Antioxidant Activity of Tocopherols and Phenolic Compounds of Virgin Olive Oil

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ABSTRACT: The antioxidant effects of hydrophilic phenols and tocopherols on the oxidative stability in virgin olive oils and in purified olive oil have been evaluated. Total hydrophilic phenols and the oleosidic forms of 3,4-dihydroxyphenolethanol (3,4-DHPEA) were correlated ($r = 0.97$) with the oxidative stability of virgin olive oil. On the contrary, tocopherols showed low correlation ($r = 0.05$). Purified olive oil with the dialdehydic form of elenolic acid linked to 3,4-DHPEA, an isomer of oleuropeine aglycon, and 3,4-DHPEA had good oxidative stability. A synergistic effect was observed in the mixture of 3,4-DHPEA and its oleosidic forms with α-tocopherol in purified olive oil by the Rancimat method at 120°C.
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KEY WORDS: α-Tocopherol, antioxidant, oxidation, phenols, virgin olive oil.

Phenolic compounds present in virgin olive oil can be classified into a lipophilic group (tocopherols) (1,2) or a hydrophilic group, including phenolic acid (cinnamic and benzoic acid derivatives), 3,4-dihydroxyphenylethanol (3,4-DHPEA), *p*-hydroxyphenylethanol (*p*-HPEA), and the oleosidic forms of 3,4-DHPEA and *p*-HPEA (oleuropeine glucoside and ligstroside derivatives) (3–6).

Oleosidic forms of 3,4-DHPEA and *p*-HPEA, in particular the dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol (3,4-DHPEA–EDA), an isomer of oleuropeine aglycon (3,4-DHPEA–EA), and the dialdehydic form of elenolic acid linked to *p*-HPEA (*p*-HPEA–EDA) have been identified, quantitatively, as the most important secoroidoid compounds of virgin olive oil by Montedoro *et al.* (6).

The antioxidant activity of 3,4-DHPEA, *p*-HPEA, and phenyl-acids (caffeic acid, *p*-coumaric acid, ferulic acid, syringic acid, and vanillic acid) has been studied, and the high antioxidant activity of 3,4-DHPEA has been demonstrated (7–12). Antioxidant activity of the oleosidic forms of 3,4-DHPEA and *p*-HPEA has been reported by a few authors (13,14).

Rapid methods have been used to evaluate the oxidative stability of oils and to compare the antioxidant activity of several compounds (10,15–18). The Rancimat apparatus (19) correlates well with the active oxygen method and with peroxide development during oil storage at room temperature (20–22).

In this study, the antioxidant effect of hydrophilic phenols and α-tocopherol were evaluated in both virgin olive oils and purified olive oil by Rancimat analysis. Different sets of data are reported: (i) linear regression analysis among total hydrophilic phenols, total tocopherols, and oxidative stability of virgin olive oils; (ii) partial least squares regression analysis (PLS) among oxidative stability and phenyl-acids, 3,4-DHPEA, *p*-HPEA and their oleosidic forms in virgin olive oil; (iii) the antioxidant activity of 3,4-DHPEA–EDA and 3,4-DHPEA–EA, extracted from virgin olive oil, evaluated in a purified olive oil and compared with 3,4-DHPEA, *p*-HPEA, and α-tocopherol; (iv) the synergistic effect of the mixtures of α-tocopherol with 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA.

MATERIALS AND METHODS

Materials. Forty-eight virgin olive oil samples, collected from different Italian regions, and refined, bleached, and deodorized olive oil were used. Olive oil was purified by passing 300 g of oil through a chromatographic column, packed with a series of 20 g activated silicic acid (100 mesh; BDH, Poole, United Kingdom), 10 g activated charcoal (BDH) and celite (2:1), 40 g powdered sugar and celite (2:1), and 20 g of activated silicic acid, as described by Lee and Min (23). Purified olive oil did not contain peroxides, free fatty acids, hydrophilic phenols, and tocopherols. α-Tocopherol was obtained from Sigma Chemical Co. (St. Louis, MO), *p*-HPEA was obtained from Janssen Chemical Co. (Beerse, Belgium), and 3,4-DHPEA was synthesized according to the procedure of Baraldi *et al.* (24). 3,4-DHPEA–EDA and 3,4-DHPEA–EA were isolated from virgin olive oil by preparative high-performance liquid chromatography (HPLC) according to the procedure of Montedoro *et al.* (6). HPLC or analytical-grade reagents and solvents were supplied by Carlo Erba (Milano, Italy).

