JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Potato Peel Extract—a Natural Antioxidant for Retarding Lipid Peroxidation in Radiation Processed Lamb Meat

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The effective utilization of potato peel, a waste generated in large quantities by the food industry, as an antioxidant was investigated. Potato peel extract (PPE) exhibited high phenolic content (70.82 mg of catechin equivalent/100 g), chlorogenic acid (27.56 mg/100 g of sample) being the major component. The yield of total phenolics and chlorogenic acid increased by 26 and 60%, respectively, when the extract was prepared from $\gamma$ irradiated (150 Gy) potatoes. PPE showed excellent antioxidant activity as determined by $\beta$-carotene bleaching and radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The suitability of PPE for controlling lipid oxidation of radiation processed lamb meat was also investigated. PPE (0.04%) when added to meat before radiation processing was found to retard lipid peroxidation of irradiated meat as measured by TBA number and carbonyl content. The antioxidant activity of PPE was found to be comparable to butylated hydroxytoluene (BHT).

**KEYWORDS: Lamb meat; oxidation; antioxidants; phenolics; potato peel extract**

## INTRODUCTION

Radiation processing is one of the most effective technologies that can extend shelf-life and eliminate pathogenic bacteria in raw meat and meat products. However, meat on irradiation may undergo pronounced oxidative changes that influence the sensory quality of meat (*1*). Development of lipid peroxidation in irradiated meat is influenced by several factors such as packaging, storage, and other processing conditions before and after irradiation.

The rate and extent of oxidative deterioration can be reduced by various means including curing, packaging in a vacuum or modified atmosphere, and adding antioxidants. Although synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) have been used extensively, recent studies have implicated them to have toxic effects (*2*). These findings together with consumer interest in natural food additives have reinforced the need for effective antioxidants from natural sources as an alternative to prevent deterioration of food during processing and storage.

The antioxidant activity of a variety of extracts from natural sources has been investigated in meat model systems (*3*) and in few storage experiments with meat (*4*, *5*). Incorporation of such extracts in meat not only preserves the wholesomeness of the food but also reduces the risk to humans of developing chronic diseases such as artherosclerosis and cancer (*6*). The potato is one of the most commonly consumed vegetables throughout the world. Potato production worldwide stands at 293 million metric tons. A large quantity of potato peel waste is generated, which contains many phenolic compounds, some in free form and others that are bound (*7*). Chu et al. (*8*) have reported that in potatoes, about 40% of the phenolics is in the bound form mainly in the form of $\beta$-glycosides that survive upper gastrointestinal digestion and are absorbed in the colon and exhibit healthful functionalities. A concoction of phytochemicals such as phenolic acids, flavonoids, coumarins, carotenoids, and terpenes are present in potatoes (*9*). Phenolic compounds have many biological activities. These include chelation of metals, scavenging of active oxygen species, and antioxidant activity. Phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. The antioxidant property of potato peel extract (*10*−*13*) has been reported, but its use as an antioxidant in irradiated meat to control lipid peroxidation has not been studied.

Therefore, the present work was undertaken to examine the utilization of potato peel, a waste material, as a source of natural antioxidants and its effectiveness in reducing lipid peroxidation of radiation-processed meat.

## MATERIALS AND METHODS

**Chemicals.** BHT, DPPH, $\beta$-carotene, chlorogenic acid, gallic acid, and caffeic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Thiobarbituric acid (TBA) was obtained from BDH Chemicals Ltd. (Poole, England). HPLC grade methanol from Merck, India was used. All other reagents used were of analytical grade and procured

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10.1021/jf048270e CCC: $30.25 
Published on Web 02/11/2005

from Qualigens Fine Chemicals (Mumbai, India) and S. D. Fine Chemicals (Mumbai, India).

**Preparation of Potato Peel Extract.** Potato (*Solanum tuberosum*) cultivar Kufri chandramukhi was purchased from the local market and cleaned with tap water, and the peel was removed. The potato peel (50 g) was homogenized with distilled ethanol (300 mL) and filtered through cheesecloth, and the filtrate was centrifuged at 12 100*g* for 20 min. The supernatant was filtered through Whatman No. 1. filter paper, the filtrate thus obtained was concentrated using a rotary evaporator (Buchi Rotavapor, Switzerland), and the yield was determined gravimetrically.

