Effect of Natural Antioxidants in Virgin Olive Oil on Oxidative Stability of Refined, Bleached, and Deodorized Olive Oil

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ABSTRACT: The factors influencing the oxidative stability of different commercial olive oils were evaluated. Comparisons were made of (i) the oxidative stability of commercial olive oils with that of a refined, bleached, and deodorized (RBD) olive oil, and (ii) the antioxidant activity of a mixture of phenolic compounds extracted from virgin olive oil with that of pure compounds and α-tocopherol added to RBD olive oil. The progress of oxidation at 60°C was followed by measuring both the formation (peroxide value, PV) and the decomposition (hexanal and volatiles) of hydroperoxides. The trends in antioxidant activity were different according to whether PV or hexanal were measured. Although the virgin olive oils contained higher levels of phenolic compounds than did the refined and RBD oils, their oxidative stability was significantly decreased by their high initial PV. Phenolic compounds extracted from virgin olive oils increased the oxidative stability of RBD olive oil. On the basis of PV, the phenol extract had the best antioxidant activity at 50 ppm, as gallic acid equivalents, but on the basis of hexanal formation, better antioxidant activity was observed at 100 and 200 ppm. α-Tocopherol behaved as a prooxidant at high concentrations (>250 ppm) on the basis of PV, but was more effective than the other antioxidants in inhibiting hexanal formation in RBD olive oil. *o*-Diphenols (caffeic acid) and, to a lesser extent, substituted *o*-diphenols (ferulic and vanillic acids), showed better antioxidant activity than monophenols (*p*- and *o*-coumaric), based on both PV and hexanal formation. This study emphasizes the need to measure at least two oxidation parameters to better evaluate antioxidants and the oxidative stability of olive oils. The antioxidant effectiveness of phenolic compounds in virgin olive oils can be significantly diminished in oils if their initial PV are too high.

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KEY WORDS: Antioxidants, hexanal, hydroperoxide decomposition, hydroperoxide formation, olive oil, oxidative stability, peroxide value, phenolic compounds, tocopherols.

Pressed olive oils obtained from the fruit of *Olea europaea* L. are known to be more resistant to oxidation than other edible oils because of their lower unsaturation and their unsaponifiable components, including tocopherols and phenolic compounds (1). Seed oils contain more tocopherols than olive oils, but great amounts of phenolics are lost during oil processing (2). If the acidity due to free fatty acids is lower than 1% (expressed as oleic acid), the olive oil is labelled as "extra virgin." If the acidity ranges from 1 to 3%, the oil is called "virgin." If the acidity is higher than 3%, the oil has to be refined, and the refined oil is usually blended with extra virgin olive oil. These mixtures are designated as "olive oil" or "pure olive oil."

Since virgin olive oils are not refined, the phenolic compounds are partly preserved, and these compounds are reportedly responsible for their higher stability to autoxidation (3). The phenolic compounds form part of the polar fraction obtained by extraction with a methanol–water mixture (4), and are determined in the extract by the Folin-Ciocalteu colorimetric method (5). This fraction is very complex, and it is not yet clear which particular substances are responsible for the antioxidant activity (6). Several authors have investigated different high-performance liquid chromatography (HPLC) separation methods to identify the polar fraction components (7,8).

The extent of oxidation in oils has been frequently evaluated by measuring peroxide value (PV). This index is related to the hydroperoxides, the primary oxidation products, which are unstable and readily decompose to form mainly mixtures of volatile aldehyde compounds. Because these compounds are directly responsible for rancid flavors (9), they are considered important markers of oxidative rancidity. Several methods have been reviewed to measure the oxidative stability of edible oils (10), but relatively few studies have evaluated the effect of antioxidants on oxidative stability on the basis of volatile formation.

This paper reports a study of the oxidative stability of different commercial olive oils by monitoring primary oxidation products—PV—and secondary oxidation products—hexanal—by static headspace gas chromatography (GC). The antioxidant activity of known phenolic and nonphenolic compounds also was tested as references.

MATERIALS AND METHODS

Materials. Olive oils were either purchased at a local market [extra light olive oil (ELOO#1); pure olive oil (POO#2); extra virgin olive oil (EVOO#3); and extra virgin olive oil,

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(EVOO#4)] or supplied commercially [refined, bleached, and deodorized olive oil (RBDOO); Beatrice/Hunt Wesson Co., Fullerton, CA]. They were kept frozen in dark glass bottles under nitrogen. Silica cartridges were supplied by Waters (Millford, MA). All chemicals and solvents used were either of analytical or HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ). Phenol standards (purity >97%) and Folin-Ciocalteu reagent were from Sigma Chemical Co. (St. Louis, MO).

Extraction of phenols. Aliquots of 5 g virgin olive oil dissolved in 5 mL of *n*-hexane were extracted three times with 10 mL of a methanol/water mixture (80:20, vol/vol). The pooled extracts were washed with 10 mL of *n*-hexane and dried with anhydrous sodium sulfate, and the solvent was removed with a rotating evaporator under vacuum. The residue was redissolved in ethanol and analyzed for phenolic content by the Folin-Ciocalteu method (11).

