Antioxidant Activity of Almonds and Their By-products in Food Model Systems

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ABSTRACT: Antioxidant activities of almond whole seed, brown skin, and green shell cover extracts, at 100 and 200 ppm quercetin equivalents, were evaluated using a cooked comminuted pork model, a β-carotene-linoleate model, and a bulk stripped corn oil system. Retention of β-carotene in a β-carotene–linoleate model system by almond whole seed, brown skin, and green shell cover extracts was 84–96, 74–83, and 71–93%, respectively. In a bulk stripped corn oil system, green shell cover extract performed better than brown skin and whole seed extracts in inhibiting the formation of both primary and secondary oxidation products. In a cooked comminuted pork model system, green shell cover and brown skin extracts inhibited the formation of TBARS, total volatiles, and hexanal more effectively than did the whole seed extract. HPLC analysis revealed the presence of caffeic, ferulic, *p*-coumaric, and sinapic acids as the major phenolic acids in all three almond extracts examined.

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KEY WORDS: Almond extracts, antioxidant effects, β-carotene–linoleate model system, cooked pork model, phenolic acids, stripped corn oil.

Various methods have been used since antiquity to inhibit lipid oxidation and off-flavor development in food systems. The practices of hydrogenation of unsaturated FA (1), removal of oxygen through vacuum packaging (2), use of superoxide scavengers such as glucose oxidase and ascorbic acid oxidase (3), removal or sequestering of metal ions (4), irradiation (5), refrigeration and freezing (6), and use of antioxidants are among approaches that are commonplace for the control of lipid oxidation (7). Addition of spices or plant constituents in a powder form or as whole leaves or stems has been practiced from the very early days, although the mechanisms of action and active components involved are not always fully understood or identified. The bioactive phenolic compounds acting as antioxidants are substances that, when present at low concentrations compared with that of an oxidizable substrate, significantly delay or inhibit oxidation of that substrate (8). These antioxidants are now added intentionally to foods to prevent lipid oxidation and are either synthetic or natural in their origin. Synthetic antioxidants that are approved for use in food include phenolic compounds such as BHA, BHT, propyl gallate, TBHQ, and nonphenolics such as erythorbic acid, ascorbic acid, and ascorbyl palmitate (7). Natural antioxidants include naturally occurring amino acids and dipeptides (9), protein hydrolyzates (10), water-soluble proteins (11), phospholipids (12), inorganic salts (13), tocopherols and their derivatives, carotenoids, ascorbic acid, antioxidant enzymes, and a large number of phenolic compounds (8) of edible and nonedible plant parts.

Antioxidants present in food commodities provide protection against oxidative attack by intercepting singlet oxygen, decreasing the oxygen concentration, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products of oxidation to nonradical compounds, and chain-breaking substances to prevent continuous hydrogen abstraction from substrates (14). However, natural antioxidants may be depleted during the processing of foods. Food producers use antioxidants derived from natural or synthetic sources to extend the shelf life and improve the quality of processed foods. Having a variety of antioxidants in the diet would be more effective against oxidation as opposed to large amounts of just one or two compounds. Nuts are one of the most important sources of natural antioxidants that are known to possess health-promoting properties (15). Almonds, when incorporated in the diet, have been reported to reduce colon cancer risk in rats (16) and increase HDL cholesterol and reduce LDL cholesterol levels in humans (17). Almonds and their hulls and shells possess powerful free radical scavenging capacities (18), and these activities could be due to the triterpenoids, flavonoids, and phenolic acids (19–21) that are present in almond by-products. The production of almond hulls, which are mainly used in livestock feed, is estimated to exceed 6 million tons annually (19), thus being a potentially good source from which to extract antioxidants that are present, if any, in high quantities. Different solvent extractions are well known to provide different types of compounds because of their variable chemical nature and sensitivity toward extraction or hydrolysis methods. As seen with almonds, diethyl ether (19), methanol (21), ethyl acetate, and *n*-butanol (20), which have been used to extract phenolic compounds in almond skins or hulls, have resulted in different components in their extractions. The objectives of this study were to investigate the antioxidant efficacies of almond whole seed, brown skin, and outer green shell cover using different food model systems and to identify the phenolic acids present in their ethanolic extracts.