**Determination of Total Phenolics.** Total phenolic content in the extracts was determined using a Folin−Ciocalteu reagent (*14*). An aliquot (100 $\mu$L) of extract was mixed with 0.75 mL of Folin−Ciocalteu reagent (diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. Sodium bicarbonate (6%, 0.75 mL) was added to the mixture and incubated at room temperature for 90 min. The absorbance was measured at 725 nm using spectrophotometer (Varian DMS 100, Australia). A standard curve was plotted using different concentrations of catechin, and the amount of total phenolics was calculated as catechin equivalents in mg/100 g of potato peel.

**Measurement of Chlorogenic Acid.** Chlorogenic acid content was determined colorimetrically by the method of Griffiths et al. (*15*). Briefly, the diluted extract was vortexed with 2 mL of urea (0.17 M) and acetic acid (0.10 M). To this, 1 mL of sodium nitrite (0.14 M) was added, followed by 1 mL of sodium hydroxide (0.5 M) after incubation at room temperature for 2 min. The suspension was then centrifuged at 2250*g* for 10 min. An aliquot of the supernatant was taken, and the absorbance of the cherry red complex formed was read at 510 nm. A standard curve was prepared using different concentrations of chlorogenic acid, and results were expressed as mg of chlorogenic acid/100 g of potato peel.

**Reducing Activity.** The reducing power of the extracts was determined according to the method of Oyaizu (*16*). A total of 2.5 mL of the extract in ethanol was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. 10% TCA (2.5 mL) was added, and the mixture was centrifuged at 650*g* for 10 min. The upper layer (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm.

**Determination of Antioxidant Activity.** For the evaluation of antioxidant capacity, the method of a thermally induced $\beta$-carotene bleaching assay was used, as described by Velioglu et al. (*17*) with some modifications. The assay reagent was prepared when a solution of $\beta$-carotene (1 mg/mL in chloroform, 1 mL), linoleic acid (40 $\mu$L), and Tween 80 (400 $\mu$L) was mixed. Chloroform was removed under a stream of nitrogen. Then, 100 mL of distilled water was added and vigorously agitated to give stable emulsion. The aliquots (4.5 mL) of the $\beta$-carotene emulsion and appropriately diluted PPE (0.5 mL) were transferred into tubes. Absorbance was measured immediately at 470 nm to measure the zero time absorbance. The tubes were then incubated in a water bath at 50 °C to induce autoxidation. The absorbance at 470 nm was recorded after 60 min. Antioxidant coefficient (AAC) was calculated as

$$\mathrm{AAC} = \frac{A_{s(60)} - A_{c(60)}}{A_{c(0)} - A_{c(60)}} 100$$

where $A_{S(60)}$ is the absorbance of the antioxidant mixture at $t = 60$ min, $A_{C(60)}$ is the absorbance of the control at $t = 60$ min, and $A_{C(0)}$ is the absorbance of the control at $t = 0$ min. BHT (0.02%) was used as a standard.

**Radical Scavenging Activity Using DPPH Assay.** The DPPH assay was performed according to the method of Yamaguchi et al. (*18*). An aliquot of the extract (200 $\mu$L) was mixed with 800 $\mu$L of Tris-HCl buffer (100 mM, pH 7.4). To this was added 1 mL of 500 $\mu$M DPPH in ethanol (final concentration of 250 $\mu$M), and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 20 min in the dark, and the absorbance was taken at 517 nm. % DPPH scavenging activity was calculated as

$$\frac{(\text{control absorbance} - \text{extract absorbance})}{(\text{control absorbance})} 100$$

**Identification of Phenolic Acids of PPE by HPLC.** The extract was passed through a 0.22 $\mu$m filter (Millipore, Westboro, MA) before being injected into a Waters high-performance liquid chromatograph (HPLC). The HPLC system consisted of a Waters 515 HPLC pump, a Waters 717 plus auto sampler, a Waters 996 photodiode detector, and a Waters Novo Pack C 18 reverse phase column (column size 3.9 × 150 mm). The mobile phase was methanol/water/acetic acid 34:65:1 (v/v/v) at a flow rate of 1 mL/min. Individual phenolic acids were identified by the retention time of sample chromatographic peaks being compared with those of authentic standards using the same HPLC operating conditions and also by the extract being spiked with the standard components.

