±5.1 efg*	44.4 ± 3.0 ef*	51.6 ± 0.5 d*	63.4 ± 1.1 cd*	–1.7 ± 4.3 e*	–19.9 ± 1.5 f*
luteolin	55.7 ±2.5 c*	66.5 ± 0.0 bc*	95.5 ± 0.6 a*	98.0 ± 0.0 a*	80.8 ± 2.8 a*	95.0 ± 0.4 a*
luteolin-7-*O*-glucoside	66.1 ±5.5 b	64.4 ± 0.4 c	96.5 ± 0.7 a*	98.1 ± 0.0 a*	–1.4 ± 5.9 e	–4.3 ± 2.1 def
oleuropein	57.2 ± 11.9 bc	65.9 ± 4.9 c	82.4 ± 6.9 b	89.9 ± 1.4 b	81.7 ± 4.0 a*	95.7 ± 0.9 a*
rutin	82.1 ± 0.8 a*	86.8 ± 0.4 a*	78.3 ± 1.0 b*	97.3 ± 0.2 a*	1.6 ± 15.8 de	–20.6 ± 16.1 f
tyrosol	25.8 ± 1.5 e	24.0 ± 8.6 gh	52.1 ± 6.7 d	52.9 ± 1.4 de	17.3 ± 4.6 cd	0.3 ± 2.5 cde
vanillic acid	12.7 ± 6.5 fgh	20.4 ± 8.4 hi	30.9 ± 1.3 e	35.2 ± 22.4 ghi	–6.3 ± 2.0 ef	–7.7 ± 5.0 def
vanillin	20.2 ± 0.7 ef*	29.5 ± 1.2 g*	23.7 ± 4.2 ef	38.7 ± 8.1 fgh	–7.8 ± 9.2 ef	–16.3 ± 4.7 ef
verbascoside	58.2 ± 5.2 bc	70.4 ± 3.1 bc	76.5 ± 5.5 b	86.5 ± 0.6 b	34.7 ± 3.0 c	50.7 ± 11.7 b

	liposome model, % inhibition of CD[b]		liposome model, % inhibition of hexanal		methyl linoleate, % inhibition of CD	
phenolic isolate[c]	4.2 μg/mL	8.3 μg/mL	4.2 μg/mL	8.3 μg/mL	4.2 μg/mL	8.3 μg/mL
3,4-DHPEA-EDA	56.0 ± 8.4 c	38.4 ± 8.9 f	62.9 ± 13.5 c	51.1 ± 7.9 e	89.5 ± 1.8 a*	95.5 ± 0.6 a*
p-HPEA-EDA	4.9 ± 3.5 h*	30.5 ± 5.0 g*	17.2 ± 2.3 fg*	44.8 ± 5.7 efg*	0.9 ± 21.1 de	–19.4 ± 24.6 f
lignans	52.4 ± 1.6 c*	74.2 ± 0.4 b*	75.5 ± 3.4 b*	88.6 ± 0.4 b*	1.8 ± 10.0 de	13.8 ± 3.1 c
p-HPEA-EA	20.2 ± 2.6 efg*	38.6 ± 2.9 f*	21.4 ± 8.8 efg*	48.0 ± 5.4 ef*	–4.4 ± 7.3 ef	3.0 ± 16.0 cd
3,4-DHPEA-EA	–4.7 ± 3.4 h*	50.2 ± 3.9 de*	11.1 ± 1.4 gh*	70.7 ± 2.5 c*	34.3 ± 11.8 c	67.3 ± 1.2 b
peak RT 36	44.7 ± 2.2 d*	56.0 ± 3.2 d*	58.0 ± 2.9 cd*	71.1 ± 2.1 c*	54.3 ± 8.7 b*	87.3 ± 2.5 a*
peak RT 38	16.6 ± 3.5 fg*	37.9 ± 2.4 f*	29.8 ± 8.9 e*	65.1 ± 4.0 c*	33.4 ± 0.4 c	60.9 ± 12.9 b
peak RT 39	39.6 ± 4.1 d	37.9 ± 3.3 f	53.9 ± 9.2 cd	53.5 ± 5.0 de	–20.0 ± 7.1 f*	10.2 ± 1.1 cd*
peak RT 42	5.7 ± 3.3 h	5.9 ± 4.6 j	6.3 ± 0.9 h	9.7 ± 3.4 j	5.0 ± 0.2 de	3.6 ± 2.8 cd

[a] SD, standard deviation; values in the same column at the same concentration followed by different letters are significantly different ($p < 0.05$). An asterisk (*) indicates that the antioxidant activity is significantly different between different concentrations ($p < 0.05$). [b] CD, conjugated diene hydroperoxides. [c] For abbreviations of the phenolic isolates see **Scheme 1** and **Figure 1**.

presence of a hydroxytyrosol moiety in its structure. During oxidation of liposomes (hydrophilic) and methyl linoleate (lipophilic) peak RT 36 exhibited a behavior similar to that of 3,4-DHPEA-EDA. Tentative identification of peak RT 36 included the presence of a hydroxytyrosol moiety linked to elenolic acid, but the ring structure (closed or open) and the number of aldehydic groups remain not yet elucidated.