Antioxidant addition. Weighed quantities of *p*-HPEA, 3,4-DHPEA, 3,4-DHPEA–EDA, 3,4-DHPEA–EA and α-tocopherol were dissolved in 3 mL of a 1:2 vol/vol mixture of methanol/chloroform to obtain the desired final molar con-

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centration (1 mM, 0.8 mM, 0.6 mM, 0.4 mM, and 0.2 mM) when added to 30 g of purified olive oil. The phenolic compounds were mixed with olive oil by stirring at 25°C for 20 min under nitrogen in the dark; organic solvents were removed in a rotary evaporator under nitrogen flow at 35°C. The antioxidant activity was measured in triplicate, and purified olive oil was used as control. The antioxidant activity of mixtures of α-tocopherol (0.1 mM and 0.25 mM) and 3,4-DHPEA, 3,4-DHPEA–EDA, and 3,4-DHPEA–EA (0.1 mM and 0.25 mM) in purified olive oil were singly compared, in triplicate.

Chemical analysis. The total hydrophilic phenols were evaluated colorimetrically at 765 nm with the Folin Ciocalteau reagent (25). Total tocopherols were evaluated by HPLC according to Carpenter (26). The HPLC analysis of phenyl-alcohols, phenyl-acids, and secoroidoid compounds extracted from virgin olive oils was carried out according to Montedoro *et al.* (25). The measurement of free acidity and the peroxide number of olive oil was carried out according to European Official Methods of Analysis (27).

Measurement of oxidative stability. Stability of virgin olive oils and purified olive oil was evaluated by Rancimat (Metrohm Co., Basel, Switzerland) analysis at 120°C with an air flow of 20 L/h; the results are expressed as induction time (hours) (10,20).

Statistical analysis. Linear regression analysis was carried out with the Statgraphic program (version 6) to evaluate the relationships among total hydrophilic phenols, total tocopherols, and induction time of virgin olive oil (28). Partial least squares regression analysis (PLS) was employed to analyze the relationships among phenyl-acids, 3,4-DHPEA, *p*-HPEA, and their oleosidic forms in virgin olive oils and the induction time of oils by means of the chemometric package SIMCA-S v. 5.1 (29). PLS performs simultaneous projections on orthogonal latent variables of X (phenyl-acids, 3,4-DHPEA, *p*-HPEA, and their oleosidic forms) and Y (induction time) spaces by maximizing the correlation between X and Y. Cross-validation was used to eliminate overfitting (30). The latent variables obtained (PLS components) are a linear combination of original variables and represent lines in X and Y spaces in the directions of the maximum variability of data. The PLS results are displayed graphically in two types of plots: in the first one, the coordinates (scores) of the projections of the samples (objects) on the latent variables are plotted to show the goodness of fit of the relation between X (t_1) and Y (u_1); in the second one, the loading-plot, displayed as *"wc"* weights, represents the combined weights of X (w) and Y (c) and shows the correlation of hydrophilic phenols and oil induction time.

RESULTS

Oxidative stability of the 48 virgin olive oils correlated highly ($r = 0.97$) with the concentration of total hydrophilic phenols, while total tocopherols showed no significant correlation ($r = 0.05$) (Fig. 1).