**Meat Irradiation.** The leg and rib region of the lamb meat was taken from the same animal, and all the visible fat was removed from it. Meat from both regions was minced separately in a food processor (Sumeet, India), and each was divided into three lots. To one lot, PPE (4 mL of 1% PPE in 100 g of meat) was added. The final concentration of PPE in meat was 0.04%. To the second lot, BHT (0.04%) was added, and in the third lot, no antioxidant was added. The samples were then packed in polythene pouches and irradiated at 2.5 and 5 kGy. Nonirradiated samples were kept as controls in all three lots. For each treatment, duplicate samples were analyzed. Three such independent experiments were carried out. The packed samples were subjected to $\gamma$ irradiation at melting ice temperature (1−3 °C) in a Package Irradiator (Nordion Intl. Inc. Canada) with a $^{60}$Co source at a dose rate of 3 kGy $h^{-1}$. The samples received minimal doses of 2.5 or 5 kGy with an overdose ratio of 1.3. Dosimetry was performed by a cerric−cerrous dosimeter calibrated against a Fricke's dosimeter. Dosimetry intercomparison was carried out with the National Standards established by the Radiological Physics and Advisory Division (RP&AD), Bhabha Atomic Research Centre (BARC), Mumbai, India.

**Measurement of Lipid Peroxidation.** Thiobarbituric acid-reactive substances produced from lipid peroxidation were determined using the method of Alasnier et al. (*19*). A 4 g portion of each sample was blended with 16 mL of 5% trichloroacetic acid (TCA) and BHT (10 $\mu$g BHT/g of lipids). It was then filtered through a Whatman filter (No. 4). Equal amounts of filtrate and 0.02 M TBA were heated in a boiling water bath for 30 min and cooled, and absorbance was measured at 532 nm. The intensity of the colored complex is a measure of the malonaldehyde concentration. 1,1,3,3-Tetraethoxypropane was employed as the standard for malonaldehyde. TBARS were expressed as mg of malonaldehyde per kg of meat. Oxidative rancidity was also measured by the carbonyl content being determined by the method of Lappin and Clark (*20*). To an appropriately diluted sample (1 mL), carbonyl free methanol (1 mL), 2,4-dinitro phenyl hydrazine (1 mL), and a drop of concentrated hydrochloric acid were added. The mixture was then heated in a water bath (50 °C) for 30 min. The tubes were cooled, and potassium hydroxide (5 mL) was added. A black mixture rapidly changing to a wine red one was obtained, and the absorbance was measured at 480 nm. A methanolic solution of acetophenone was used to obtain the standard curve. Results were expressed as $\mu$mol/g of meat.

**Statistical Analysis.** All results are given as mean ± standard deviation. Differences between variables were tested for significance by one-way ANOVA with Tukey's post-test using the GraphPad InStat version 3.05 for Windows 95, GraphPad Software, San Diego. Differences at $p < 0.05$ were considered to be significant.

## RESULTS AND DISCUSSION

**Quantification of Phenolic Compounds.** Phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators; hence, their total amount in PPE was determined. **Table 1** depicts the total phenolic content of PPE, and it was found to be 70.8 mg in terms of

**Table 1.** Total Phenolics and Chlorogenic Acid Content of Potato Peel Extract[a]

| dose (kGy) | total phenolics[b] | chlorogenic acid[c] |
|---|---|---|
| 0 | 70.8 ± 4.2 | 27.6 ± 1.3 |
| 2.5 | 54.6 ± 2.5 | 26.2 ± 1.4 |
| 5 | 53.6 ± 1.1 | 27.3 ± 1.1 |

[a] Each value is the mean ± standard deviation of three replicate experiments. [b] Expressed as mg of catechin equivalents/100 g of potato peel. [c] Expressed as mg of chlorogenic acid/100 g of potato peel.