Total phenols (Folin-Ciocalteu). Folin-Ciocalteu reagent (1 mL) diluted 1:10 with distilled water was added to 0.2 mL of phenolic extract. The color was developed with 0.8 mL of 7.5% sodium carbonate. After 30 min, the absorbance was read at 765 nm, using gallic acid as a standard for the calibration curve. Results of duplicate analyses are expressed as gallic acid equivalents (GAE).

Tocopherols. Tocopherols were analyzed in duplicate by normal-phase HPLC and fluorimetric detection (12).

Fatty acid composition. Duplicate aliquots of the olive oils were converted to methyl esters and analyzed by GC with a flame-ionization detector (13). Free fatty acids were determined titrimetrically in duplicate (13).

Oil chromatography. One gram of ELOO#1 diluted in 1 mL of *n*-hexane was applied onto a two-gram Sep-pak silica cartridge previously washed with 6 mL of *n*-hexane. The sample was eluted with ten 1-mL fractions of 10% diethyl ether in hexane (vol/vol). Polar oxidation products started to elute in the sixth fraction obtained after 6 mL along with the triacylglycerols (TAG). Recovery of pure TAG was about 88%. Purity of the different fractions was evaluated by thin-layer chromatography (TLC) on silica gel plates. After developing with 20% diethyl ether in hexane (vol/vol), the plates were checked under an ultraviolet (UV) lamp for oxidation products and charred with sulfuric acid to visualize the spots.

Oxidation experiments. Refined and virgin olive oils were compared with RBDOO as control. These oils (10 g) were weighed in 50-mL screw-capped Erlenmeyer flasks and oxidized in the dark in a shaker oven (Lab-Line Instrument Inc., Melrose Park, IL) set at 60°C. Phenolic antioxidants were added to the RBDOO in ethanolic solution and the solvent removed under nitrogen. Samples were withdrawn for duplicate PV and volatile analyses. The ferric thiocyanate method was used to determine PV (14).

Volatile analysis. Static headspace GC for volatile analysis was performed with a gas chromatograph equipped with an H-6 headspace sampler (Perkin Elmer 3B, Norwalk, CT) and a flame-ionization detector. Oil samples (0.10 g) were weighed in 6-mL headspace vials sealed with a Teflon liner cap. Samples were then equilibrated for 20 min at 110°C and pressurized for 30 s before injection onto a capillary column DB-1701 (30 m x 0.32 mm x 1 μm thickness; J&W, Folsom, CA). Oven temperature was set at 75°C, the injector at 180°C, and the detector at 200°C. The carrier gas was helium set at 20 cm/s linear velocity.

Statistical analyses. The data are presented as mean ± standard deviation of duplicate determinations and are representative of two oxidation experiments. Statistical significance within sets of data was determined by one-way analysis of variance (15). Significance level was in all cases $P < 0.001$. The Minitab computer program (release 9-; Minitab Inc., Addison-Wesley Publishing Co., Reading, MA) was used for statistical treatment of the data.

RESULTS

Analyses of five different types of olive oils used for this study showed the major fatty acids varying in the following ranges: oleic acid (65–79%), palmitic acid (9–15%), linoleic acid (5–14%), and stearic acid (2–4%) (Table 1). Tocopherol content varied in the virgin olive oils from 74 ppm (EVOO#3) to 233 ppm (EVOO#4). Total phenolic compounds ranged from 8 ppm in the RBD olive oil (RBDOO) to 534 ppm as GAE in EVOO#3. PV ranged from 0.4 in RBDOO to 32.5 meq/kg in EVOO#4. Small amounts of hexanal ranged from 0.1 (RBDOO) to 1.0 mmol/kg (EVOO#4), and free fatty acid content from 0.0 in RBDOO to 0.5% oleic acid in EVOO#4.

Oil oxidation was monitored by two methods to measure different types of products: the PV to estimate hydroperoxides, as initial products, and volatile analysis to measure hexanal, a major decomposition product of linoleate hydroperoxides (16), as a marker of rancid flavors. Although linoleic acid is not the major unsaturated fatty acid in olive oil, it is the most susceptible fatty acid to oxidation. The hexanal peak observed by headspace GC proved to be the most consistent among replicates and with oxidation time and was, therefore, chosen to follow oxidation. On the other hand,oleic is the major fatty acid in olive oil, but it oxidizes at a rate fifty times slower than linoleic acid (17). The volatile product heptanal formed from oleate hydroperoxides (9) was found only in trace amounts after oxidation for 15 d at 60°C.

