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Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts

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Abstract

Almond (*Prunus amygdalus*) skins are agricultural by-products that are a source of phenolic compounds. Phenolic compounds from gamma-irradiated almond skins were extracted with 40% ethanol. Total phenolic content was determined using the Folin–Ciocalteu (F–C) method. Almond skin extracts (ASE): soybean oil (1:4 v/v) mixtures containing 0.08% $FeCl_3$ were prepared. Antioxidant activity was determined by conjugated dienes and trienes (CD and CT, respectively) measurements, peroxide value (PV), Trolox® equivalent antioxidant capacity (TEAC) and Photochemiluminescence (PCL). Phenolic content yield ($p < 0.05$) was higher in ASE irradiated at doses greater than 4 kGy (trial I) or 12.7 kGy (trial II) compared to the control. Increased antioxidant activity was observed in TEAC assay and PCL with lipid-soluble antioxidant capacity reagents in ASE irradiated above 4 kGy (trial I) and 12.7 kGy (trial II) compared to 0 kGy. Gamma irradiation of almond skins thus increased the yield of total phenolic content as well as enhanced antioxidant activity of extracts.

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Keywords: Almond skins; Antioxidant activity; Phenolic content; Gamma irradiation; Lipid oxidation

1. Introduction

During processing of almonds, large amounts of less valuable by-products such as almond skins are produced. Almond skins, resulting from the hot water blanching process, are ground into animal feed or burned as fuel in processing plants (Board on Agriculture, 1983). The skins constitute about 4% of the almond fruit, and are a readily available source of phenolics (Chen, Milbury, Lapsley, & Blumberg, 2005). Other by-products from the agriculture industry including almond hulls, grape pomace and rice hulls have also been shown to be good sources of phenolic compounds with antioxidant activity (Heim, Tagliaferro, & Bobilya, 2002; Lee, Kim, Nam, & Ahn, 2003; Murthy, Singh, & Jayaprakasha, 2002; Takeoka & Dao, 2003). These phenolic compounds inhibit lipid oxidation by scavenging free radicals, chelating metals, activating antioxidant enzymes, reducing tocopherol radicals and inhibiting enzymes that cause oxidation reactions (Heim et al., 2002).

Phenolic compounds can range in size from monomers to long-chain polymers such as tannins, and usually exist bound to carbohydrates or as part of repeating subunits of high molecular weight polymers. Various phenolic compounds have been detected in almond by-products. Four different flavone glycosides: isorhamnetin rutinoside, isorhamnetin glucoside, kaempferol rutinoside and kaempferol glucoside have been reported in almond seedcoats (Frison-Norrie & Sporns, 2002; Wijeratne, Abou-Zaid, & Shahidi, 2006a). Other investigators have likewise identified phenolic compounds in almond skins including quercetin glycosylated to glucose, galactose and rhamnose, kaempferol, naringenin, catechin, protocatechuic acid, vanillic acid and a benzoic acid derivative (Chen et al., 2005; Sang et al., 2002). Phenolic compounds act as antioxidants by scavenging free radicals and chelating metals in

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foods (Heim et al., 2002). These natural antioxidants could be used in food products to extend shelf-life and impart the image of wholesomeness to consumers.

Gamma irradiation is approved by the Food and Drug Administration and the United States Department of Agriculture to preserve various food products. Various forms of Irradiation have been shown to influence the phenolic content of foods and their by-products. Far-infrared has also been used to release low-molecular-weight phenolics with antioxidant activity from food by-products for instance rice hulls and sesame meal extracts (Lee et al., 2003; Lee, Jeong, Kim, Nam, & Ahn, 2005). Gamma irradiation (10 kGy) increased phenolic acid content in cinnamon and clove while phenolic content in nutmeg remained unaltered (Cantos, Garcia-Viguera, Pascal-Teresa, & Tomas-Barberan, 2000; Variyar, Bandyopadhyay, & Thomas, 1998). Gamma irradiation in combination with 0.1% sodium metabisulfite during packaging and 2000 ppm SO_2 during extraction of phenolics inhibited loss of anthocyanin in grape pomace (Ayed, Yu, & Lacroix, 1999).