**Table 2.** Yield of Total Phenolics and Chlorogenic Acid from $\gamma$ Irradiated Potatoes[a]

| assay | nonirradiated potatoes | irradiated potatoes | % increase in yield |
|---|---|---|---|
| mg of phenolics in terms of catechin equiv/100 g of sample | 87.5 ± 0.5 | 110.7 ± 6.0 | 26.57 |
| mg of chlorogenic acid/100 g of sample | 22.7 ± 1.3 | 36.3 ± 3.9 | 60.29 |

[a] Each value is the mean ± standard deviation of three replicate experiments.

catechin equivalent per 100 g of the sample. Rodriguez de Sotillo et al. (*21*) have reported the total phenolic content in potato peel waste to be 48 mg/100 g. Kahkonen et al. (*22*) have reported 4.3 mg of gallic acid equivalents of phenolics per g of dry potato peel. Major differences in total phenolic content have been reported depending on the color and variety of the potato cultivars (*23*). The yukon gold variety had a total phenolic content of 23.8 mg/100 g, while in the Russet Norkotah variety, it was 52.7 mg/100 g. The selection of the solvent and the extraction method also affected the concentration of the total phenolics. Rodriguez de Sotillo et al. (*21*) have reported that the total phenolic acid content of PPE using methanol at 4 °C was 41.6 mg/100 g; using water at 25 °C was 32.1 mg/100 g, and by the water reflux method, it was found to be 48 mg/100 g. Methanol being toxic is not a preferred solvent. In this study, distilled ethanol was used for the extraction.

Potato tubers contain free and conjugated phenolic acids, the major constituent being chlorogenic acid (*10*). The chlorogenic acid content in PPE was found to be 27.6 mg/100 g of sample (**Table 1**). Rodriguez de Sotillo et al. (*21*) found the chlorogenic acid content in potato peel waste to be 20.5 mg/100 g. Brandl and Herrmann (*24*) analyzed 10 varieties of potato cultivated under the same conditions for the occurrence of chlorogenic acids by high performance liquid chromatography and found that depending on the variety, chlorogenic acid content varied from 22 to 71 ppm.

The main objective of the study was to investigate the potential of PPE as a natural antioxidant in radiation processed meat. To be used as an antioxidant, PPE would be added to meat before irradiation (2.5 and 5 kGy). Since the antioxidant potential of PPE depends on its phenolic and chlorogenic acid content, the effect of irradiation on PPE was determined. When PPE was irradiated at 2.5 kGy, the total phenolic content reduced by 29% (**Table 1**). However, when it was irradiated at 5 kGy, there was no further significant ($p < 0.05$) decrease in total phenolic content. The chlorogenic acid content of PPE did not reduce significantly when irradiated at these doses. Since irradiation of PPE did not affect the chlorogenic acid content, the major phenolic acid of PPE, it can be used as an antioxidant for minimizing rancidity in irradiated meat.

Various stresses, such as irradiation, wounding, nutrient deficiencies, herbicide treatment, or microbial infection are known to induce synthesis of phenolics in tubers. Craft and Audia (*25*) and Zucker (*26*) reported the accumulation of chlorogenic acid and other polyphenols at the sites of infection of potatoes by certain microorganisms, sites of mechanical damage, and bruised potatoes that were exposed to light. Therefore, the total phenolic content from PPE of radiation-processed potatoes was determined. The potatoes were irradiated at 150 Gy, and after about 20 h the peel was removed, and PPE was prepared. The yield of phenolics and chlorogenic acid increased (**Table 2**) significantly ($p < 0.05$). Total phenolics were 26% more in PPE prepared from $\gamma$ irradiated potatoes than from control potatoes, while the chlorogenic acid content increased by 60%. Other workers have also reported increases in total phenolic constituents of potato tubers subjected to $\gamma$ irradiation (*27*, *28*). Also, increases in the total phenol content have also been reported in irradiated mushrooms (*29*). These results show that the selection of appropriate stresses can enhance the nutritional and functional value of potatoes. Phenolics are secondary metabolites produced by the plant, and its increased biosynthesis is a typical reaction of plant tissue to irradiation and other stress factors (*30*). Increases in the concentration of phenolics due to irradiation have been attributed to enhanced phenylalanine ammonia−lyase (PAL) activity (*29*, *31*). PAL (EC 4.3.1.5) catalyzes the first connected step in the biosynthesis of a diverse range of phenyl proponoid derived secondary products in plants such as flavonoids, isoflavonoids, coumarins, and lignins (*32*). It has been reported that chlorogenic acid content of $\gamma$ irradiated potatoes varies during storage (*33*). Within 6−24 h after irradiation, there was a steep increase in chlorogenic acid followed by a decline, and 3 days after irradiation, it was lower in irradiated than nonirradiated tubers. Hence, when extracts are prepared from irradiated potato tubers, the period of storage prior to extraction is of great importance. Large quantities of potatoes are being irradiated commercially, especially in Japan for sprout inhibition (*34*). The waste peel generated by the potato processing plants can thus be utilized as a source of a natural antioxidant having a higher yield of phenolic and chlorogenic acid thereby resulting in value addition.

