Antiradical Activity of Extracts of Almond and Its By-products

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ABSTRACT: Antioxidant activities of ethanolic extracts of whole almond seed, brown skin, and green shell cover were evaluated using different free radical trapping assays. Trolox equivalent antioxidant capacity assay revealed that the total antioxidant capacities of brown skin and green shell cover extracts were 13 and 10 times greater than that of the whole seed extract at the same extract concentration. The free radical-scavenging activity of extracts of brown skin and green shell cover also exceeded that of the whole seed. The scavenging activity of superoxide radical by different almond extracts ranged from 76 to 97% at 100 ppm and 85 to 99% at 200 ppm. The corresponding reduction of hydrogen peroxide concentration was 59–66% (100 ppm) and 86–91% (200 ppm). The hydroxyl radical-scavenging capacities at 100 and 200 ppm were 16 and 42% for whole seed, 57 and 100% for brown skin, and 40 and 56% for green shell extracts, respectively. A 100% scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was observed for brown skin and green shell extracts at 100 and 200 ppm concentrations, respectively, and whole seed extracts scavenged 21 (at 100 ppm) and 73% (at 200 ppm) of the DPPH radical.

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KEY WORDS: Almond, antioxidant, DPPH, EPR, free radical scavenging, hydroxyl, superoxide, TEAC.

Living cells aerobically generate energy by reducing molecular oxygen (O_2) to water by a process that involves cytochrome c oxidase-catalyzed reactions and flavin enzymes. Transfer of four electrons to this oxygen generates superoxide radical ($O_2^{\bullet-}$), which acts in activated animal phagocytes to kill bacteria (1). However, aerobic organisms are susceptible to the damaging actions of $O_2^{\bullet-}$, hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) that are formed during the metabolism of oxygen if not neutralized.

Free radicals that are generated in the human body as part of its metabolic function may lead to oxidative stress when produced excessively or not adequately neutralized, which may in turn cause tissue damage (2). Inflammatory cells such as eosinophils, neutrophils, monocytes, and macrophages become activated during inflammation and produce oxygen radicals (3). These radicals play an important role in asthma and bronchial hyperresponsiveness (2). Rheumatoid arthritis also is believed to be initiated by free radicals (4,5).

Superoxide dismutase (SOD) is responsible for removing $O_2^{\bullet-}$ by converting it to H_2O_2, which is less toxic to tissues. Hydrogen peroxide, present in aerobic cells as a metabolite in low concentrations, is generated by nonenzymatic and SOD-catalyzed dismutation reactions (6). H_2O_2 is also the substrate for two enzymes, catalase and glutathione peroxidase, that catalyze the conversion of H_2O_2 to water and oxygen, thus displaying a detoxification mechanism. Moreover, H_2O_2 is converted by myeloperoxidase in neutrophils to hypochlorous acid (1). The biologically significant reaction of H_2O_2 is its spontaneous conversion, catalyzed by Fe^{2+} (Fenton reaction), to the highly reactive $HO^{\bullet}$, which reacts instantaneously with any biological molecule (BH) from which it can abstract a hydrogen atom. The resulting free radical ($B^{\bullet}$) is more stable and hence longer-lived than $HO^{\bullet}$. When the human body fails to counteract the effects of free radicals through the prevention mechanisms afforded by the antioxidant systems in the body, they may result in carcinogenic, atherosclerotic, and other physiologically unfavorable conditions. To balance the levels of reactive oxygen species (ROS) and to defend the cellular components from damage, enzymes such as SOD, catalase, and glutathione peroxidase play a major role in defense mechanisms (7). These enzymes detoxify hydrogen peroxide and hydroperoxides to water or their corresponding alcohols. Among the plant-derived antioxidants, flavonoids possess potent activities in scavenging ROS and reducing the risk associated with cardiovascular diseases (8,9). Plant phenolics have been shown to enhance the *in vivo* activity of liver glutathione-S-transferase and NAD(P)H-quinone reductase, which are enzymes involved in detoxification of carcinogens and xenobiotics in the body (10).

Phenolic compounds, commonly found in both edible and nonedible plant parts, display multiple biological effects, including radical-scavenging activity. Nuts and extracts of their hulls are able to reduce risks of cancer, diabetes, and other chronic diseases (11); such properties may be attributed to the effect of their bioactive compounds such as flavonoids, isoflavones, and other phenolics (12). No particular antioxidant can exert all possible antioxidant mechanisms in order to combat oxidation. The types of free radical reactions that can be neutralized by an antioxidant are therefore important in determining their usefulness.