While the phenolic composition of almond skins and its antioxidant effect in model systems and in pork has been reported (Chen et al., 2005; Sang et al., 2002; Siriwardhana & Shahidi, 2002; Wijeratne et al., 2006a; Wijeratne, Amarowicz, & Shahidi, 2006b), to the best of our knowledge no studies incorporating almond skins to retard lipid oxidation in vegetable oils or determination of effect of gamma irradiation on almond skin properties have been published. The present study was thus designed to determine the effect of gamma irradiation on total phenolic content yield and antioxidant activity of almond skin extracts (ASE).

2. Materials and methods

2.1. Materials

Two trials were conducted. In trial I, almond skins of unknown variety were supplied by Blue Diamond Growers, Sacramento, CA. For trial II, almond skins of the Non-pareil variety from the 2004 season were supplied by Campos Brothers, Caruthers, CA. Soybean oil was obtained from a local grocery store. HPLC grade acetic acid, ethanol, sodium hydroxide, phenolphthalein and isooctane were purchased from Fisher Scientific (Fairlawn, NJ). Folin–Ciocalteu (F–C) phenol reagent, sodium carbonate, 0.1 N sodium thiosulfate, potassium iodide, sodium lauryl sulfate (SDS), starch indicator, C, 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic acid (Trolox®), ferric chloride and quercetin were purchased from Sigma Chemical Co (St. Louis, MO). ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt) were purchased from ICN/CAPPEL (Aurora, OH). Water-soluble antioxidant capacity (ACW) and lipid-soluble antioxidant capacity (ACL) kits were purchased from AnalytikJenaAG (The Woodlands, TX).

3. Methods

3.1. Preparation of almond skin extract

Almond skins were irradiated at IBA/SteriGenics, Tustin, CA, at ambient temperature with target irradiation levels from 0 to 16 kGy. At each dose level, five bags containing about 25 g of almond skins were irradiated. The IBA/Sterigenics facility uses a radiochromic dye dosimetry system to determine the dose absorbed by the irradiated product. The dosimeters are periodically calibrated by the National Institute of Standards and Technology (Gaithersburg, MD). A pair of dosimeters was placed at regions of maximum and minimum dose areas that were predetermined specifically for containers used. After completion of irradiation, the irradiated and non-irradiated almond skins were transported back to the Chapman University laboratory and frozen at − 80 °C until they were subjected to the extraction process.

Almond skin extracts were prepared by a modified method from Waterman and Mole (1994). Briefly, 50 ml of 40% ethanol were added to 2.5 g of almond skins. The extraction solvent was chosen because 40–80% ethanol has been shown to be effective in extracting phenolic compounds (Suzuki et al., 2002). The mixture was blended (Sunbeam Oster blender model 6640, Boca Raton, FL) three times for 3 min each with a 5 min cool down period between each blending step to avoid any potential overheating. The solutions were vacuum-filtered with Whatman No. 1 filter paper. Clear filtrates were aliquoted and analyzed.

3.2. Determination of total phenolic content by Folin–Ciocalteu method

Total phenolic content of ASEs was determined as described by Singleton and Rossi (1965) using quercetin as a standard. ASE (0.5 ml) and 30 ml of HPLC grade water were placed in a 50 ml volumetric flask. To the mixture, 2.5 ml of Folin–Ciocalteu phenol reagent were added. After 5 min, 7.5 ml of 20% sodium carbonate were mixed into the solution. The solution was brought up to volume with HPLC grade water. After stoppering the flask and inverting it several times, the solutions were incubated for 90 min. Absorbance was recorded at 760 nm using a DU 800 UV–VIS spectrophotometer (Beckman Coulter, Fullerton, CA). Quercetin standard solutions (0–0.8 mg/ml) were prepared in a similar manner.

To correct for any possible interference of ascorbic acid in the total phenolic assay, ascorbic acid content in ASE was determined with the 2,6-dichloroindophenol titrimetric AOAC method 967.21 (AOAC-International, 2000).