**Identification of the Phenolic Acids in PPE.** The major phenolic acids in crude PPE were identified by HPLC (**Figure 1**). The major peaks identified by comparison with authentic standards corresponded to chlorogenic acid, caffeic acid, and gallic acid. Rodriguez de Sotillo et al. (*21*) also found that the phenolic content of potato peel determined by HPLC consisted of 50% chlorogenic acid, 41% gallic acid, 8% protocatechic acid, and 0.2% caffeic acid. The major constituent of crude PPE was chlorogenic acid. Other studies (*10*, *21*) have also reported the same phenolic acids and chlorogenic acid as the major constituent in the peels of tubers of different potato cultivars. The HPLC profile of irradiated PPE was the same as that of the nonirradiated PPE. This observation substantiates our earlier observation, wherein it was seen that quantitatively, chlorogenic acid content did not change due to irradiation of PPE.

**Antioxidant Activity of PPE.** Heat-induced oxidation of an aqueous emulsion system of $\beta$-carotene and linoleic acid was employed as an antioxidant test reaction. In the absence of an antioxidant, as the $\beta$-carotene molecules lose their conjugation, their characteristic orange color is lost, which can be monitored spectrophotometrically. **Figure 2** shows the antioxidant activity of PPE determined by this model system. At a concentration of 0.1%, PPE had an antioxidant activity coefficient (AAC) of 550, while BHT (0.02%) had an AAC of 418.

The DPPH free radical scavenging activity of PPE is shown in **Figure 3**. The DPPH radical has been widely used to test the free radical scavenging ability of various natural products and has been accepted as a model compound for free radicals

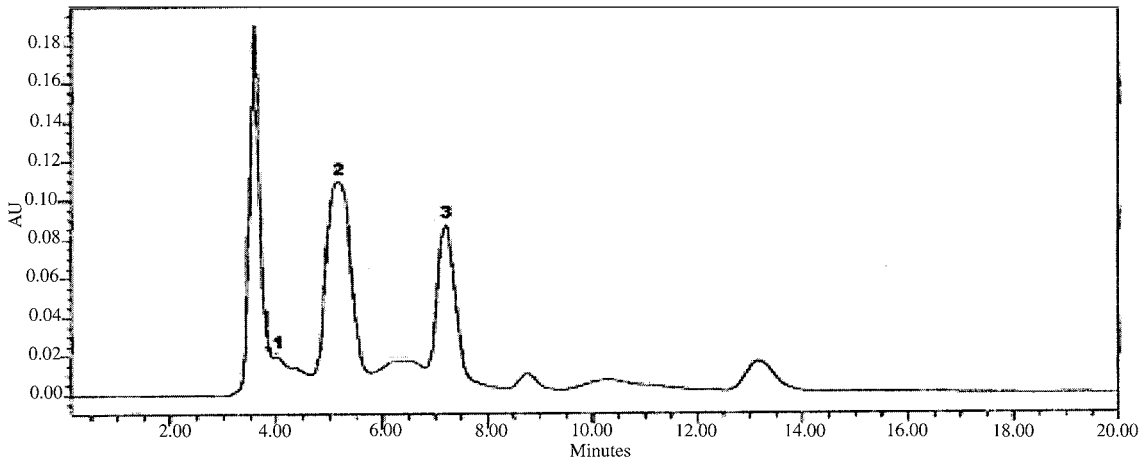


**Figure 1.** HPLC profile of crude PPE. Peaks: 1-gallic acid (4.02); 2-chlorogenic acid (5.16); and 3-caeffic acid (7.20). Figures in parentheses indicate retention time in min.