The occurrence, content, and type of phenolics in oilseeds and nuts is dictated by the species involved. Phenolic acids, phenylpropanoids, and flavonoids as well as lignans and tannins are often found in canola, rapeseed, and mustard (13–16), soybean (17), sunflower (18), flax (14,19), sesame (14,20), borage (21), and evening primrose (22). In almond hulls, the presence of catechin, protocatechuic acid, and a prenylated benzoic acid, 2-prenyl-4-*O*-β-D-glucopyranosyloxy-4-hydroxybenzoic acid, was reported (23). In hazelnuts, gallic acid, *p*-hydroxybenzoic acid, sinapic acid, and quercetin

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as well as caffeic acid or epicatechin were tentatively identified (24).

The objective of this study was to investigate the free radical scavenging activities of extracts of whole almond seed, brown skin, and the outer green shell cover. This information would be important in using almond during preparation of medical or health foods as potential curative agents for specific disease conditions.

MATERIALS AND METHODS

Materials. Almond seeds, brown skin, and green shell covers were obtained from the Almond Board of California (Modesto, CA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Acros Organics (Fair Lawn, NJ). Reagents sodium carbonate, hexane, and ethanol were purchased from Fisher Scientific Co. (Nepean, Ontario, Canada). Catechin, ferrous sulfate, mono- and dibasic sodium phosphate, nitro blue tetrazolium, 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH), Folin and Ciocalteau's phenol reagent, vanillin, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide, xanthine oxidase, hypoxanthine, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario).

Methods. (i) Preparation of crude phenolic extracts. Almond seed, skin, and shells were ground in a coffee grinder (Black and Decker Canada Inc., Brockville, Ontario) for 10 min and then defatted by blending with hexane (1:5 wt/vol, 5 min × 3) in a Waring blender (Model 33BL73) at ambient temperature. Phenolic compounds present in defatted samples were extracted using 80% ethanol (6 g sample/100 mL solvent) under reflux condition in a thermostated water bath at 80°C for 30 min. The resulting slurries were centrifuged at $4000 \times g$ for 5 min and the supernatants collected. The residue was reextracted under the same condition, and the supernatants were combined. The solvent was then removed under vacuum at 40°C and the resulting slurry lyophilized for 72 h at –48°C and 46×10^{-3} mbar (Freezone 6, Model 77530; Labconco Co., Kansas City, MO).

(ii) Determination of total phenolics content. Almond crude extracts were dissolved in methanol to obtain a concentration of 1 mg/mL for seed and 0.5 mg/mL for skin and shell extracts. The content of total phenolics was determined according to a modified version of the procedure described by Singleton and Rossi (25). Folin and Ciocalteau'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 solution of sodium carbonate 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 at $4000 \times g$ for 5 min. Absorbance of the supernatants was read at 725 nm. A blank sample for each extract was used for background subtraction. Content of total phenolics in each extract was determined using a standard curve prepared for catechin. Total extracted phenolics were expressed as mg catechin equivalents/g extract.

(iii) Qualitative detection of vanillin-positive compounds in almond extracts. One milliliter of a methanolic solution of crude extracts was mixed with 5 mL of a freshly prepared 0.5% vanillin solution in 4% hydrochloric acid and allowed to stand for 20 min at 30°C. A positive test was indicated by a characteristic pink color of the solution (26).

(iv) Measurement of total antioxidant capacity by Trolox equivalent antioxidant capacity (TEAC) assay. Total antioxidant capacity was determined according to the method described by van den Berg *et al.* (27). All solutions were prepared in a 0.1 M phosphate buffer (pH 7.4) solution containing 0.15 M sodium chloride (PBS). A solution of ABTS radical anion ($ABTS^{\bullet-}$) was prepared by mixing 2.5 mM AAPH with 2.0 mM $ABTS^{2-}$ stock solution. The solution was heated for 12 min at 60°C, protected from light, and stored at room temperature. Blank measurements of $ABTS^{2-}$ stock solution (decrease in the absorption at 734 nm due to the solvent) were made and values recorded each time. To measure the antioxidant capacity, almond extracts were dissolved in PBS solution at a concentration of 2 mg/mL and diluted so as to fit in the range of the assay values. A standard curve was prepared by measuring the reduction in absorbance of the $ABTS^{\bullet-}$ solution at different concentrations of Trolox over a period of 6 min, as the change in absorbance after 6 min of assay was marginal. The absorbance values were corrected for the solvent. TEAC values for the almond extracts were determined in the same way; 40 µL of the extract solution was mixed with 1960 µL of the radical solution, and the absorbance was monitored for 6 min. The TEAC of an extract represents the concentration of a Trolox solution that has the same antioxidant capacity as the extract. TEAC values were determined as follows:

$$\Delta A_{\text{Trolox}} = \left(A_{t=0\ \text{Trolox}} - A_{t=6\ \text{min Trolox}}\right) - \Delta A_{\text{solvent (0–6 min)}} \quad [1]$$

$$\Delta A_{\text{Trolox}} = m \cdot [\text{Trolox}] \quad [2]$$

$$\text{TEAC}_{\text{extract}} = \left(\Delta A_{\text{extract}}/m\right) \cdot d \quad [3]$$

where ΔA = reduction of absorbance, A = absorbance at a given time, m = slope of the standard curve, [Trolox] = concentration of Trolox, d = dilution factor.

(v) Hydrogen peroxide-scavenging assay. A modified version of the method described by Ruch *et al.* (28) was used to determine the hydrogen peroxide-scavenging ability of almond extracts. Extracts were dissolved in 3.4 mL of a 0.1 M phosphate buffer (pH 7.4) solution and mixed with 600 µL of a 43 mM solution of hydrogen peroxide (prepared in the same buffer). Catechin was used as the reference compound. Final concentrations of extracts and standards were 100 or 200 ppm. The concentration of hydrogen peroxide was measured by reading the absorbance values at 230 nm of the reaction

mixtures at 0 min and then at 10, 20, 30, and 40 min. For each extract concentration, a separate blank sample devoid of hydrogen peroxide was used for background subtraction. Reduction of absorbance in a hydrogen peroxide solution alone due to its degradation was recorded and values were corrected accordingly. The concentration of hydrogen peroxide in the assay medium was determined using a standard curve, and hydrogen peroxide-scavenging capacities of the extracts were calculated using the following equation:

$$H_2O_2\text{-scavenging capacity (\%)} = 100 - \left(\frac{H_2O_2 \text{ concentration of medium containing the additive}}{H_2O_2 \text{ concentration of the control medium}}\right)\times 100 \quad [4]$$

(vi) Hydroxyl radical-scavenging assay. A modified version of the method described by Shi *et al*. (29) was used to determine the hydroxyl radical-scavenging ability of almond extracts. Hydroxyl radicals were generated *via* iron-catalyzed Haber–Weiss reaction and spin-trapped with DMPO. The resultant DMPO-OH adduct was detected using an EPR spectrometer (Bruker ESP 300, Bruker Instruments, Inc., Billerica, MA). Almond extracts and catechin were dissolved in 0.1 M phosphate buffer (pH 7.4) so that a 200 µL aliquot would afford 100 or 200 ppm of phenolics in the final assay medium (final volume was 800 µL). Extracts (200 µL) were mixed with 200 µL of 0.075 mM DMPO, 200 µL of 2.5 mM ferrous sulfate, and 200 µL of 2.5 mM hydrogen peroxide. All solutions were prepared in a 0.1 M phosphate buffer (pH 7.4) solution. After 3 min, 100 µL of the mixture was taken into a syringe and transferred into a quartz capillary tube. The spectrum was recorded in the EPR spectrometer set at 8×10^5 receiver gain, 1.0 G modulation amplitude, 167.77 s sweep time, 3460 G center field, 100 G sweep width, and 0.655 s time constant. Hydroxyl radical-scavenging capacities of the additives were calculated using the following equation:

$$OH^{\bullet}\text{-scavenging capacity (\%)} = 100 - \left(\frac{\text{EPR signal intensity for the medium containing the additive of concern}}{\text{EPR signal intensity for the control medium}}\right)\times 100 \quad [5]$$

(vii) Superoxide radical-scavenging assay. A modified version of the method explained by Nishikimi *et al*. (30) was employed. Superoxide radicals were generated *via* an enzymatic reaction. The reaction mixture contained 1 mL of 3 mM hypoxanthine, 1 mL of xanthine oxidase (100 mIU), 1 mL of 12 mM diethylenetriaminepentaacetic acid, 1 mL of 178 µM nitro blue tetrazolium, and 1 mL of the extracts (final concentration of the phenolics in the reaction mixture was 100 or 200 ppm as catechin equivalents). The reference antioxidant was catechin. All solutions were prepared in a 0.1 M phosphate buffer (pH 7.4) solution. The absorbance values of the mixtures at 560 nm were recorded at 0 min, and thereafter every 10 min for up to 60 min. Superoxide radical-scavenging capacities (after 10 min of assay) of the additives were calculated using the following equation:

$$\text{superoxide radical-scavenging capacity (\%)} = 100 - \left(\frac{\text{absorbance of medium containing the additive}}{\text{absorbance of the control medium}}\right)\times 100 \quad [6]$$