3.3. Preparation of almond extract-soybean oil mixture

Antioxidant activity in trial II only was determined by preparing mixtures of soybean oil with or without ASE. Oxidation in soybean oil mixtures was initiated with ferric

chloride. Eighty milliliters of soybean oil, 20 ml of ASE and 1.0 ml of 0.08% ferric chloride (w/v) were blended (Sunbeam Oster blender model 6640, Boca Raton, FL) for 2 min. A negative control was prepared with 80 ml vegetable oil, 20 ml 40% ethanol and 1.0 ml of 0.08% ferric chloride (w/v). Positive controls were prepared by replacing 20 ml of almond skin extract with 20 ml Trolox® (1.275 mg/L). The amount of Trolox® added was dependent on the average total phenolic content of ASE. Mixtures were kept in the dark for 8 days at room temperature and periodically analyzed.

3.4. Conjugated dienes and trienes

The CD and CT were determined by mixing 30 mg almond extract-soybean oil samples with 50 ml isooctane, and absorbance measured at 234 nm and 268 nm, respectively, using a DU 800 spectrophotometer (Beckman Coulter, Fullerton, CA) (Kulås & Ackman, 2001). Samples were evaluated each day for the first 3 days after sample preparation and every other day thereafter. CD values were calculated with the following formula:

$$CD = (A/C) * d$$

where A is the absorbance of sample, C is the concentration in g of oil in 100 ml solvent and d is the length of cell in cm (Jeon, Kamil, & Shahidi, 2002).

3.5. Peroxide value

Peroxide values were determined periodically over an 8-day period as outlined in AOCS (1997) Method Cd 8b-90.

3.6. Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC) was determined as outlined by Re et al. (1999) in both trials. A synthetic free radical $ABTS^{*+}$ (2,2′-azino-di-[3-ethyl-benzthiazoline sulfonate (6)] diammonium salt) was allowed to form in the dark for 16 h. The $ABTS^{*+}$ stock solution was diluted with 40% ethanol to an absorbance of 0.70 ± 0.02 at 734 nm (DU 800 spectrophotometer, Beckman Coulter, Fullerton, CA). Absorbance values of extracts were then monitored every min for 6 min at 734 nm (Re et al., 1999).

3.7. Photochemiluminescence

PCL analysis for trial II was carried out as described by Lee et al. (2004) and AnalytikJenaAG (2000). ASEs were diluted with reagents from water-soluble antioxidant capacity (ACW) and lipid-soluble antioxidant capacity (ACL) kits. For ACW analysis ASEs were diluted 1:50 (v:v) in 40% ethanol, and 40 μl were added to analysis solution. For ACL analysis, ASEs were diluted 1:100 (v:v) in 40% ethanol and 50 μl was used in analysis. Antioxidant ability of ASE was compared to Trolox® and ascorbic acid standards provided in the ACL and ACW kits. Standard sample volumes for ACW and ACL measurements ranged from 5 to 20 μl. Measurement time for ACW was 100–500 s and for ACL 180 s. Calibration and measurements for ACW were based on the difference in lag time between sample and blank. The ACL calibration and measurements were based on inhibition (Lee et al., 2004).

3.8. Statistical analysis

A minimum of two replications for each dose level treatment were carried out. Averages and least significant differences were calculated using the SAS system version 9.1.3 (Cary, NC). A P value of <0.05 was considered significant.

4. Results and discussion

The $D_{max/min}$ ratios for each target dose in trial I were 1.0 for average irradiation doses of 0, 2.05, 4.05, 8.0, and 12 kGy. The $D_{max/min}$ ratios for each target dose in trial II were 1.0 for average irradiation doses of 0, 2.8, 4.8, 8.8, 12.7, and 16.3 kGy. The soybean oil used in this study was not stripped of endogenous antioxidants, so antioxidant results presented may be an interaction between the almond skin extracts and any endogenous antioxidants that may have been present in the oil.

4.1. Phenolic content via Folin–Ciocalteu

Almond skins from Blue Diamond (trial I) showed an increase in phenolic content at irradiation levels of 4.05 kGy and above while almond skins from Campos Brothers (trial II) showed an increase in phenolic content at irradiation levels of 12.7 kGy and above (Table 1). The amount of ascorbic acid in the ASE as determined by the 2,6-dichloroindophenol titrimetric method 967.21 (AOAC-International, 2000) was low in trial I and was below detection limits for trial II (data not shown). So,

Table 1
Total phenolic content of almond skin extracts determined with Folin–Ciocalteu method