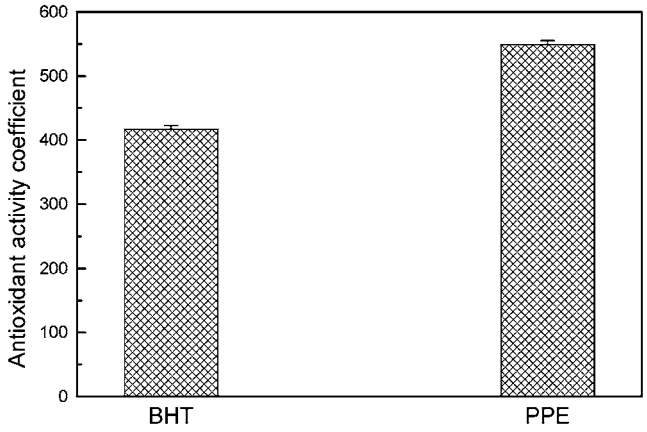


**Figure 2.** Antioxidant activity of PPE by $\beta$-carotene bleaching assay. Values are the mean ± standard deviation of three replicate experiments.

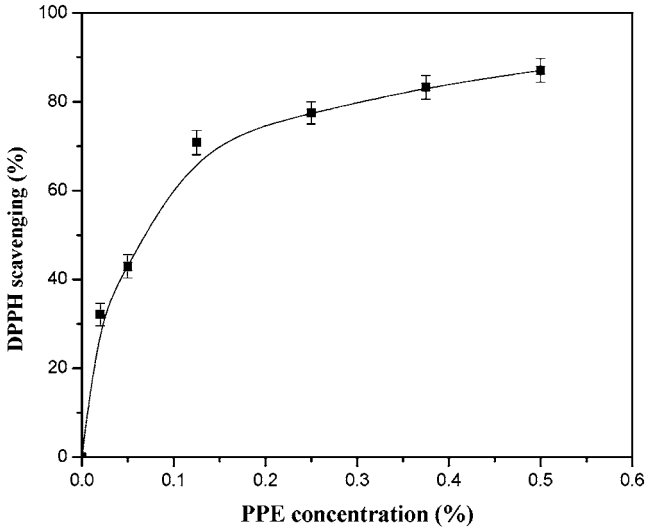


**Figure 3.** Antioxidant activity of PPE by DPPH colorimetric assay. Values are the mean ± standard deviation of three replicate experiments.

originating in lipids (*35*). The % DPPH scavenging activities of the extracts were concentration dependent. Significant DPPH radical scavenging activity was evident at all the tested concentrations of PPE. At 0.1%, PPE gave 60% activity, while at 0.5%, it gave 85% activity by this method. The DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability; the more the number of hydroxyl groups, the

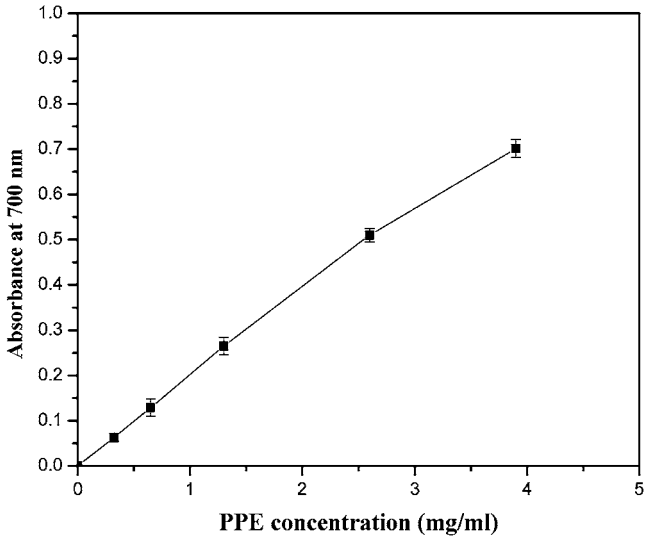


**Figure 4.** Reducing power of potato peel extract. Values are the mean ± standard deviation of three replicate experiments.

higher the possibility of free radical scavenging ability (*36*). A linear correlation between radical scavenging activity and polyphenolic content has been reported in an extensive range of vegetables and fruits (*37*).