(viii) DPPH radical-scavenging assay. A DPPH radical-scavenging assay was performed using a modified version of the method described by Santiago *et al*. (31). An aliquot of 100 µL of a 0.5 mM solution of DPPH in ethanol was added to 100 µL of a solution containing almond extracts so that the concentration of phenolics in the final assay medium was either 100 or 200 ppm as catechin equivalents. Contents were mixed, and after 1 min 100 µL of the mixture was drawn into a syringe and transferred to a quartz capillary tube. The spectrum was recorded in the EPR spectrometer set at 2×10^5 receiver gain, 1.0 G modulation amplitude, 167.77 s sweep time, 3460 G center field, 100 G sweep width, and 0.655 s time constant. The reference antioxidant was catechin. DPPH radical-scavenging capacities of the additives were calculated using the following equation:

$$\text{DPPH radical - scavenging capacity (\%)} = 100 - \left(\frac{\text{EPR signal intensity for the medium containing the additive}}{\text{EPR signal intensity for the control medium}}\right)\times 100 \quad [7]$$

RESULTS AND DISCUSSION

All almond extracts afforded a positive vanillin test, thus indicating the presence of condensed tannins. Therefore, the total phenolic contents were reported as catechin equivalents and the antioxidative activities of extracts were compared with it.

The highest yield of the ethanolic extract of 41 ± 3% (w/w) (mean ± SD) was afforded by the green shell cover and the lowest, 8% ± 0.1%, by the brown skin. Almond whole seed produced a yield of 19 ± 2% (w/w). However, despite its low yield, the brown skin had the highest content of phenolics. The total phenolic contents of seed, skin, and shells were 8.1 ± 0.87, 87.8 ± 1.75, and 71.1 ± 1.74 mg catechin equivalents/g ethanolic extract, respectively.

Free radicals possess an unpaired electron, which makes them highly reactive. Antioxidants neutralize free radicals by donating a hydrogen atom to them. Attempts have been made to evaluate the effectiveness of antioxidants in scavenging free radicals such as ABTS radical anion ($ABTS^{\bullet-}$) and $DPPH^{\bullet}$, hydroxyl $HO^{\bullet}$, and superoxide $O_2^{\bullet-}$ radicals (27–32). In all these methods antioxidant efficacies are measured at room temperature, thus eliminating any risk of thermal degradation of the molecules being tested.

The TEAC assay was employed to evaluate the antioxidant capacities of polar compounds in a preparation as the experiment is performed in an aqueous buffer. The antioxidant capacity of the extracts is determined through the measurement of the absorbance of $ABTS^{\bullet-}$ at 734 nm in the presence of extracts and compared with that of Trolox, a water-soluble vitamin E analog. The TEAC of a compound determines its antioxidant capacity relative to that of Trolox on a mole basis. TEAC of an unknown mixture represents the concentration of

a Trolox solution that has the same activity as the mixture under investigation. The almond extracts were tested at a concentration of 2 mg/mL and diluted accordingly to achieve a readable absorbance after addition of the free radical solution. Trolox reacts instantaneously with $ABTS^{\bullet-}$, and the reaction is completed by the first minute (Fig. 1). On the other hand, the scavenging of $ABTS^{\bullet-}$ by the three almond crude extracts continued beyond the first minute. This type of reaction also has been reported by van den Berg *et al.* (32) for a series of flavonoids. These authors suggested that total TEAC (TEAC after 6 min) could be used in predicting antioxidative capacities of structurally related compounds, as it correlates positively with the inhibition of lipid peroxidation and thus protection against doxorubicin-induced cardiotoxicity (32). TEAC after 6 min was therefore used to evaluate the antioxidant capacities of almond extracts (Table 1). As TEAC can be used to provide a ranking order of the antioxidant capacity of unknown mixtures (27), the data indicated that the capacities of almond extracts at the same extract concentration were in the order of seed < shell < skin. Moreover, TEAC values of brown skin extract and green shell cover extract were 13 and 10 times greater than that of seed extract, respectively. The total phenolic content of the extracts showed a similar pattern; hence, at a given extract concentration skin and shell extracts would be more effective antioxidants than seed extracts.