MATERIALS AND METHODS

Almond seeds, skin, and shell covers were obtained from the Almond Board of California (Modesto, CA). Corn oil stripped

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of its natural antioxidants was purchased from Acros Organics (Morris Plains, NJ). Sodium carbonate, sodium chloride, hexane, methanol, ethanol, TCA, sulfuric acid, hydrochloric acid, glacial acetic acid, butanol, isooctane, and chloroform were purchased from Fisher Scientific Co. (Nepean, Ontario, Canada). α-Tocopherol, sodium hydroxide, 2-TBA, 1,1,3,3-teramethoxyparopane, β-carotene, linoleic acid, Tween 40 (polyoxyethylene sorbitan monopalmitate), BHA, quercetin, ferrous sulfate, Folin and Ciocalteu's phenol reagent, mono- and dibasic sodium phosphate, vanillin, and tetramethylmurexide were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). Pentanal, hexanal, heptanal, and 2-heptanone were obtained from Aldrich Chemical Company (St. Louis, MO). Hexamethylenetetramine was purchased from J.T.Baker Inc. (Phillipsburg, NJ).

Preparation of almond seed, skin, and outer shell cover samples. Almond whole seed, brown skin, and green shell covers were ground in a coffee grinder (Black & Decker Canada Inc., Brockville, Ontario, Canada) for 10 min and then defatted by blending with hexane (1:5 wt/vol, 5 min × 3) in a Waring blender (Model 33BL73; Dynamics Corp. of America, New Hartford, CT) at room temperature. Defatted powders were air-dried for 12 h and then stored in vacuum-packaged polyethylene pouches at –20°C until used.

Preparation of crude phenolic extracts. Preliminary studies and close scrutiny of the literature data were used to determine extraction conditions to obtain the highest amounts of phenolic extracts from almonds and their co-products. Thus, phenolic compounds in defatted almond samples were extracted using 80% ethanol (6 g/100 mL) under reflux conditions in a thermostated water bath at 80°C for 30 min. The resulting slurries were centrifuged for 5 min at 4000 × *g* (ICE Centra MS; International Equipment Co., Needham Heights, MA) and the supernatants were collected. The residue was reextracted under the same conditions, and the supernatants were collected. The solvent from the combined supernatants was removed under vacuum at 40°C, and the resulting concentrated solutions were lyophilized for 72 h at –48°C and 46×10^{-3} mbar (Freezone 6, Model 77530; Labanco Co., Kansas City, MO).

Determination of total phenolics content. Extracts were dissolved in methanol to obtain a concentration of 1 mg/mL for seed extract and 0.5 mg/mL for brown skin and green shell cover extracts. The content of total phenolics was determined according to a modified version of the procedure described by Singleton and Rossi (22). Folin and Ciocalteu's reagent (0.5 mL) was added to centrifuge tubes containing 0.5 mL of the extracts. Contents were mixed and 1 mL of a saturated sodium carbonate solution was added to each tube. Volume was adjusted to 10 mL with distilled water and the contents were thoroughly mixed. Tubes were allowed to stand at ambient temperature for 45 min and then centrifuged for 5 min at 4000 × *g* (ICE Centra MS; International Equipment Co.). Absorbance of the supernatants was read at 725 nm. A blank sample was used for background subtraction in each case. The content of total phenolics in each extract was determined using a standard curve prepared for quercetin. Total extracted phenolics were expressed as milligrams of quercetin equivalents per gram of extract.

Determination of the content of hydrophilic and hydrophobic phenolics. The crude extract was fractionated into its hydrophilic and hydrophobic components by mixing 5 g extract with 100 mL water and 100 mL butanol in a 250-mL separatory funnel. The mixture was allowed to stand at 4°C for 12 h. The separated layers were removed and desolventized using a rotary evaporator (Büchi, Flawil, Switzerland) at 40°C. The resulting concentrated solution was lyophilized for 72 h at –48°C and 46×10^{-3} mbar (Freezone 6, Model 77530; Labanco Co.). The weight of each fraction was recorded and the content of phenolics determined as explained above.

β-Carotene-linoleate model system. A solution of 5 mg/10 mL β-carotene was prepared in chloroform, and 2 mL of this solution were pipetted into a 100-mL round-bottomed flask. After chloroform was removed under vacuum using a rotary evaporator at 40°C, 40 mg of linoleic acid, 400 mg of Tween 40 emulsifier, and 100 mL of aerated distilled water were added to the flask with vigorous shaking. Aliquots of 4.8 mL of this emulsion were transferred into a series of tubes containing 100 or 200 μL of the extracts (in methanol) so that the final concentration of phenolics in the assay media was 100 or 200 ppm. The total volume was adjusted to 5 mL with methanol. BHA and quercetin were used for comparative purposes. Immediately after the addition of the emulsion to each tube, the zero-time absorbance was measured at 470 nm using a Hewlett-Packard diode array spectrophotometer (Model 8452A; Hewlett-Packard Co., Mississauga, Ontario, Canada). Subsequent absorbance readings were recorded over a 2-h period at 15-min intervals by keeping the samples in a water bath at 50°C. Blank samples devoid of β-carotene were prepared for background subtraction (23). The content of β-carotene in assay media was determined using a standard curve prepared using β-carotene.