**Figure 4** depicts the reducing power of PPE as a function of its concentration. In this assay, the reductants present in the extract cause reduction of $Fe^{3+}$ to the $Fe^{2+}$ form, which can be monitored spectrophotometrically. The reducing power of PPE increased with an increase in concentration and at 4 mg/mL displayed a good reducing power of 0.7, thus indicating its potential as an antioxidant. The reducing power of a compound is related to the electron-transfer ability of that compound; therefore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Several authors (*38*, *39*) have observed a direct correlation between antioxidant activities and reducing power of certain plant extracts, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.

**Effect of PPE on Lipid Peroxidation of Lamb Meat.** As PPE demonstrated good antioxidant activity; its efficacy in retarding lipid peroxidation of lamb meat was examined. Lipid peroxidation was measured in terms of the TBA number and carbonyl value that were affected ($p < 0.05$) by storage time and treatment. On irradiation (2.5 and 5 kGy), there was a dose dependent increase in TBA and carbonyl values (**Figures 5**−**8**).

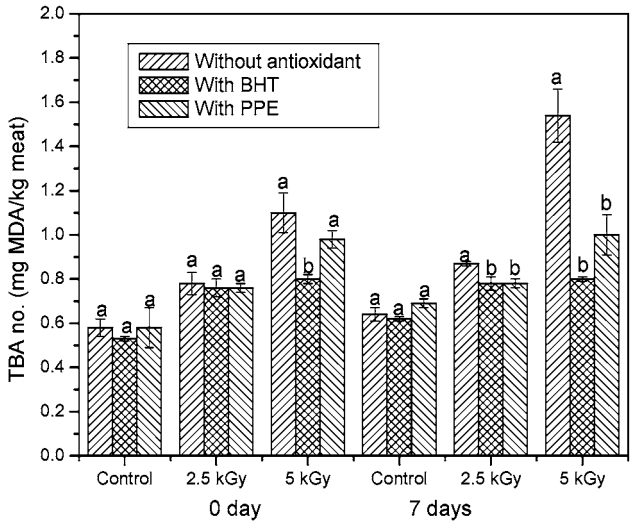


**Figure 5.** Effect of PPE and BHT on the formation of thiobarbituric acid-reactive substances in lamb leg meat stored at 0–3 °C. Data represent means ± standard deviation. Means with the same letter (a or b) are not significantly different ($p > 0.05$).

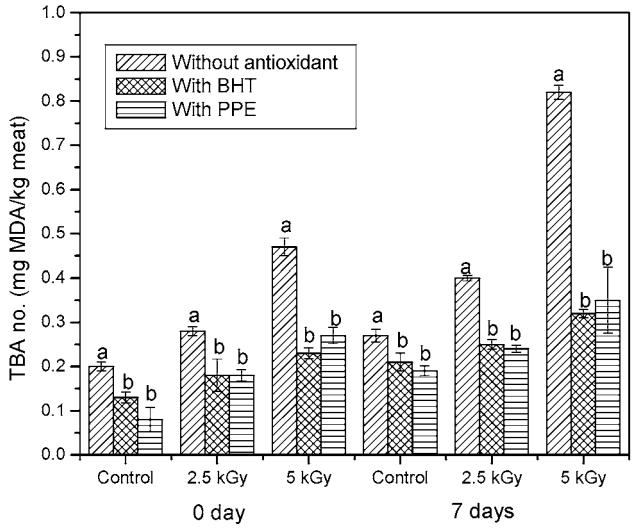


**Figure 6.** Effect of PPE and BHT on the formation of thiobarbituric acid-reactive substances in lamb rib meat stored at 0–3 °C. Data represent means ± standard deviation. Means with the same letter (a or b) are not significantly different ($p > 0.05$).