The concentrations of extracts tested for their free radical-scavenging activities were 100 and 200 ppm phenolics as catechin equivalents and are referred to as 100 and 200 ppm in the text.

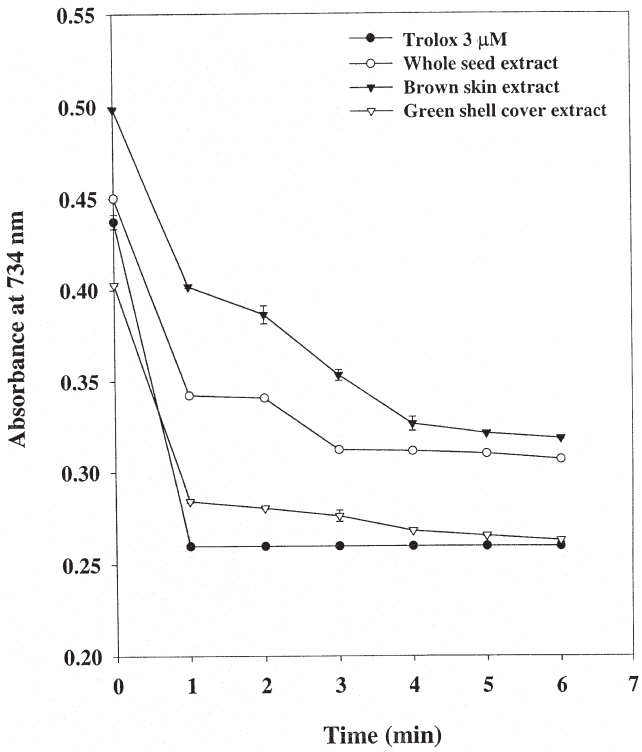

FIG. 1. Reaction of Trolox (3 μM) and almond extracts with the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical anion ($ABTS^{\bullet-}$) as exhibited by the reduction of absorbance of $ABTS^{\bullet-}$ at 734 nm. Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

TABLE 1
Trolox Equivalent Antioxidant Capacity (TEAC) Values of Almond Extracts[a]

Extract	Concentration (mg/mL)	Dilution factor	TEAC
Whole seed extract	2	1	4.21 ± 0.24[a]
Brown skin extract	2	10	52.9 ± 0.88[c]
Green shell cover extract	2	10	41.1 ± 0.65[b]

[a]Results are mean values of three determinations ± SD. Means in a column sharing the same superscript are not significantly ($P > 0.05$) different from one another. Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

Hydrogen peroxide exhibits weak activity in initiating lipid peroxidation; however, its potential to produce highly ROS, such as hydroxyl radical through Fenton reaction, is very high (2). Hydrogen peroxide is poorly reactive in aqueous solutions at physiological concentrations, is toxic to cells at 10–100 μM levels, and can cross biological membranes rapidly to form cytotoxic hydroxyl radicals (1). The hydrogen peroxide-scavenging activity of almond ethanolic extracts was concentration dependent, and within the first 10 min most of the hydrogen peroxide was scavenged, as shown in Table 2. The rates of hydrogen peroxide scavenging of seed, skin, and shell up to the first 10 min of assay were 0.375, 0.392, and 0.411 mM H_2O_2/min at 100 ppm, and 0.539, 0.571, and 0.565 mM H_2O_2/min at 200 ppm, respectively. Hydrogen peroxide-scavenging activity of the seed was inferior to other extracts. At 100 ppm all additives showed significantly different ($P < 0.05$) scavenging capacities, but at 200 ppm both the skin and shell extracts showed 91% scavenging activity, which was not different from that of catechin. Thus, hydrogen peroxide-scavenging activity of almond extracts would contribute to their inhibition of lipid peroxidation and thereby protect cells from oxidative damage.