Commercial olive oils. On the basis of PV, the oxidative stability of several commercial olive oils varied significantly ($P < 0.001$), with the oil having the lowest initial PV being the most stable (Fig. 1A and Table 2). Although the virgin olive oils (EVOO#3 and EVOO#4) contained phenolic compounds in concentrations ranging from 518 to 534 ppm, they were significantly less stable ($P < 0.001$) than the RBDOO containing only 8 ppm phenolics. The differences in oxidative stability between these oils reflect their initial PV, which was much lower in the RBDOO than in the virgin olive oils. The greater oxidative stability of EVOO#3 than EVOO#4 can be explained by its lower initial PV and free fatty acids (Table 1). Evidently, the antioxidant activity expected from the phenolic compounds in these virgin olive oils was reduced by the oxidation products contributing to their initial PV.

TABLE 1
Analysis of α-Tocopherol, Total Phenols, Fatty Acid Composition, Free Fatty Acids, Initial Peroxide Value, and Hexanal Contents of the Olive Oils (OO) Used for the Oxidation Experiments[a]

Type of olive oil	Refined, bleached, and deodorized	Refined extra *light*	Refined pure	Extra virgin	Extra virgin
Identification	RBDOO	ELOO#1	POO#2	EVOO#3	EVOO#4
Origin	California	Italy	Italy	Spain	California
α-Tocopherol (ppm)	81.4 (1.6)	167.0 (1.6)	147.5 (5.4)	74.0 (0.1)	233.0 (9.2)
Total phenols (ppm gallic acid equivalents)	8.1 (0.8)	63.4 (0.0)	81.2 (2.1)	534.4 (23.9)	518.1 (16.2)
Fatty acid composition (%)					
$C_{16:0}$	9.0 (0.01)	14.8 (0.04)	14.5 (0.1)	8.9 (0.06)	14.9 (0.05)
$C_{16:1n-7}$	0.5 (0.004)	1.8 (0.005)	1.7 (0.01)	0.5 (0.005)	1.3 (0.007)
$C_{18:0}$	3.5 (0.02)	2.3(0.003)	2.4 (0.03)	3.4 (0.002)	2.6 (0.0007)
$C_{18:1n-9}$	76.9 (0.04)	65.0 (0.01)	66.2 (0.04)	79.4 (0.03)	71.7 (0.03)
$C_{18:2n-6}$	8.5 (0.02)	14.1 (0.008)	13.3 (0.007)	5.1 (0.002)	5.7 (0.003)
$C_{18:3n-3}$	0.6 (0.01)	0.8 (0.003)	0.7 (0.008)	0.7 (0.0001)	1.2 (0.003)
$C_{20:0}$	0.5 (0.001)	0.5 (0.001)	0.5 (0.005)	0.5 (0.004)	0.6 (0.002)
$C_{20:1n-9}$	0.3 (0.01)	0.3 (0.008)	0.3 (0.01)	0.3 (0.01)	0.4 (0.01)
$C_{33:0}$	0.1 (0.007)	0.2 (0.006)	0.2 (0.01)	0.2 (0.007)	0.2 (0.001)
Free fatty acids (% oleic aicd)	0.0 (0.0)	0.1 (0.0001)	0.3 (0.001)	0.3 (0.002)	0.5 (0.001)
Peroxide value (meq/kg)	0.4 (0.2)	11.0 (0.7)	12.7 (1.4)	15.6 (0.1)	32.5 (1.1)
Hexanal (mmol/kg)	0.1 (0.0)	0.2 (0.001)	0.3 (0.008)	0.3 (0.02)	1.0 (0.002)

[a]Mean (standard deviation in parentheses), n = 2.

On the basis of hexanal formation, the olive oils were difficult to compare because of their wide differences in initial hexanal values. Initially, the control RBD olive oil had the lowest hexanal value which increased after an induction period of 5 d (Fig. 1B). Although the ELOO#1, the POO#2, and the EVOO#3 showed similar initial hexanal formation, an induction period of 7 d was apparent with the first two samples, while the sample EVOO#3 showed no induction period. The initial high hexanal level of virgin olive oil EVOO#4 did not change during oxidation, and appeared to have reached a maximum value. Even though in the first days of the study all samples had similar (ELOO#1) or significantly higher (POO#2, EVOO#3, and EVOO#4) hexanal content than the RBD olive oil, after 11 d of oxidation, the hexanal content of EVOO#3 was significantly lower ($P < 0.001$) than the control RBDOO, while ELOO#1, POO#2, and EVOO#4 were not significantly different from the control (Table 2).

Effect of olive oil phenolic extracts. The combined phenolic extracts from the virgin olive oils EVOO#3 and EVOO#4 were added to the RBD olive oil at different levels to determine their antioxidant effect. Based on PV formation, these phenolics were more effective in inhibiting hydroperoxide formation at 50 ppm (as GAE) than at 100 and 200 ppm after 11 and 15 d oxidation (Fig. 2A and Table 3). In contrast, based on hexanal formation, phenolics were significantly more effective antioxidants at 100 and 200 ppm than at 50 ppm (Fig. 2B and Table 3). Similar results were previously obtained with tocopherols, which at high concentrations, were shown to be more effective in inhibiting hexanal formation than hydroperoxide formation (18). The addition of phenolic extracts to pure TAG (obtained from chromatographed ELOO#1) decreased the PV after 6 to 12 d, and the hexanal values after 12 d oxidation at 60°C, compared to the control (Fig. 3).