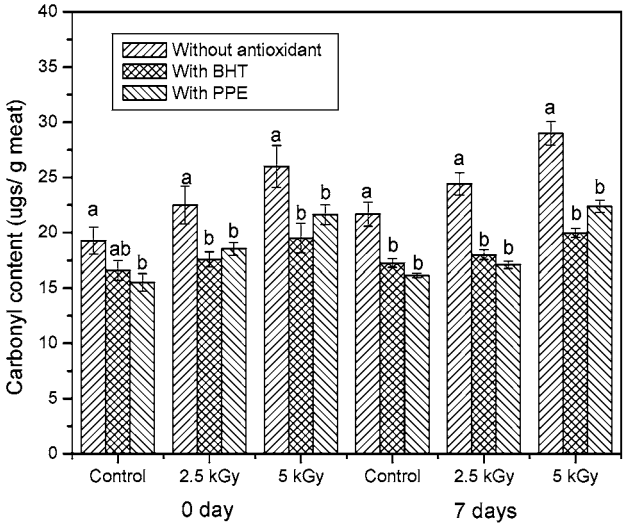


**Figure 7.** Effect of PPE and BHT on the carbonyl content of lamb leg meat stored at 0–3 °C. Data represent means ± standard deviation. Means with the same letter (a or b) are not significantly different ($p > 0.05$).

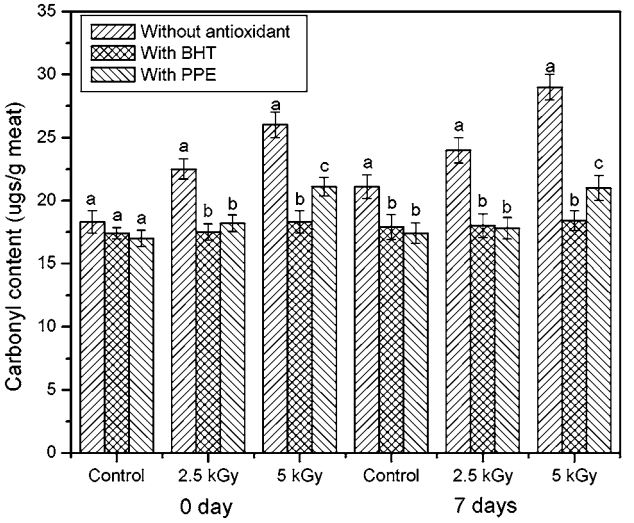


**Figure 8.** Effect of PPE and BHT on the carbonyl content of lamb rib meat stored at 0–3 °C. Data represent means ± standard deviation. Means with the same letter (a, b, or c) are not significantly different ($p > 0.05$).

There are many reports on the increase in lipid peroxidation of radiation processed meat and meat products. Luchsinger et al. (*40*) found that the TBA values for aerobically packaged pork chops increased with irradiation dose or storage time. The presence of oxygen affects the rate of oxidation (*41*).

To establish the antioxidant potential of crude extract of PPE, it was added to the meat prior to irradiation. Lowered ($p < 0.05$) TBA values and carbonyl content (**Figures 5**−**8**) indicated that lipid peroxidation was retarded in lamb meat by the addition of PPE before irradiation. Initially, when PPE was added to meat prior to irradiation (5 kGy), the TBA number reduced by 12% as compared to meat irradiated without PPE. On storage (at 0−3 °C for 7 days), the TBA number of irradiated (5 kGy) meat containing PPE was 54% less in comparison to the irradiated (5 kGy) meat not containing PPE. Similarly, in the case of rib meat, addition of PPE prior to irradiation resulted in a reduction of the TBA number by 74%. A similar trend was observed in the carbonyl content also. Initial carbonyl content of irradiated meat containing PPE was 20% less than the corresponding sample not containing PPE. On storage, a 30% reduction was obtained in PPE containing irradiated meat. Thus, the efficacy of PPE in retarding oxidative rancidity in meat samples was evident and comparable to that of BHT.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen, or decomposing peroxides (*42*). There are few reports of the use of PPE as an antioxidant. Rodriguez de Sotillo et al. (*7*) found that freeze-dried extracts of potato peel waste prevented oxidation in sunflower oil. Mansour and Khalil (*13*) have reported that freeze-dried extract of potato peel was effective in controlling lipid oxidation and color changes during cold storage in beef patties, although to a lesser extent as compared to ginger rhizomes and fenugreek seeds.

PPE retards lipid peroxidation of radiation-processed meat without affecting its flavor/aroma, thereby improving its storage quality. Hence, the possibility for utilizing waste potato peel as a promising source of a potent antioxidant for radiation-processed meat should be explored further in product development.

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**Received for review October 19, 2004.**

JF048270E