Cooked comminuted pork model system. Ground pork (1.5 kg) was mixed with 20% (w/w) deionized water in Mason jars (height 10 cm, i.d. 6 cm). Almond extracts (100 and 200 ppm based on phenolics content as well as BHA, α-tocopherol, and quercetin) were added separately to meat (100 g) that was then thoroughly homogenized. A control sample containing no extract was also prepared. Samples were cooked in a thermostated water bath at 80 ± 2°C (internal temperature of 72 ± 2°C) for 40 min while stirring every 5 min with a glass rod. After cooling to room temperature, meat systems were homogenized in a Waring blender for 30 s, transferred into plastic bags, and then stored in a refrigerator at 4°C for 7 d (13). Samples for the analyses of headspace gases and TBARS were drawn on days 0 and 7.

Determination of TBARS in meat. Samples were analyzed for TBARS on days 0 and 7, according to the method of Siu and Draper (24) with modifications. Two grams of each sample was weighed in a centrifuge tube to which 5 mL of a 10% (wt/vol) solution of TCA was added and vortexed (Fisher Vortex Genie 2; Fisher Scientific, Nepean, Ontario, Canada) at high speed for 2 min. An aqueous solution (0.02 M) of TBA (5 mL) was then added to each centrifuge tube, followed by further vortexing for

30 s. The samples were subsequently centrifuged at 3000 × *g* for 10 min and the supernatants were filtered through a Whatman No. 3 filter paper. Filtrates were heated in a boiling water bath for 45 min, cooled to room temperature in ice, and the absorbance of the resultant pink-colored chromogen read at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane as a precursor of the malonaldehyde. The TBARS values were then calculated using the standard curve and expressed as milligrams malonaldehyde equivalents per kilogram sample.

Static headspace GC analysis. A Perkin-Elmer 8500 gas chromatograph and an HS-6 headspace sampler (Perkin-Elmer Corporation, Montreal Québec, Canada) were used for volatile analyses of cooked comminuted lean pork samples. A high-polarity SUPELCOWAX 10 fused-silica capillary column (30 m × 0.32 mm i.d., 0.10 mm film thickness; Supelco Canada Ltd., Oakville, Ontario, Canada) was used. The carrier gas (helium) was used at an inlet column pressure of 17.5 psig with a split ratio of 7:1. The oven temperature was maintained at 40°C for 5 min and then ramped to 100°C at 20°C/min and held there for 5 min. The injector and FID temperatures were adjusted to 280°C and held there throughout the analysis (13).

For headspace analysis, 4.0 g of homogenized meat was transferred to 5-mL glass vials. The vials were then capped with Teflon-lined septa, crimped, and kept at −60°C (Ultra Low; Revco, Inc., West Columbia, SC) until used. To avoid heat shock after removal from storage, frozen vials were tempered at room temperature for 30 min and then preheated in the HS-6 magazine assembly at 90°C for a 45-min equilibrium period. Pressurization time of the vial was 6 s, and the volume of the vapor phase drawn was approximately 1.5 mL. Chromatographic peak areas were expressed as integrator count units. Individual volatile compounds were identified by comparing relative retention times of GC peaks with commercially available standards. Quantitative determination of dominant aldehydes was accomplished using 2-heptanone as an internal standard (13).

Bulk stripped corn oil model system. Extracts and standards (BHA, α-tocopherol, quercetin) and oil (5 g) were mixed well in 30-mL capped glass tubes (13 × 2 cm i.d.) so that the final concentrations of phenolics were 100 or 200 ppm. Samples were placed in a forced-air convection oven (Thelco, Model 2; Precision Scientific Co., Chicago, IL) at 60°C for 7 d. Samples for analyses of conjugated dienes and TBARS were drawn on days 0 and 7. The method used for determining volatiles was the same as that employed for the analysis of a meat model system, but only 200 mg of oil was used. TBARS were determined by a direct method using the AOCS (25) procedure.

Determination of conjugated dienes. Conjugated dienes of samples were measured according to the IUPAC method (26). Oil was weighed (0.02–0.04 g) into a 25-mL volumetric flask, diluted, and made up to the mark with isooctane (2,2,4-trimethylpentane). The solution was thoroughly mixed and its absorbance read at 243 nm using a Hewlett-Packard diode array spectrophotometer (Model 8452A; Agilent, Palo Alto, CA). Pure isooctane was used as the reference. Conjugated dienes were calculated as CD = $A/(c \cdot d)$, where A is the absorbance of the solution at 234 nm; c is the concentration of the oil in g/100 mL; and d is the length of the cell (cm).