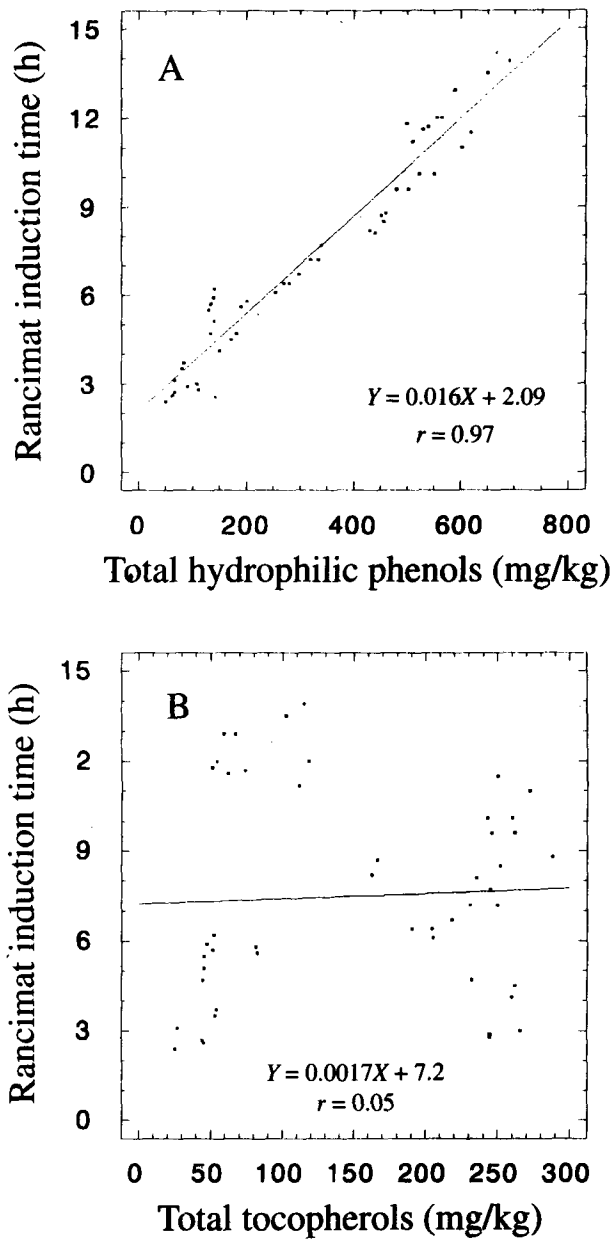

FIG. 1. Linear regression analysis for (A) total hydrophilic phenols, (B) total tocopherols and the induction times of 48 virgin olive oil samples.

The PLS model explained 86% of the total variance of data Y with the first component. The score-plot of u_1 vs. t_1 demonstrated good linearity of objects along the diagonal of the plot, thus showing that there is a good correlation between the variables X (phenyl-acids, 3,4-DHPEA, *p*-HPEA, and their oleosidic forms) and Y (induction time), while the loading-plot of wc_1 showed that only 3,4-DHPEA–EDA and 3,4-DHPEA–EA have an high positive influence on Y (Fig. 2).

The results obtained in purified olive oil showed no significant differences between the antioxidant activity of 3,4-DHPEA and its oleosidic forms, while α-tocopherol demonstrated low antioxidant activity with respect to 3,4-DHPEA, 3,4-DHPEA–EDA, and 3,4-DHPEA–EA (Fig. 3 and Table 1).

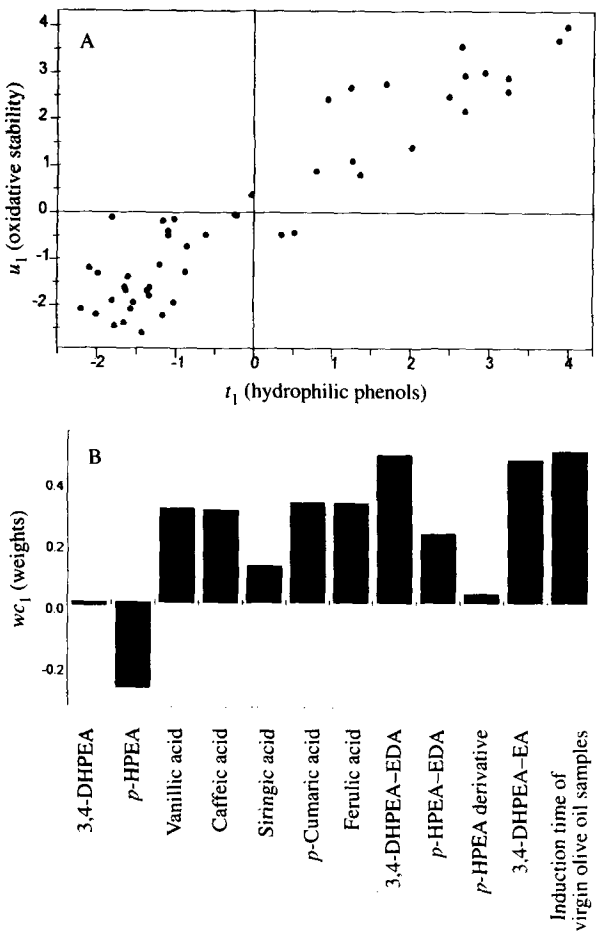

FIG. 2. Score-plot (A) and loading-plot (B) of partial least squares regression analysis between the concentrations of hydrophilic phenols of virgin olive oil, evaluated by high-performance liquid chromatography, and oxidative stability (induction time of virgin olive oil samples): u_1 and t_1 represent the object (samples) projections in the Y space (autoxidation stability) and in the X space (concentration of hydrophilic phenols), respectively; wc weights represent the combined weights of X (w) and Y (c) and show the correlation of hydrophilic phenols and oil induction time.