Effect of pure phenolic compounds. Different phenols (Fig. 4) reported to be present in olive oil (19,20) were evaluated for their effects on the antioxidant activity of olive oil (Fig. 4). These phenolic compounds were added separately and at different concentrations to equal aliquots of RBD olive oil. Based on PV, antioxidant efficiency after 15 d oxidation decreased in the following order (Fig. 5A and Table 3): (i) at 50 ppm: phenolic extract ≈ caffeic acid > *p*-coumaric acid > cinnamic acid ≈ vanillic acid; (ii) at 100 ppm: caffeic acid > ferulic acid ≈ *p*-coumaric acid ≈ vanillic acid ≈ *o*-coumaric acid > cinnamic acid > phenolic extract > α-tocopherol; (iii) at 500 ppm: ferulic acid > *o*-coumaric acid > α-tocopherol.

Based on PV, the antioxidant activity of the phenolic extract from crude olive oils was much greater at 50 ppm (52% inhibition) than at 100 ppm (9% inhibition). Although the activity of ferulic acid was similar at concentrations of 100 and 500 ppm, *o*-coumaric acid and α-tocopherol were less effective at 500 ppm than at 100 ppm. The *o*-diphenols (caffeic acid and the hydroxymethoxy derivatives, vanillic and ferulic acids) seemed to perform best at 100 ppm, but there were no significant differences between the two concentrations used at the end of the oxidation. The monophenols, *p*- and *o*-coumaric acids, and the nonphenol, cinnamic acid, were significantly more effective at the lowest concentration tested. α-Tocopherol was the least effective phenolic compound tested and showed prooxidant activity at 500 ppm after 11 and 15 d of oxidation (Table 3).

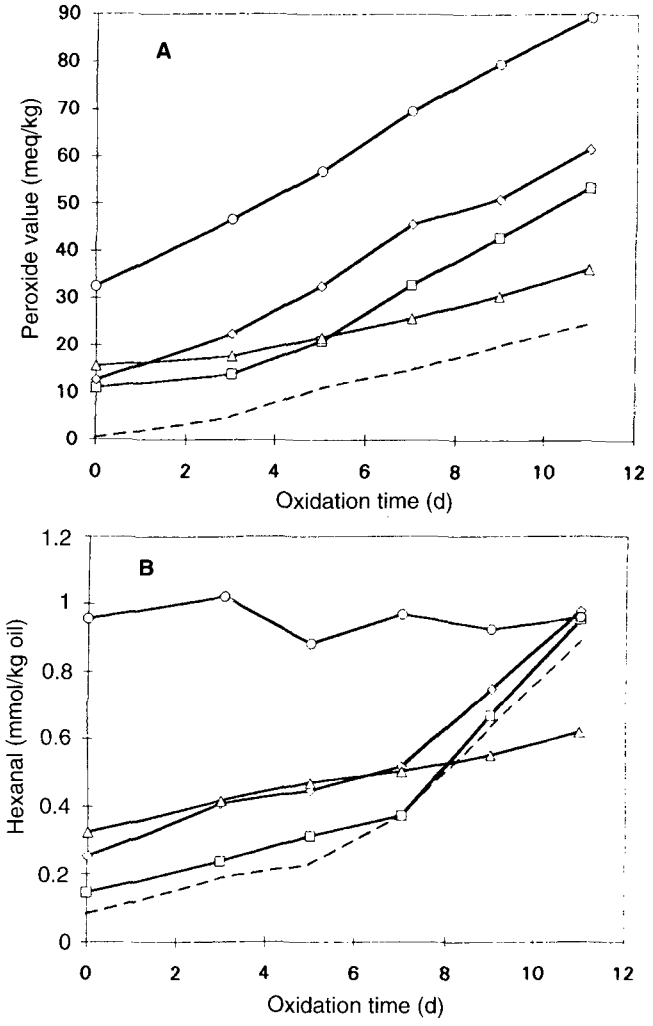

FIG. 1. Oxidative stability of commercial olive oils at 60°C: (A) peroxide value (n = 2); (B) hexanal formation (n = 2). RBD olive oil (----) = refined, bleached, and deodorized olive oil, ELOO#1 (-□-) = extra light olive oil, POO#2 (-◇-) = pure olive oil, EVOO#3 (-△-) = extra virgin olive oil, and EVOO#4 (-○-) = extra virgin olive oil.