Treatment	Quercetin equivalents (ppm)
Trial I (almond skins from Blue Diamond Growers)	
0 kGy (control)	3.69 ± 0.06^{d}
2 kGy	3.53 ± 0.16^{d}
4 kGy	5.34 ± 0.20^{a}
8 kGy	4.40 ± 0.24^{b}
12 kGy	4.50 ± 0.20^{c}
Trial II (almond skin from Campos Brothers)	
0 kGy (control)	1.91 ± 0.14^{b}
2.8 kGy	1.95 ± 0.04^{b}
4.8 kGy	1.85 ± 0.11^{b}
8.8 kGy	1.75 ± 0.07^{b}
12.7 kGy	2.29 ± 0.20^{a}
16.3 kGy	2.23 ± 0.16^{a}

Values are means of at least four replications ± standard deviation. Numbers followed by the same letter are not significantly different.

total phenolic content expressed as quercetin equivalents was not corrected for vitamin C activity in trial II. Gamma irradiation increased the total phenolics yield in almond skin extracts in trial I by about 45% at dose levels of 4 kGy and above and in trial II by about 20% at dose levels of 12 kGy compared to the control. This data indicate that gamma irradiation is capable of affecting the phenolic composition in almond skins. The ability of gamma irradiation to increase phenolic content in plant material has been observed in soybeans and spices. Soybean samples treated with gamma irradiation at levels ranging from 0.5 to 5 kGy had increased free (aglycone) phenolic content (Variyar, Limaye, & Sharma, 2004). Research on the effect of gamma irradiation on spices showed that some spices such as clove and nutmeg had increased phenolic content while spices such as cardamom and cinnamon did not exhibit greater phenolic content with irradiation (Variyar et al., 1998). The differences in effects were attributed to the different phenolic compounds present in the various spices. Clove and nutmeg have appreciable amounts of hydrolysable tannins, which may be more susceptible to gamma-irradiation compared to the condensed tannins present in cinnamon and other spices. The increased phenolic content in gamma-irradiated ASE in both trials could be attributed to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation.

4.2. Conjugated dienes and conjugated trienes

Conjugated Dienes (CD) and trienes (CT) values monitor bond shifts that take place during oxidation and are determined at 234 and 268 nm, respectively (Deiana et al., 2002). On day 0 all samples had CD values less than 356 except for samples treated with 4.8 kGy irradiation which had a higher CD value of 507. After a day of incubation, ASEs treated with 16.3 kGy had significantly lower CD values than the control and Trolox samples (Table 2). Oxidation was inhibited to a greater extent by 16.3 kGy ASE in soybean oil on day 2 of analysis (Table 2). There was a general increase in CD values of samples over the 8-day period of analysis. Similar to CD, an increase in absorbance at 268 nm can be used as an indicator of lipid oxidation, because conjugated unsaturated fatty acids have increased ultraviolet absorption at 234 and 268 nm (Gray, 1978). CT values displayed a similar trend to CD values (data not shown).

4.3. Peroxide value

On day 0 (start of lipid oxidation reaction) peroxide values were around 10 meq peroxide/kg for all samples (Fig. 1). The values did not change a day after incubation except for control sample that increased to 14 meq peroxide/kg sample on day 1. On days 2 and 3, the control sample containing no ASE or Trolox exhibited significantly higher PV. There was a decrease in PV after the first 2 days of incubation for the control sample whereas PV decreased after day 5 for other samples. The decrease in PV over time could be attributed to breakdown of primary initiation products of oxidation including peroxides. In general, lipid oxidation as determined by PV was the highest for the control from days 0 to 3, which is in agreement with CD values. A study on fish oil oxidation has shown that PV measurements correlate well to CD (Kulås & Ackman, 2001). Peroxide values indicate that soybean oil without Trolox or ASE underwent lipid oxidation at a higher rate. The addition of ASE lowered lipid oxidation due to the presence of phenolic compounds present in the almond skins, however, no significant irradiation effect between the various doses was observed.