HPLC analysis of almond crude extracts. Free phenolic acids and those liberated from soluble esters and glycosides were isolated from the extracts according to the method described by Naczk and Shahidi (27). An aqueous suspension of the extract (800 mg in 20 mL water) was adjusted to pH 2 (6 M HCl), and free phenolic acids were extracted five times into 20 mL diethyl ether using a separatory funnel. The ether extract was evaporated to dryness under vacuum at room temperature. The aqueous solution was neutralized and then lyophilized. The residue was dissolved in 20 mL of 2 M NaOH and hydrolyzed for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, phenolic acids released from soluble esters were extracted from the hydrolyzate five times into 30 mL diethyl ether using a separatory funnel. To the water solution was added 15 mL of 6 M HCl, and the solution obtained was placed under a nitrogen atmosphere and hydrolyzed for 1 h in a water bath at 100°C. Phenolic acids released from soluble glycosides were separated from the hydrolyzate five times into 45 mL diethyl ether. After ether evaporation, the dry residue was dissolved in 10 mL methanol and filtered through a 0.45-μM nylon filter. A Shimadzu HPLC system (Mandel Scientific Co., Ltd., Guelph, Ontario, Canada) was used (two LC 10AD pumps, SPD M10A diode array detector, SACL AA system controller, CTO 10AS column oven) for the separation of phenolic acids. The separation conditions were as follows: prepacked LiChrospher 100 RP-18 column (5 μM, 4 × 250 mm; Merck, Darmstadt, Germany); mobile phase, water–acetonitrile–acetic acid (88:10:2, by vol); flow rate, 1 mL/min: injection volume, 20 μL; detector, 320 nm; and column temperature, 30°C. All phenolic acids were quantified using the external standard method, and the quantification was based on peak area. Calibration curves of the standards were made by diluting stock solutions in methanol to yield 10–80 μg/L of phenolic acids. Identity and purity of the sample peaks were monitored by comparing spectral data of the sample peaks with that of the phenolic acid standards and monitoring retention times and symmetry of the peaks.

Statistical analysis. All experiments were carried out in triplicate. The significance of differences among mean values was determined at $P \leq 0.05$ using ANOVA followed by Tukey's least honest significant difference test.

RESULTS AND DISCUSSION

Phenolic contents of almond extracts. The total phenolic contents of almond whole seed, brown skin, and green shell cover extracts were 8 ± 1, 88 ± 2, and 71 ± 2 mg quercetin equivalents/g ethanolic extract, respectively. The weight ratios of hydrophilic to hydrophobic phenolics were 1:2, 3:8, and 2:5 for whole seed, brown skin, and green shell cover extracts, respectively. Generally, the hydrophobic phenolic contents of the extracts were two to three times higher than those of their hydrophilic counterparts. The antioxidant activity of the extracts

depends on the type of oxidizable substrate and the composition of the model system used. Activity in a particular model system is generally influenced by the partitioning properties of the antioxidants between the lipid and aqueous phases. Both hydrophobic and hydrophilic compounds present in a mixture of antioxidants are responsible for the observed antioxidant activity (28). Because a crude plant extract contains a mixture of compounds with different polarities, they can be expected to exhibit varying antioxidant capacities, depending on the model system used. Therefore, to gain a better understanding of the mechanisms involved in their antioxidant activities, various model systems were used to evaluate the efficacy of almond extracts; the results obtained are presented and discussed in the following sections.

β-Carotene–linoleate model system. In this model system, β-carotene is subjected to bleaching because of oxidation by free radicals formed from linoleic acid. By neutralizing the linoleate free radical, the presence of a phenolic antioxidant hinders the extent of β-carotene bleaching. Because the change in β-carotene content after 120 min was marginal, the retention of β-carotene over 120 min of assay was used to evaluate the efficacies of the extracts. The initial mass of β-carotene in the assay media (5 mL) was 75 ± 2 μg. The control sample devoid of any additive lost 98% of its initial β-carotene after 120 min of assay. We observed that the amount of β-carotene in the control, as measured by the absorbance at 470 nm, was reduced exponentially. In contrast, in the presence of additives, this reduction followed a second order polynomial pattern, exhibiting a significantly ($P \leq 0.05$) high retention of β-carotene compared with the control. The retention was more pronounced at the 200 ppm level compared (Fig. 1) with that at the 100 ppm level (Fig. 2). The highest retention (98% of the initial content) of β-carotene was observed for quercetin at 200 ppm, and the activities of whole seed extract and BHA at the same concentrations were comparable to that of quercetin. Brown skin extract, which had the lowest activity, was still able to retain 83% of the initial β-carotene content.