The antioxidant activity of 3,4-DHPEA and its oleosidic forms was also evaluated in mixtures with α-tocopherol; the data summarized in Figure 4 demonstrated the synergistic effect of the mixtures.

DISCUSSION

Data showed that oxidative stability of virgin olive oil correlated mainly with the concentration of hydrophilic phenols ($r = 0.97$) and, in particular, with the oleosidic forms of 3,4-DHPEA. The PLS results confirmed observations, reported in previous papers, about the low antioxidant activity of phenyl-acid and *p*-HPEA (7–12) and showed, for the first time, that the oxidative stability of virgin olive oil is not due to 3,4-DHPEA in the nonlinked form but is closely correlated to the concentration of the oleosidic forms of 3,4-DHPEA. These forms originate, on account of the glucosidase enzymatic activity, from the oleuropeine glucoside contained in the fruits (31). The lack of correlation between 3,4-DHPEA and the oxidative stability of virgin olive oil is probably due to the low concentration of this compound in the oil (Table 2). In fact, the data obtained from the purified olive oil demonstrated that the presence of ester links with different derivatives of elenolic acid (EA and EDA) did not modify the antioxidant activity of 3,4-DHPEA.

Although no correlation ($r = 0.05$) was observed between the oxidative stability of virgin olive oils and the concentration of total tocopherols, the α-tocopherol, which is quantitatively the most important tocopherol in this oil, showed synergistic activity in mixtures with 3,4-DHPEA and its oleosidic forms in purified olive oil. Similar results were observed by Boguth *et al.* (32) by combining α-tocopherol with butylated hydroxytoluene. The scavenger effect on the peroxyl radicals, observed by Schuler (33) for α-tocopherol, synergizes the activity of 3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA.

Linear regression analyses of total hydrophilic phenols or total tocopherols with induction time, evaluated by Rancimat analysis, only showed significant correlation for the total phenols.

TABLE 1
Slope (m) and Intercept (q) of the Straight Line and Squared Correlation Coefficient (r^2) Calculated from the Relationship Between Antioxidant Activity and the Concentration of Phenolic Compounds

	m[a]	q[a]	r^2
3,4-DHPEA	10.60 ± 1.33	5.50 ± 0.88	0.955
p-HPEA	−0.25 ± 0.05	3.93 ± 0.03	0.893
3,4-DHPEA-EDA	11.20 ± 1.09	4.90 ± 0.72	0.972
3,4-DHPEA-EA	11.65 ± 1.60	4.87 ± 1.06	0.946
α-Tocopherol	2.45 ± 0.61	5.03 ± 0.41	0.839

[a]Mean ± standard error; 3,4-DHPEA, 3,4-dihydroxyphenylethanol; *p*-HPEA, *p*-hydroxyphenylethanol; 3,4-DHPEA-EDA and 3,4-DHPEA-EA, the dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol and an isomer of oleuropeine aglycon, respectively.

TABLE 2
Concentration (mg/kg) of Hydrophilic Phenols of 48 Virgin Olive Oil Samples

Phenols	Average	Range
3,4-DHPEA	4.5	0.0–25.4
p-HPEA	25.7	0.1–123.1
Vanillic acid	0.2	0.0–0.8
Caffeic acid	0.1	0.0–1.0
Siringic acid	0.5	0.0–2.3
p-Cumaric acid	0.2	0.0–0.6
Ferulic acid	0.7	0.0–2.4
3,4-DHPEA-EDA	67.1	0.0–351.2
p-HPEA-EDA	25.6	0.0–79.8
p-HPEA derivative[a]	43.8	0.0–113.4
3,4-DHPEA-EA	27.7	0.0–83.5

[a]Montedoro *et al.* (Ref. 6). See Table 1 for abbreviations.