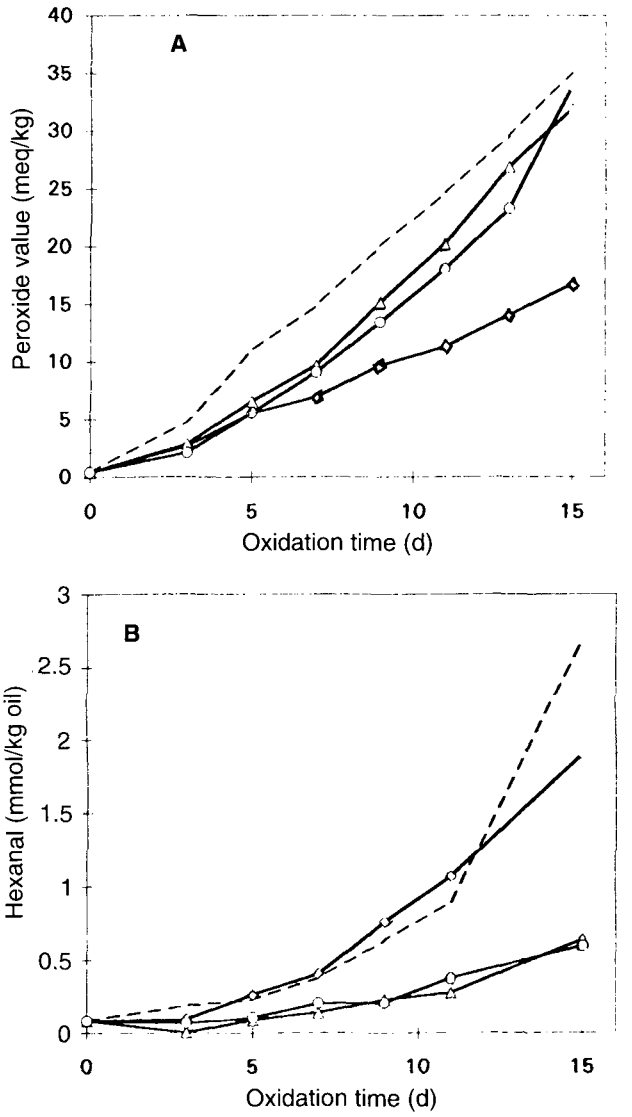

FIG. 2. Effect of phenolic extracts from virgin olive oil on oxidative stability of a refined, bleached, and deodorized olive oil at 60°C: (A) peroxide value (n = 2); (B) hexanal formation (n = 2). RBD olive oil = refined, bleached, and deodorized olive oil, GAE = gallic acid equivalents. ----, RBD olive oil; -◇-, + phenolic extract (50 ppm GAE); -△-, + phenolic extract (100 ppm GAE); -○-, + phenolic extract (200 ppm GAE).

Based on hexanal formation, antioxidant efficiency, after 15 d oxidation, decreased in the following order (Fig. 5B and Table 3): (i) at 50 ppm: caffeic acid > vanillic acid ≈ cinnamic acid > *p*-coumaric acid > phenolic extract; at 100 ppm: α-tocopherol > caffeic acid > ferulic acid ≈ cinnamic acid ≈ phenolic extract > *o*-coumaric acid > vanillic acid ≈ *p*-coumaric acid; at 500 ppm: ferulic acid > α-tocopherol > *o*-coumaric acid.

TABLE 2
Formation of Hydroperoxides (peroxide value) and Hexanal in Commercial Olive Oils (means ± standard deviation, n = 2)[a]

Sample	Peroxide value		Hexanal	
	Day 3	Day 11	Day 3	Day 11
Control (RBD olive oil)	4.8 ± 0.0a	24.7 ± 1.3a	0.2 ± 0.0a	0.9 ± 0.0b
ELOO#1	13.8 ± 0.1b	53.4 ± 1.7c	0.2 ± 0.0ab	0.9 ± 0.0b
POO#2	22.3 ± 0.2d	61.6 ± 3.2d	0.4 ± 0.0bc	1.0 ± 0.1b
EVOO#3	17.6 ± 0.0c	36.2 ± 0.2b	0.4 ± 0.1c	0.6 ± 0.0a
EVOO#4	46.5 ± 0.1e	89.3 ± 1.4e	1.0 ± 0.1d	0.9 ± 0.1b

[a]Values within each column followed by the same letter are not significantly different ($P < 0.001$). See Table 1 for abbreviations.

On the basis of hexanal formation, caffeic acid showed no significant differences in antioxidant activity between 50 and 100 ppm, but vanillic acid was more effective at 50 ppm. The phenolic extracts from virgin olive oil were more effective at 100 and 200 ppm than at 50 ppm in inhibiting hexanal formation but less effective in inhibiting PV formation. Monophenols and the nonphenolic cinnamic acid were more effective at 100 ppm. α-Tocopherol was a very good inhibitor of hexanal formation at 100 and 500 ppm, even though on the basis

TABLE 3
Inhibition of Hydroperoxide (peroxide value) and Hexanal Formation by Phenolic Compounds Added to RBD Olive Oil (percent mean inhibition ± standard deviation, n = 2)[a,b]