The β-carotene–linoleate model is similar to an oil-in-water emulsion system, and variations in activities could be attributed to differences in the proportion of hydrophobic and hydrophilic compounds present in each extract. The hydrophobic antioxidants are likely to perform more efficiently than hydrophilic antioxidants in preventing oxidation in oil-in-water emulsion systems by orienting in the oil and at the oil–water interface. This is true when considering the effectiveness of β-carotene retention by quercetin and BHA, which are lipophilic in nature. However, this pattern was not observed with the almond extracts. Whole seed extract, with the highest proportion of hydrophilic compounds compared with extracts of brown skin and green shell cover, was the most effective, whereas brown skin extract, which had the highest proportion of hydrophobic compounds, was least effective in preventing the bleaching of β-carotene. Even though the hydrophilic compounds are diluted in the aqueous phase, if present in high quantities, they still can reduce the concentration of oxygen-derived free radicals in the aqueous phase. Some of the phenolic compounds may exist in their glycosylated form, which would be measured as hydrophilic compounds, but the glycosylated phenolic components have not always been able to render antioxidative activity (29). Another possibility is the presence of potent hydrophobic antioxidants in the extract, which even in small concentrations could effectively inhibit lipid oxidation. The variation may also be due to linoleic acid, which behaves in a different manner when compared with other lipids. Although nonpolar compounds generally tend to remain within the oil phase in an emulsion, linoleic acid has been observed to form micelles that would allow it to move between phases in an emulsion system (30). As such, hydrophobic as well as hydrophilic groups of compounds could exert protective effects against linoleate oxidation and thereby prevent the bleaching of β-carotene.

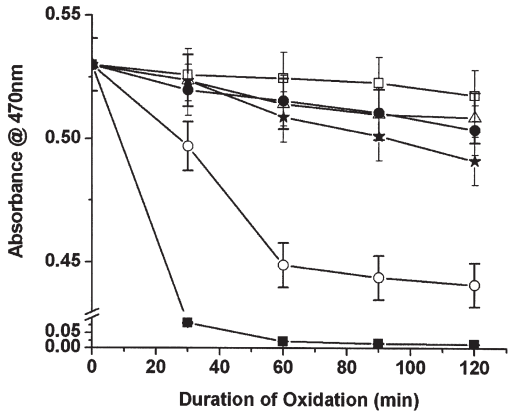

FIG. 1. Retention of β-carotene in a β-carotene–linoleate model system at 50°C for 120 min by almond extracts at 200 ppm, as quercetin equivalents. (Symbols are: ■, control; □, quercetin; ●, BHA; △, whole seed extract; ○, brown skin extract; ★, green shell cover extract).

Cooked comminuted pork model system. All three almond extracts showed higher activity at a higher concentration in lowering TBARS, hexanal, and total volatiles levels of meat samples upon storage. The control with no additives showed

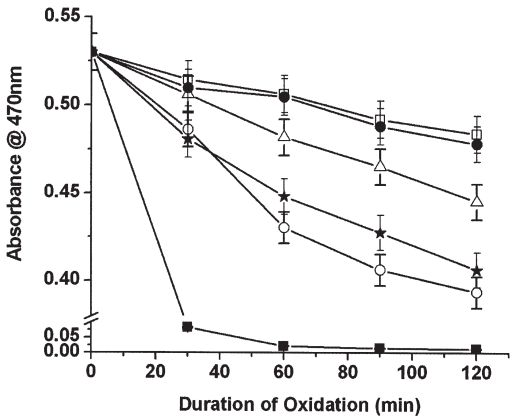

FIG. 2. Retention of β-carotene in a β-carotene–linoleate model system at 50°C for 120 min by almond extracts at 100 ppm, as quercetin equivalents. (Symbols are: ■, control; □, quercetin; ●, BHA; △, whole seed extract; ○, brown skin extract; ★, green shell cover extract).

TABLE 1
TBARS (as mg malonaldehyde equivalents/kg sample), Hexanal Content (mg/kg sample), and Total Volatiles Content (mg/kg sample) of Almond Extracts and Reference Antioxidants at 200 ppm as Quercetin Equivalents in a Meat Model System on Days 0 and 7 of Storage at 4°C[a]