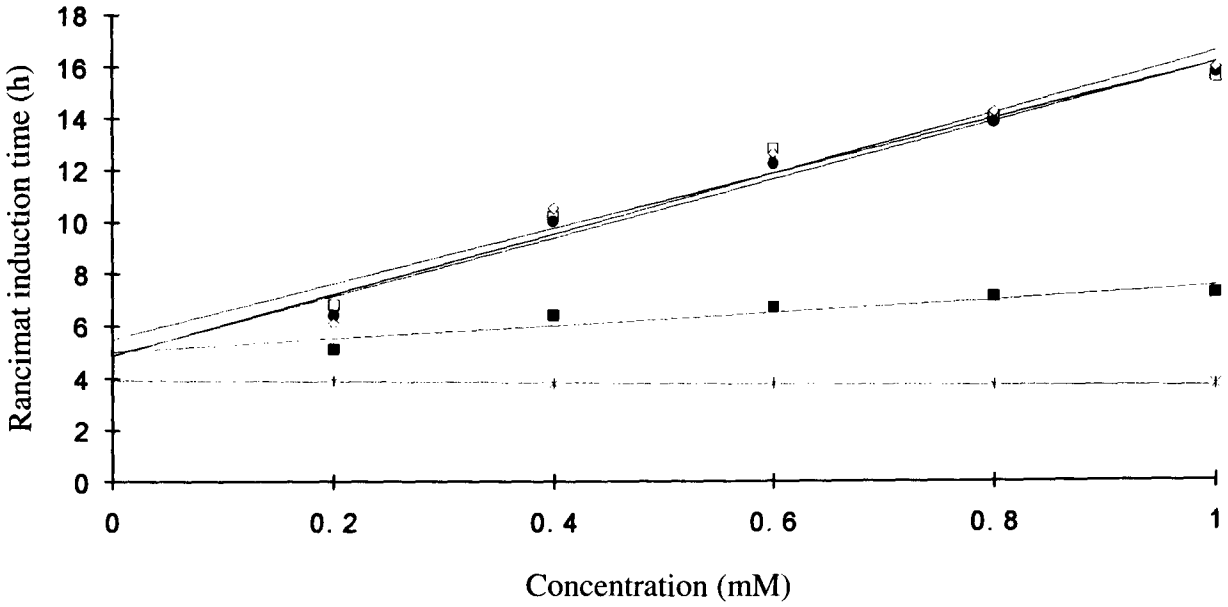

FIG. 3. Antioxidant activity of 3,4-dihydroxyphenylethanol (3,4-DHPEA) (□), dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA–EDA) (○) an isomer of oleuropeina aglycon (3,4-DHPEA–EA) (◇), α-tocopherol (■), and *p*-HPEA (*) measured in purified olive oil oxidized at 120°C by Rancimat. Each value is the mean of three experiments; the slopes and intercept for straight lines are reported in Table 1.

Oleosidic forms of 3,4-DHPEA demonstrated high correlations with the induction time of virgin olive oil.

The results obtained in purified olive oil confirmed the high antioxidant activity of 3,4-DHPEA–EDA and 3,4-DHPEA–EA.

Preliminary studies performed on the mixtures of α-tocopherol and 3,4-DHPEA, 3,4-DHPEA–EDA, and 3,4-DHPEA–EA showed the synergistic effect of the mixtures in purified olive oil.

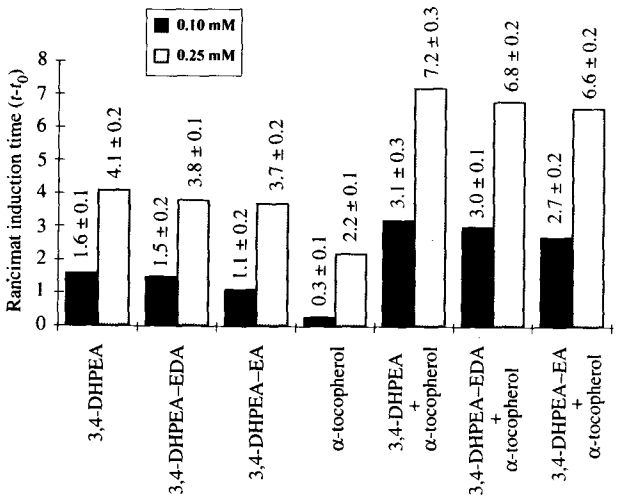

FIG. 4. Synergistic effect of 3,4-DHPEA, 3,4-DHPEA–EDA, and 3,4-DHPEA–EA in mixture with α-tocopherol. Each value is the mean of three experimental values ± standard deviation; t = induction time of purified olive oil added to antioxidant compounds; t_0 = induction time of purified olive oil. See Figure 3 for abbreviations.

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