The superoxide radical is a powerful oxidizing agent that can react with biological membranes and induce tissue damage (2). It also decomposes to singlet oxygen, hydroxyl radical, or hydrogen peroxide (33). In this work, superoxide radical was enzymatically generated using a hypoxanthine/xanthine oxidase system. The spectrophotometric measurement of the ink blue color of reduced nitro blue tetrazolium in the presence of superoxide anion was used as a means of evaluating the scavenging activity of the extracts. The continuous development of the ink blue color in the control showed the generation of superoxide radical. All three almond extracts showed a concentration-dependent scavenging activity. Almond green shell cover extract gave a 99% radical-scavenging activity, which was comparable with that of catechin after 10 min assay. Almond skin and seed extracts at 200 ppm scavenged superoxide radical by 95 and 85%, respectively. This superoxide radical-scavenging potential of extracts, especially that of the almond skin and shell, would be one of the major mechanisms contributing to their antioxidant capacities.

TABLE 2
Free Radical-Scavenging Capacities[a] of Almond Extracts

	% Hydrogen peroxide-scavenging capacity[b]		% Superoxide radical-scavenging capacity[b]		% DMPO-OH-scavenging capacity[c]		% DPPH radical-scavenging capacity[d]	
Extract	100 ppm	200 ppm	100 ppm	200 ppm	100 ppm	200 ppm	100 ppm	200 ppm
Catechin	90 ± 2^{c}	94 ± 2^{b}	100 ± 0^{c}	100 ± 0^{c}	35 ± 2^{b}	72 ± 3^{c}	100 ± 0^{c}	100 ± 0^{b}
Whole seed extract	59 ± 2^{a}	86 ± 1^{a}	76 ± 1^{a}	85 ± 1^{a}	16 ± 2^{a}	42 ± 2^{a}	21 ± 2^{a}	73 ± 2^{a}
Brown skin extract	63 ± 1^{a,b}	91 ± 1^{b}	89 ± 1^{b}	95 ± 1^{b}	57 ± 3^{c}	100 ± 0^{c}	100 ± 0^{c}	100 ± 0^{b}
Green shell cover extract	66 ± 1^{b}	91 ± 1^{b}	97 ± 1^{c}	99 ± 1^{c}	40 ± 2^{b}	56 ± 1^{b}	35 ± 2^{b}	100 ± 0^{b}

[a]Data are reported as mean ± SD (n = 4). Values followed by the same superscript in the same column are not significantly ($P > 0.05$) different.
[b]Values are after 10 min of assay.
[c]Values are after 3 min of assay. DMPO, 5.5-dimethyl-1-pyrroline-*N*-oxide.
[d]Values are after 1 min of assay. DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Hydroxyl radicals, generated by Fenton-driven Haber–Weiss reactions, may be detected by EPR. The intensity of the EPR signal is proportional to the concentration of free radicals in the system (34). The highly reactive state of the radicals makes them unstable and short-lived and therefore they must be trapped into a stable form in order to be detected by EPR. The spin-trapping technique is widely used in this context. In this method, diamagnetic compounds, usually nitroso or nitrone derivatives, are reacted with radicals to convert them to a stable form (35). Owing to the short lifetimes of the hydroxyl radical, the formed hydroxyl radicals were therefore spin-trapped with DMPO to form DMPO-OH adduct. The intensity of the signal was reduced when the extracts or catechin were added but did not completely disappear at the 100 ppm level for any of them. In Table 2 the reduction of DMPO-OH adducts by almond extracts, calculated from the reduction of intensity of the signals after addition of each extract, are presented. The minimum needed for a 100% inhibition was exhibited by skin extract and catechin at 200 and 260 ppm, respectively. Intensity reduction at levels higher than 200 ppm of seed and shell extracts could not be measured owing to the presence of insoluble particles. However, seed extract showed the lowest activity, and skin extract showed the highest.

The use of the DPPH free radical is advantageous in evaluating antioxidant efficacies because it is more stable than the hydroxyl and superoxide radicals. The EPR spectrum of the DPPH radical and the resulting spectrum after addition of extracts were evaluated. Catechin and almond skin extract at 100 ppm, and shell extract at 200 ppm showed a 100% scavenging of DPPH radicals (Table 2). The lowest concentrations needed for a complete scavenging of DPPH radicals by catechin and by seed, skin, and shell extracts were 35, 210, 50, and 120 ppm, respectively. These results show that skin extract scavenges organic free radicals more effectively than other almond extracts. As proposed by Blois (36), hydrogen donation from an antioxidant involves the decoloration of DPPH radicals. This confirms the hydrogen-donating ability of almond extracts.

Different assays used for examining free radical-scavenging activity of almond extracts revealed that the three almond extracts did not perform similarly. The overall activity of almond extracts thus may depend on the type of individual phenolic compounds present in each extract, their relative activities, and possible synergistic and antagonistic effects brought about by different interactions among compounds involved.

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