	Peroxide value			Hexanal		
Sample	Day 3	Day 11	Day 15	Day 3	Day 11	Day 15
Control (RBD olive oil)	0.0 ± 0.3g	0.0 ± 5.3f	0.0 ± 0.8f	0.0 ± 3.0e	0.0 ± 3.3i	0.0 ± 2.5j
+Phenolic extract (50 ppm)	44.4 ± 0.9d,e	54.2 ± 1.0a	52.4 ± 2.0a	50.8 ± 1.5c	−19.9 ± 0.4j	28.8 ± 0.3i
+Phenolic extract (100 ppm)	41.1 ± 5.0e	18.0 ± 0.3d	9.0 ± 3.1e	100.0 ± 0.0a	69.0 ± 0.9c	76.2 ± 1.4d,e
+Phenolic extract (200 ppm)	55.9 ± 3.1c	27.0 ± 3.7c,d	3.5 ± 2.2e,f	65.2 ± 5.3b	58.0 ± 5.6d,e	77.9 ± 2.0c,d
+*p*-Coumaric (50 ppm)	42.6 ± 0.3d,e	35.7 ± 1.2b	38.9 ± 0.0b	32.6 ± 3.8d	17.4 ± 0.2h	37.5 ± 2.7h
+*p*-Coumaric (100 ppm)	46.7 ± 3.3d	31.5 ± 1.9b,c	34.5 ± 0.2b,c	51.1 ± 4.9c	27.0 ± 1.3g	47.1 ± 2.4g
+Caffeic (50 ppm)	49.4 ± 2.8d	52.3 ± 0.3a	47.0 ± 0.6a	100.0 ± 0.0a	78.5 ± 2.6b	89.6 ± 2.5b
+Caffeic (100 ppm)	38.7 ± 2.5e	54.0 ± 0.1a	52.0 ± 0.2a	100.0 ± 0.0a	82.4 ± 6.6b	90.4 ± 2.1b
+Vanillic (50 ppm)	27.9 ± 3.3f	28.2 ± 0.6c	22.6 ± 6.6c,d	100.0 ± 0.0a	64.3 ± 1.2c,d	73.8 ± 3.8d,e
+Vanillic (100 ppm)	63.4 ± 4.0b	38.5 ± 0.1b	33.8 ± 3.5b,c	100.0 ± 0.0a	50.6 ± 4.2e,f	20.8 ± 3.6g
+Cinnamic (50 pm)	29.0 ± 0.7f	24.9 ± 4.5c	27.7 ± 0.6c	100.0 ± 0.0a	59.4 ± 1.4d	73.1 ± 2.3e
+Cinnamic (100 ppm)	−47.2 ± 0.7h	8.8 ± 1.5e	21.0 ± 2.9d	63.6 ± 5.3b	62.1 ± 1.7c,d	77.5 ± 0.6c,d
+*o*-Coumaric (100 ppm)	84.2 ± 0.4a	21.1 ± 1.3d	28.2 ± 0.1c	100.0 ± 0.0a	45.7 ± 5.4f	63.9 ± 3.6f
+*o*-Coumaric (500 ppm)	47.8 ± 1.5d	23.4 ± 0.0c	6.0 ± 3.9e,f	100.0 ± 0.0a	−1.7 ± 0.8i	32.3 ± 0.5h,i
+Ferulic (100 ppm)	85.2 ± 1.0a	34.4 ± 0.9b,c	40.1 ± 1.1b	100.0 ± 0.0a	62.4 ± 0.6c,d	81.2 ± 0.3c
+Ferulic (500 ppm)	66.8 ± 0.1b	33.3 ± 0.4b,c	36.8 ± 1.4b	100.0 ± 0.0a	65.3 ± 2.2c,d	82.7 ± 1.1c
+α-Tocopherol (100 ppm)	80.8 ± 0.7a	0.4 ± 0.6f	0.3 ± 1.8f	100.0 ± 0.0a	90.6 ± 0.6a	95.3 ± 0.3a
+α-Tocopherol (500 ppm)	63.5 ± 1.2b	−174.1 ± 0.6g	−158.9 ± 3.0g	100.0 ± 0.0a	51.8 ± 5.1e,f	75.9 ± 2.5d,e

[a]% Inhibition = [(C − S)/C] × 100; C = hydroperoxide or hexanal formed in control, and S = hydroperoxide or hexanal formed in sample. Negative values represent prooxidant activity. See Table 1 for abbreviation.

[b]Values within each column followed by the same letter are not significantly different ($P < 0.001$).

of PV it had no antioxidant activity at 100 ppm and had prooxidant activity at 500 ppm (Table 3).

DISCUSSION

The antioxidant mechanism recognized for phenolic compounds involves quenching of peroxyl (ROO•) radicals in the propagation step leading to the formation of hydroperoxides in the free radical sequence (21,22). These compounds also can inhibit further decomposition of hydroperoxides by reacting with alkoxyl radicals (RO•), which are responsible for the generation of volatile compounds such as hexanal contributing to rancidity (9). Our results showed that increasing the concentration of phenolic antioxidants to 100 ppm and above increased their inhibition of hydroperoxide decomposition but decreased their inhibition of hydroperoxide formation. Moreover, the effectiveness of the compounds varied according to the oxidation marker measured. Whereas caffeic acid was the most effective antioxidant based on either PV or hexanal formation, α-tocopherol and cinnamic acid at 100 ppm were more effective in inhibiting hexanal than PV formation. In fact, based on PV, these antioxidants, especially α-tocopherol, showed a prooxidant effect at high concentrations. In contrast, the mixture of phenols extracted from the virgin olive oils had one of the highest activities in inhibiting PV formation at 50 ppm, but one of the lowest activities in inhibiting hexanal formation.