	TBARS		Hexanal content		Total volatiles content	
Sample	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Control	1.2 ± 0.1[a]	23.2 ± 1.2[e]	6.3 ± 0.2[d]	25.7 ± 1.4[d]	25.3 ± 1.1[e]	64.2 ± 2.3[e]
Whole seed extract	1.0 ± 0.1[a]	10.6 ± 0.8[c,d]	3.3 ± 0.2[b]	7.2 ± 0.3[b,c]	15.6 ± 0.9[d]	42.9 ± 1.9[d]
Brown skin extract	1.1 ± 0.1[a]	9.3 ± 0.5[c]	4.1 ± 0.1[c]	8.3 ± 0.4[c]	12.9 ± 0.9[c]	34.7 ± 1.6[c]
Green shell cover extract	1.1 ± 0.1[a]	12.2 ± 0.5[d]	1.9 ± 0.1[a]	6.8 ± 0.2[b,c]	11.9 ± 0.8[b,c]	20.7 ± 1.5[b]
Quercetin[b]	1.0± 0.1[a]	2.5 ± 0.3[a]	2.0 ± 0.1[a]	3.3 ± 0.1[a]	6.8 ± 0.7[a]	12.4 ± 0.8[a]
α-Tocopherol[b]	1.0 ± 0.1[a]	4.8 ± 0.4[b]	2.0 ± 0.1[a]	6.5 ± 0.2[b]	10.9 ± 0.9[b,c]	18.5 ± 1.1[b]
BHA[b]	1.1 ± 0.1[a]	2.6 ± 0.2[a]	2.2 ± 0.1[a]	5.6 ± 0.1[b]	10.2 ± 0.9[b]	18.2 ± 1.0[b]

[a]Results are mean values of three determinations ± SD. Means in a column sharing the same roman superscript are not significantly ($P > 0.05$) different from one another.
[b]Reference antioxidants.

the highest values for all three indices employed. The trends by the additives in inhibiting oxidation, as evaluated by the inhibition of TBARS, hexanal, and total volatiles formation, varied slightly on different days of storage; therefore, results at the end of the storage period were used to elaborate the activity difference exerted by the additives. Almond extracts at 100 and 200 ppm levels inhibited the formation of TBARS, hexanal, and total volatiles by 2–36 and 22–74%, 20–44 and 54–76%, and 1–23 and 42–70%, respectively. Table 1 shows the activity of additives at the 200 ppm level on days 0 and 7 of storage. The inhibition of TBARS formation by brown skin extract was significantly ($P \leq 0.05$) higher than those of whole seed extract and green shell cover extract, but was significantly ($P \leq 0.05$) lower than those of the reference antioxidants, namely, quercetin, α-tocopherol, and BHA. For hexanal and total volatiles formation, the inhibition by green shell cover extract was not significantly ($P > 0.05$) different from those of BHA and a-tocopherol. The inhibition of hexanal formation at 200 ppm at the end of the storage period was in the order: quercetin > α-tocopherol ~ BHA ~ green shell cover extract > brown skin extract > whole seed extract. The inhibition of total volatiles formation by the additives followed the same order, but the observed differences between green shell cover extract and brown skin extract and between brown skin extract and whole seed extract were not significantly ($P > 0.05$) different, whereas values for green shell cover extract and whole seed extract were significantly ($P < 0.05$) different from one another. Thus, it may be concluded that green shell cover extract and brown skin extract are more efficient than whole seed extract in inhibiting lipid oxidation in a pork model system. Because both hydrophobic and hydrophilic compounds are present in substantial amounts in the extracts, they could effectively inhibit lipid oxidation.

Bulk corn oil system. Stripped corn oil was used to evaluate the efficacy of different extracts. Inhibition of the formation of conjugated dienes (primary oxidation products), hexanal, and TBARS (secondary oxidation products) in treated oil samples was used as an indicator of antioxidant activity of the additives of concern.

The conjugated diene, hexanal, and TBARS values of bulk stripped corn oil containing additives at 200 ppm on days 0 and 7 of storage are given in Table 2. The most effective additive in reducing the oxidation level was quercetin and the least active was α-tocopherol. The conjugated diene values of bulk stripped corn oil increased by four- to fivefold at the end of the 7-d storage period, whereas the control samples showed a sixfold increase. At 100 and 200 ppm levels, the additives inhibited the formation of conjugated dienes by 0–38 and 3–55%, respectively. The oxidation inhibitory activity of the extracts and controls used decreased in the order: quercetin > green shell cover extract > BHA > whole seed extract > brown skin extract > α-tocopherol. Hexanal content in oil samples increased throughout

TABLE 2
Conjugated Diene Values, Hexanal Content (mg/kg sample), and TBARS (as μmol malonaldehyde equivalents/g sample) of Almond Extracts and Reference Antioxidants at 200 ppm as Quercetin Equivalents in a Bulk Stripped Corn Oil System on Days 0 and 7 of Storage at 60°C[a]