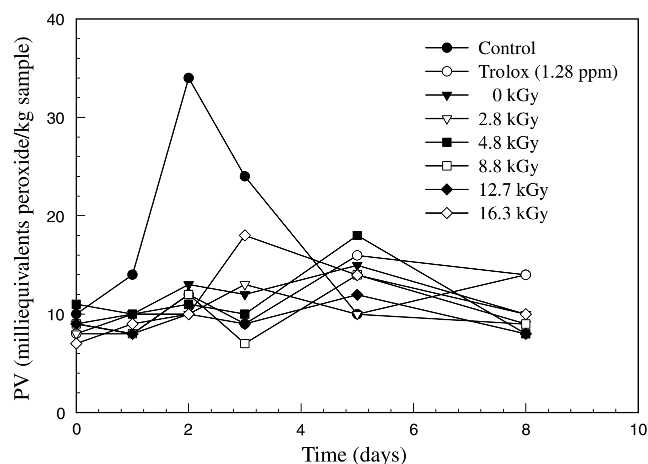

Fig. 1. The effect of almond skin extracts (trial II) on the formation of peroxides in soybean oil over an 8-day period (standard deviations for replications ranged from 0.01 to 3.56).

Table 2
Conjugated dienes values of soybean oil/almond skin extract/ferric chloride mixtures (trial II) monitored for an 8-day period

Time (day)	Control	Trolox 1.28 ppm	Almond skin extract: soybean oil (1:4) mixtures					
			0 kGy	2.8 kGy	4.8 kGy	8.8 kGy	12.7 kGy	16.3 kGy
0	337.5	355.8	320.8	312.5	506.7	331.7	295.8	283.3
1	406.7	342.5	244.2	337.5	325.8	289.2	333.3	227.5
2	393.3	240.8	395.0	398.3	375.8	371.7	369.2	291.7
3	522.5	545.8	565.0	530.0	559.2	536.7	510.8	536.7
5	403.3	420.8	380.8	449.2	391.7	410.8	402.5	435.0
8	556.7	488.3	505.0	473.3	466.7	495.0	479.2	358.3

Values are means of four replications.

4.4. Photochemiluminescence

The lipid-soluble antioxidant analysis using Trolox as a standard indicated that samples treated with 12.7 and 16.3 kGy irradiation had increased antioxidant ability (Table 3). The lipid-soluble antioxidant capacity of phenolic compounds in ASE appears to be enhanced by gamma irradiation. Increased antioxidant activity of almond skins irradiated at 12.7 and 16.3 kGy could be related to higher yield of phenolic content in these extracts (Table 1). ACW analysis using ascorbic acid as a standard did not reveal any significant effect of gamma irradiation on the antioxidant activity of ASE in aqueous systems. The standard deviations were larger for the ACW compared to ACL analysis and this may have accounted for the lack of detectable differences between the treatments for ACW. A difference between ACW and ACL has been reported in a study with soybean isoflavones (Lee et al., 2004). The researchers attributed the difference to solubility differences of soybean extracts (Lee et al., 2004). The difference in ASE results between antioxidant activity in the lipid and water systems could be a function of the polarity of the antioxidants in almond skins. Antioxidants function differently depending on the food system. In bulk oils polar antioxidants have been shown to be more effective than non-polar antioxidants. Researchers have suggested that polar antioxidants will accumulate at the surface of oil where lipid oxidation would be great due to the presence of oxygen. In an aqueous system the water-soluble antioxidants would disperse and would not be able to inhibit lipid oxidation as effectively as non-polar antioxidants (Frankel, 1998). The difference in the trends between the aqueous and lipophilic systems could thus be a function of polarity of phenolic compounds in ASE.

4.5. Trolox equivalent antioxidant capacity

The reduction of absorbance at 734 nm in the TEAC assay is an indicator of phenolic compounds ability to scavenge the synthetic $ABTS^{*+}$. All ASEs in both trials were more effective at scavenging the $ABTS^{*+}$ than Trolox. ASEs for trial I exposed to irradiation levels of 4.05 kGy