Our results agree with those of other studies, which attribute the oxidative stability of olive oil to the antioxidant activity of *o*-diphenols (5,23). In fact, caffeic acid, an *o*-diphenol derived from cinnamic acid (Fig. 4), had the highest activity for inhibiting both PV and hexanal formation at the two concentrations tested. Ferulic acid, another derivative of cinnamic acid, with one of the two *ortho* hydroxyls substituted by a methoxyl group, was also a good antioxidant, either considering PV or hexanal formation. Vanillic acid is structurally similar to ferulic acid, but derived from benzoic acid instead of cinnamic acid, it also showed good antioxidant properties, especially in inhibiting hexanal formation. Derivatives of cinnamic acid seem to be better antioxidants than the corresponding benzoic acid derivatives, especially in preventing decomposition of hydroperoxides. The nonphenolic compound, cinnamic acid, detected in virgin olive oil, showed antioxidant activity in inhibiting hexanal formation, with higher activity than its monophenol derivatives. The *o*- and *p*-coumaric acids, which are monophenols derived from cinnamic acid, showed lower antioxidant activity than the *o*-diphenols, even when partially substituted. α-Tocopherol was not as good an antioxidant for the prevention of hydroperoxide formation as the other phenolic compounds, and at high concentrations showed a prooxidant effect, a fact already reported by other investigators in bulk oil (24) and in free fatty acid systems (25). However, α-tocopherol, at a concentration of 100 ppm, was the best inhibitor of hexanal formation in

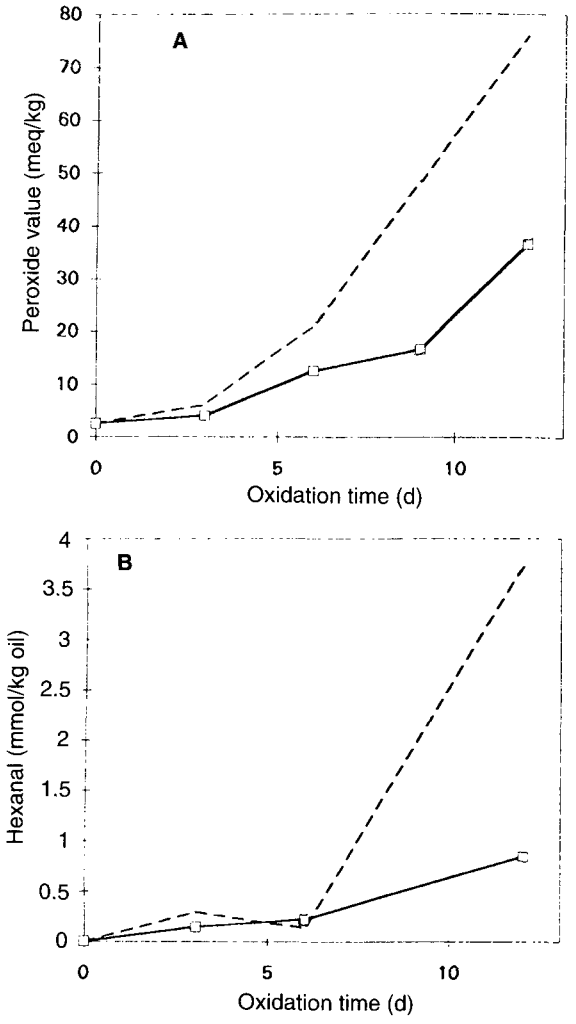

FIG. 3. Effect of phenolic extracts from virgin olive oil on oxidative stability of pure triacylglycerols (TAG) at 60°C: (A) peroxide value (n = 2); (B) hexanal formation (n = 2). See Figure 2 for other abbreviations. ----, TAG; -□-, + phenolic extract (133 ppm GAE).

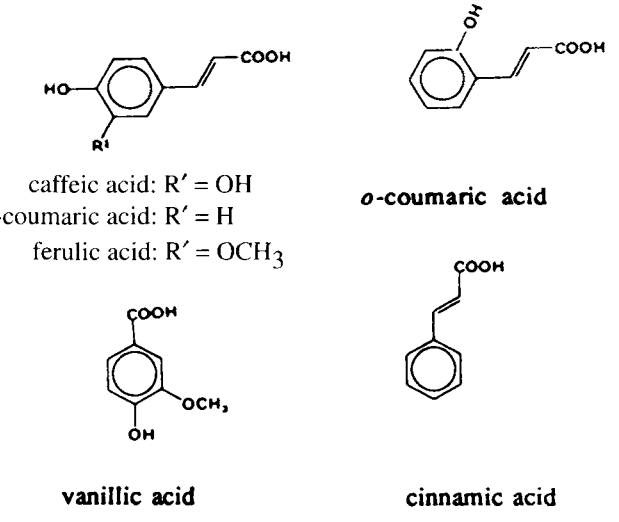

FIG. 4. Structures of the phenolic compounds used in the experiment.