	Conjugated dienes		Hexanal content		TBARS	
Sample	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Control	2.24 ± 0.12[a]	13.65 ± 0.45[e]	1.1 ± 0.1[a]	105.3 ± 6.8[d]	0.29 ± 0.02[a]	1.41 ± 0.09[f]
Whole seed extract	2.21 ± 0.10[a]	10.33 ± 0.36[c,d]	1.0 ± 0.2[a]	18.7 ± 1.1[b]	0.26 ± 0.03[a]	0.75 ± 0.04[c,d]
Brown skin extract	2.33 ± 0.15[a]	10.86 ± 0.34[d]	1.0 ± 0.1[a]	16.3 ±.1.9[b]	0.28 ± 0.01[a]	0.81 ±.0.04[d]
Green shell cover extract	2.11 ± 0.21[a]	8.35 ± 0.21[b]	1.2 ± 0.1[a]	10.2 ± 1.2[a]	0.27 ± 0.02[a]	0.66 ± 0.03[b,c]
Quercetin[b]	2.25 ± 0.12[a]	6.23 ± 0.20[a]	1.1 ± 0.1[a]	5.4 ± 0.8[a,b]	0.26 ± 0.02[a]	0.37 ± 0.05[a]
α-Tocopherol[b]	2.32 ± 0.18[a]	12.93 ± 0.35[e]	1.2 ± 0.1[a]	67.7 ± 3.5[c]	0.26 ± 0.02[a]	1.14 ± 0.05[e]
BHA[b]	2.25 ± 0.12[a]	9.83 ± 0.12[c]	1.3 ± 0.1[a]	14.7 ± 1.1[b]	0.26 ± 0.01[a]	0.57 ± 0.02[b]

[a]Results are mean values of three determinations ± SD. Means in a column sharing the same roman superscript are not significantly ($P > 0.05$) different from one another.
[b]Reference antioxidants.

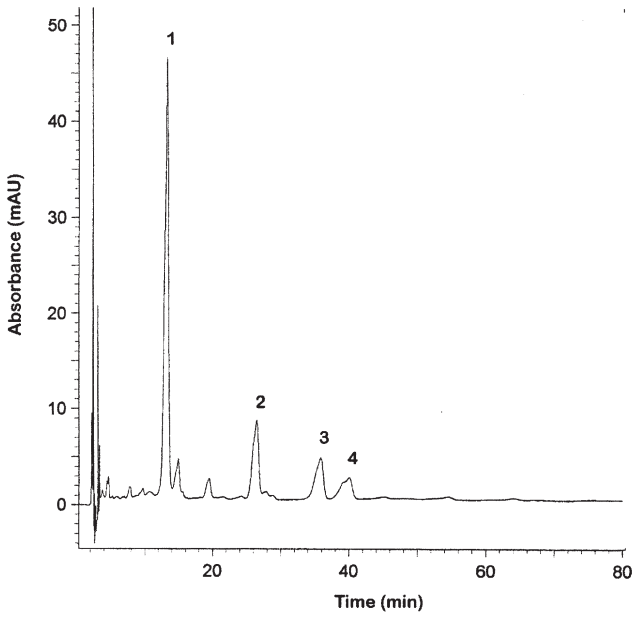

FIG. 3. HPLC profile of phenolic acids liberated from esters in almond shell cover extract detected at 330 nm. (Peaks are: 1, caffeic acid; 2, *p*-coumaric acid; 3, ferulic acid; and 4, sinapic acid).

the storage period, but there was a significant ($P \leq 0.05$) decrease in their formation when additives were used, compared with those of the control. At the higher concentration level, almond extracts were able to reduce the formation of hexanal in corn oil by 82–93%. The inhibition of hexanal formation by the additives used decreased in the order of quercetin > green shell cover extract ~ brown skin extract ~ whole seed extract ~ BHA > α-tocopherol. The TBARS values of the control showed a fivefold increase at the end of a 7-d storage period; with the addition of almond extracts, there was only a two- to threefold increase at 200 ppm. On day 7 of storage at 100 and 200 ppm levels, the additives inhibited TBARS by 28–55 and 19–74%, respectively; thus, the order of antioxidant activity, as indicated by the inhibition of TBARS, followed a trend similar to that observed for conjugated dienes, but BHA showed a significantly ($P \leq 0.05$) higher inhibition than that of green shell cover extract. These results show that green shell cover extract was more effective than whole seed or brown skin extracts in reducing both the primary and secondary oxidation of the bulk oil system. α-Tocopherol, a lipophilic antioxidant, exerted the least protection against the oxidation of bulk corn oil, which could be attributed to its uniform distribution in the bulk lipid phase rather than orienting at the oil–air interface, where better protection could be attained. However, crude extracts containing a mixture of both hydrophobic and hydrophilic compounds are better able to protect the oil against oxidation.

The foregoing results show that almond extracts exerted antioxidative effects comparable to or better than BHA, at the same concentration, in β-carotene–linoleate, bulk corn oil, and cooked comminuted pork model systems. As described next, the identified phenolic acids may be responsible, in part, for the observed antioxidant effects of the extracts examined.