Flavonoids from olive pulp and olive oil include flavonol glycosides such as rutin, flavones (apigenin and luteolin), and their glucosides (apigenin-7-glucoside and luteolin-7-*O*-gluco-

side). Structural differences between apigenin and luteolin are characterized by the presence of a hydroxyl group in the luteolin C ring, which appears in the ortho position. According to Bors et al. (*29*), luteolin and its glucosides fulfill all criteria for maximal radical scavenging activity. Thus, it is not unexpected that these phenolics expressed the highest radical scavenging activities as well as antioxidant activity toward the formation of hexanal during liposome oxidation. According to Shahidi et al. (*30*), luteolin and apigenin also may act as chelators forming ligands with the cupric ions (Cu^{2+}). Luteolin and its glucoside were equally potent in inhibiting liposome oxidation. On the contrary, only the aglycon, luteolin, was effective toward the inhibition of bulk lipid oxidation. As described by Hopia et al. (*31*), the sugar moiety in the flavonol glycoside has a significant effect on the antioxidant activity in the MeLo model. This result is contradictory to the polar paradox phenomenon (*28*). Both oleuropein and luteolin-7-glucoside bear a glucose moiety in their structure; however, they exhibit different antioxidant activities toward the oxidation of bulk lipids. The difference could be due to the different locations of the glucose moiety in their structures, because in luteolin-7-glucoside the glucose moiety substitutes for a hydroxyl group at C_7 linked to a phenolic ring. The higher activities of aglycons in the MeLo model could be explained by the higher oxidizability of these compounds compared to flavonol glycosides. In oleuropein, the glucose moiety is linked to elenolic acid, thus substituting for an aldehydic group, which is not linked to a phenolic ring and thus gives rise to a less soluble phenolic compound in the MeLo model.

A hydroxycinnamic derivative such as verbascoside present in olive pulp is ubiquitous in the Oleaceae family (*2*). Verbascoside possesses the ability to act as a hydrogen donor with a radical scavenging value of 85.3 ± 1 due to its two ortho dihydroxy structures. Antioxidant activities of verbascoside in the MeLo model and liposome model differed markedly due to different mechanisms of antioxidant action, which depended mainly on the solubility of verbascoside in bulk lipid or in water-soluble systems. Higher antioxidant activity was found in the hydrophilic model as compared to luteolin and rutin, whereas in the bulk lipid model the antioxidant activity at highest concentration (25 μM) was only moderate (50.7 ± 12).

Lignans (acetoxypinoresinol and pinoresinol) present in olive oil exhibited only a weak radical scavenging activity as well as low antioxidant activity toward the oxidation of bulk lipids, but a high antioxidant activity was found toward the oxidation of liposomes. The higher antioxidant activity in the liposome model could be due to chelating properties of lignans because cupric acetate was added as an initiator of the liposome oxidation.

Simple phenols such as tyrosol, vanillic acid, vanillin, and *p*-coumaric acid possess an aromatic ring bearing one hydroxyl substituent. Simple phenols have a very low antiradical activity with the exception of *p*-coumaric acid, which bears a propenoic acid substituent that confers to the moiety a higher proton delocalization, facilitating a scavenging activity higher than observed with simple phenols.

Part of the antioxidative effects of phenolics present in olive pulp and olive oil can be explained by their radical scavenging activity, but as the present results clearly demonstrate, the hydrogen-donating ability of antioxidants in a solvent model does not necessarily indicate their activity in a lipid environment. In this study a correlation was found between radical scavenging activities (DPPH test) and antioxidant activities toward liposome and bulk lipid oxidation. The radical scavenging properties of phenolic compounds assessed by the DPPH test seem to be explanatory factors, but not conclusive, for the MeLo and liposome models. Although correlations are moderate, when phenolic compounds are divided between the ones originating in olive pulp or in virgin olive oil, it is evident that the radical scavenging properties of phenolic compounds present in olive oil exhibit a higher correlation with the inhibition of bulk lipid oxidation compared to the liposome oxidation. On the other hand, the radical scavenging properties of phenolic compounds present in olive pulp correlate better with the inhibition of liposome oxidation than with bulk lipid oxidation.

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