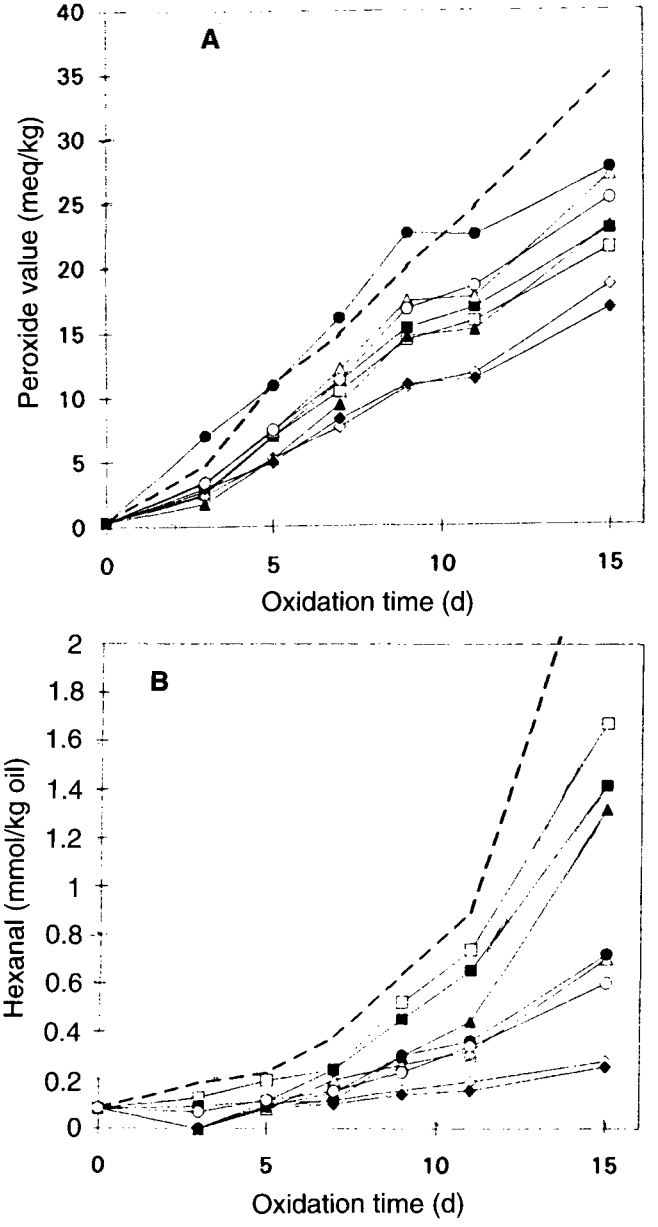

FIG. 5. Effect of pure phenolic compounds on oxidative stability of refined, bleached, and deodorized olive oil at 60°C: (A) peroxide value (n = 2); (B) hexanal formation (n = 2). ----, RBD olive oil; -□-, + *p*-coumaric (50 ppm GAE). -■-, + *p*-coumaric (100 ppm GAE); -◇-, + caffeic (50 ppm GAE); -◆-, caffeic (100 ppm GAE); -△-, + vanillic (50 ppm GAE); -▲-, + vanillic (100 ppm GAE); -○-, + cinnamic (50 ppm GAE); -●-, + cinnamic (100 ppm GAE). See Figures 1 and 2 for abbreviations.

olive oil. We have found previously that α-tocopherol behaves the same way in corn oil (18).

Hexanal formed from linoleate hydroperoxides was the major volatile found in our samples of olive oil oxidized at 60°C. On the other hand, heptanal, which is a product expected from the decomposition of oleate hydroperoxides (9), was not detected despite the high percentage of oleic acid in olive oil. To assess the influence of oxidation temperature on

volatile formation, we oxidized aliquots of the RBD olive oil at different temperatures. Heptanal formation was not detected at 60°C. However, oxidation at 100°C yielded heptanal, pentanal, heptane, and octane in addition to hexanal. In the oxidation of fish and vegetable oils, Frankel (26) found that different volatile compounds were formed according to the temperature used. These differences in volatile formation indicate that the formation and decomposition of different hydroperoxides vary significantly with temperature of oxidation. Therefore, studies of oxidative stability at high temperatures may not be extrapolated to ambient temperatures (10). The evaluation of oxidative stability should be carried out at temperatures as low as possible, depending on the oxidative susceptibility of the oil sample. Furthermore, since antioxidants show different activities toward hydroperoxide formation and decomposition, it is important that more than one method be used to monitor the oxidation process.

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