Phenolic acids in almond extracts. In general, all almond extracts had the same array of phenolic acids, i.e., caffeic, *p*-coumaric, ferulic, and sinapic acids, with retention times of 12.7, 25.3, 36.8, and 40.7 min, respectively. Figure 3 depicts a typical HPLC profile of the phenolic acids obtained for green shell cover extract. Substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids present in many foods of plant origin, and their derivatives differ in hydroxylation and methoxylation patterns of their aromatic rings (31). The phenolic acids tentatively identified in almonds were derivatives of cinnamic acid.

The majority of these phenolic acids, more appropriately known as phenyl proponoids, were found in the form of soluble esters, as shown in Table 3. Total amounts of the identified free phenolic acids in brown skin and green shell cover extracts were 16.28 ± 0.03 and 13.99 ± 0.03 μg/g extract, respectively, whereas whole seed extract contained only trace amounts. Total esterified phenolic acids in the whole seed, brown skin, and green shell cover extracts were 40.34 ± 0.05, 279.55 ± 1.01, and 967.10 ± 1.74 mg/g extract, respectively. Green shell cover extract served as a better source of phenolic acids (approximately 0.1% of the extract) compared with whole seed and brown skin extracts. Caffeic acid was the predominant phenolic acid present in brown skin extract and in green shell cover extract in the bound form, but was present only in trace amounts in the free form, a pattern observed also for fruits (31).

The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule (32). Hydroxylated cinnamic acids were found to be more effective than their benzoic acid counterparts (31). Caffeic acid,

TABLE 3
Phenolic Acid Contents of Almond Extracts[a]

	Quantity of phenolic acids in almond extracts (μg/g extract)					
	Whole seed extract		Brown skin extract		Green shell cover extract	
Compound	Free	Esterified	Free	Esterified	Free	Esterified
Caffeic	Trace	4.34 ± 0.01[b]	Trace	160.09 ± 0.95[d]	Trace	653.52 ± 1.25[d]
p-Coumaric	Trace	3.09 ± 0.01[a]	4.55 ± 0.02[b]	64.17 ± 0.35[c]	1.34 ± 0.01[a]	195.56 ± 1.22[c]
Ferulic	Trace	23.88 ± 0.05[d]	2.19 ± 0.01[a]	31.92 ± 0.15[b]	2.71 ± 0.02[b]	76.27 ± 0.56[b]
Sinapic	Trace	8.98 ± 0.03[c]	9.51 ± 0.03[c]	22.36 ± 0.56[a]	9.92 ± 0.02[c]	40.01 ± 0.44[a]

[a]Results are mean values of three determinations ± SD. Means in a column sharing the same roman superscript are not significantly ($P > 0.05$) different from one another.

which has two hydroxyl groups, was more effective than *p*-coumaric acid, which has only one hydroxyl group, in preventing oxidation of a stripped corn oil system (33). The electron-withdrawing effect of the carboxylic acid group is greatly reduced in the presence of a –CH=CH– moiety in phenyl proponoids. However, the –CH=CH– moiety contributes to the radical stabilization of these acids through resonance, which has a positive influence on their overall antioxidant efficacy (34). Ferulic acid has been reported to exhibit a lower antioxidant activity than caffeic acid (35), possibly due to its methoxylation in the *ortho* position, which could possibly result in a decrease in its radical-scavenging or transition metal-chelating ability. Hydroxylation, as in caffeic acid, in place of methoxylation has been shown to be more effective in certain cases (36,37). However, the antioxidant activity increased in the order of caffeic < *p*-coumaric < ferulic < sinapic in preventing accelerated autoxidation of methyl linoleate, indicating an increased antioxidant efficacy of phenolic acids with methoxy substitutions (38). This may be due to a better electron-donationg activity by a methoxy as compared with a hydroxyl group. The presence of methoxy substitutions in the 3- and 5-positions, as in sinapic acid, may impose a negative effect on the hydrogen-donating ability of the molecule, but the orientation of functional groups in the 3-, 4-, and 5-positions enhances the antioxidant efficacy of the molecules as metal chelators (31). A study conducted to investigate the protection exerted by monomeric hydroxycinnamates against oxidation of human LDL revealed that antioxidant activity decreased in the order of caffeic acid > sinapic acid > ferulic acid > *p*-coumaric acid (15). In studies related to *in vitro* human LDL oxidation, the antioxidant activity was improved as the number of hydroxyl and methoxy groups increased, and the presence of *o*-dihydroxy groups in the phenolic ring, as in caffeic acid, enhanced the antioxidant activity (39). Because caffeic acid has better antioxidant properties and is present in high quantities, it could be said that its presence in brown skin and green shell cover extracts may have been partly responsible for preventing lipid oxidation in the cooked pork and bulk corn oil model systems. Almond green shell cover extract, which contains a high phenolic acid content, could serve as a valuable source for phenolic acids.

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