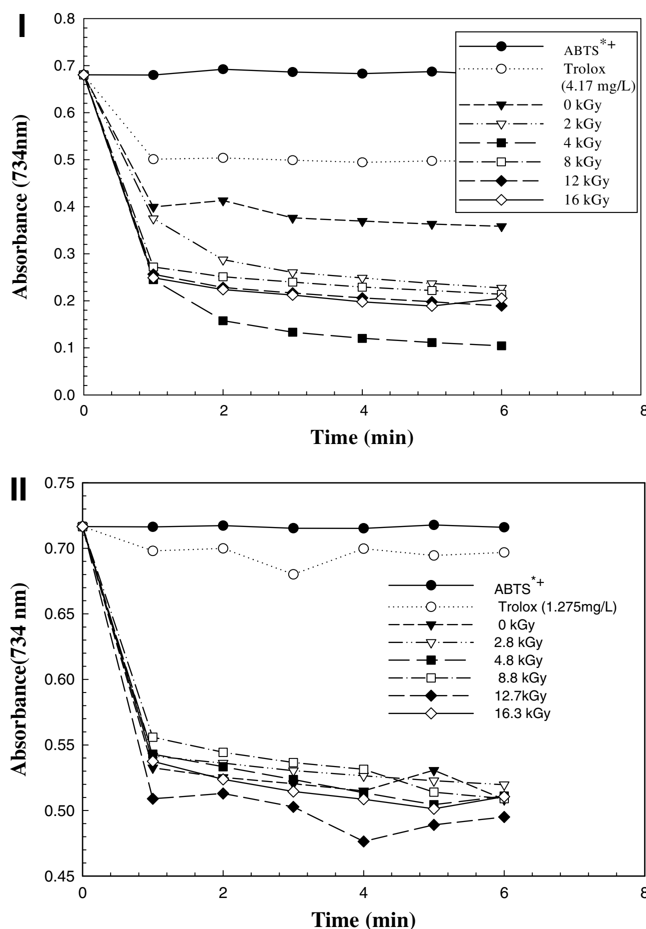

Fig. 2. The effect of almond skin extracts from (I) Blue Diamond and (II) Campos Brothers on the suppression of the absorbance of the $ABTS^{*+}$ radical over a 6-minute period (standard deviations ranged from 0.00 to 0.20 and 0.00 to 0.04 for trials I and II, respectively).

and for trial II samples exposed to irradiation levels of 12.7 kGy were most effective at reducing the absorbance of $ABTS^{*+}$ (Fig. 2). Similar findings of higher antioxidant capacity of phenolic compounds extracted from wine making by-products compared to Trolox have been observed (Gonzalez-Paramas, Esteban-Ruano, Santos-Buelga, Pascual-Teresa, & JC, 2004). Greatest scavenging ability of ASE corresponded with the higher total phenolic content of ASE irradiated at the higher dose levels. Studies employing F–C method and TEAC have shown an excellent correlation between the total phenolic content and antioxidant activity of a substance (Huang, Ou, & Prior, 2005).

Table 3
Antioxidant activity of irradiated almond skin extracts as determined by Photochemiluminescence assay

Sample (kGy)	Ascorbic equivalent (μg/ml)[A]	Trolox equivalent (μg/ml)[B]
0	128.41 ± 21.5[a]	54.075 ± 6.19[c]
2.8	139.72 ± 26.5[a]	57.944 ±1.45[b,c]
4.8	119.89 ± 2.83[a]	53.434 ± 3.22[c]
8.8	165.24 ± 3.39[a]	64.175 ± 4.92[b]
12.7	155.53 ± 2.53[a]	73.463 ± 4.79[a]
16.3	161.99 ± 58.0[a]	72.235 ± 7.38[a]

Values for ACW are means of two replications ± standard deviation.
Values for ACL are means of four replications ± standard deviation.
[A] ACW – water-soluble antioxidant capacity.
[B] ACL – lipid-soluble antioxidant capacity.

5. Conclusions

Gamma irradiation of almond skins above 4 kGy in trial I and 12 kGy in trial II increased the yield of total phenolic content as well as enhanced antioxidant activity as assessed by CD in the first three days, TEAC and ACL analyses. There was no apparent dose effect observed with PV, however, samples with almond skin extracts had a lower degree of oxidation compared to control samples with no added extracts, an indication that the phenolic compounds in

the almond skins inhibited lipid oxidation. Almond skin extracts inhibited iron catalyzed lipid oxidation of soybean oil and have potential antioxidant activity in food lipids. Future studies are planned to determine the effect of gamma irradiation on different phenolic compound classes at the molecular level, investigating the effect of gamma irradiation on individual phenolic compounds.

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