Utilization of Electron Beam Irradiated Almond Skin Powder as a Natural Antioxidant in Ground Top Round Beef

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ABSTRACT: Defatted Carmel variety almond skin powder (ASP) irradiated at 0, 10, 20, and 30 kGy was mixed with ground top round beef at 0.5% (w/w) and stored at 4°C. Color stability, peroxide values (PV), conjugated dienes (CD), thiobarbituric acid reactive species (TBARS), and hexanal were determined periodically over a 2-wk period. The L* values were not affected by time or treatment. Initially, the redness (a* value) was higher for both the negative control with no ASP (a* value of 21.83) and positive control with 0.01% BHT (a* value of 22.33) compared to samples that contained the ASP (a* values from 17.70 to 20.17) on day 1. This decrease in redness was attributed to the presence of the ASP. Similar to day 1, the a* values were not significantly different between the 2 controls over the duration of the study. All the samples with ASP exhibited lower lipid oxidation when compared to the negative control, with greatest oxidation retardation observed at 20 and 30 kGy. Over the treatment period, a 13% to 85% reduction in PV and a 40% to 80% reduction in TBARS were observed in the sample with 30 kGy ASP compared to the negative control. Generally, PV and TBARS of samples with ASP decreased with an increase in irradiation dose. While a difference due to irradiation dose was not observed in CD and hexanal content, the values were significantly lower (P < 0.05) than the controls over time. This study demonstrates that almond skin powder could be used to extend the shelf life of refrigerated ground raw beef.

Keywords: almond skin powder, beef, electron beam irradiation, lipid oxidation

Introduction

Lipid oxidation caused by exposure to oxygen, light, and metal catalysts during processing and storage is a major cause of chemical deterioration in beef meat. Synthetic antioxidants such as BHT, BHA, and TBHQ can be added to beef at 100 ppm based on fat content when used alone or 200 ppm total when used in combination (Sang and others 2002a; USDA 2006). Possible toxicity and ongoing consumer concern over synthetic antioxidants have increased interest in the use of natural antioxidants to extend the shelf life of food. Use of agricultural co-products rich in antioxidants further also increases the value of waste products. Cherry tissue, peanut skin extracts, potato peels, rice hulls, grape skins, and seeds are among the agricultural co-products that have been investigated as effective retarders of lipid oxidation in muscle foods (Mansour and Khalil 2000; Tang and others 2001; Candogan 2002; Nam and others 2004; Nissen and others 2003; Rababah and others 2004).

Here we investigate the use of almond skins, a co-product from almond blanching, as a potential natural antioxidant in raw ground beef. Almond skins, which comprise 4% of the almond fruit, are an underutilized co-product and often are discarded as waste or used as cattle feed (Sang and others 2002a, 2002b; Takeoka and Dao 2003; Dourado and others 2004; Chen and others 2005). Rich in phenolic compounds, almond skins have demonstrated 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities, Trolox equivalent antioxidant capacity, and hydroxyl radical-scavenging capacities (Sang and others 2002a; Siriwardhana and Shahidi 2002).

In food products, almond skin extract in soybean oil and comminuted cooked pork have exhibited antioxidant activity (Wijeratne and others 2006; Harrison and Were 2007). The antioxidant activity of plant products is partially attributed to phenolic compounds. Phenolic compounds present in plants are normally glycosylated. Processing methods that involve heat treatment or irradiation could liberate aglycone phenolics from their glycoside components, with a potential enhancement of phenolic-related properties, for example, antioxidant and antimicrobial capacity. The effect of various forms of irradiation on the total phenolic content and related antioxidant activity of various dehydrated agricultural products has been determined with conflicting results. Gamma-irradiation doses ranging from 1 to 20 kGy had no significant effect on the phenolic content, DPPH, superoxide, and free radical scavenging capacity of tea leaves or green tea leaf by product powders (Mishra and others 2006; Na Young and others 2006). Similarly, no difference in total phenolic content between nonirradiated controls and alcohol extracted 30 kGy irradiated dry rosemary leaves was observed (Perez and others 2007). The insignificant effect on the phenolic content was attributed to the dry nature of the products (Mishra and others 2006). In contrast to the aforementioned studies, gamma irradiation has been shown to increase the phenolic content and antioxidant effect of dry products, for example, spices, rice hulls, almond skins, and soybeans (Variyar and others 1998; Lee and others 2003; Murcia and others 2004; Varriyar and others 2004; Harrison and Were 2007). The increase in phenolic content and antioxidant activity has been attributed to the cleavage of covalent bonds that liberate and activate low molecular weight phenolic compounds from their glycosylated forms. The liberated aglycones with higher antioxidant activity in...
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various systems may be also beneficial when applied to food matrices, for example, meat (Jeong and others 2004; Variyar and others 2004; Lee and others 2006).

The experimental objectives were to determine the effect of almond skin powder on retardation of lipid oxidation in top round ground beef and whether electron beam irradiation of the almond skin powder enhanced its antioxidant property.

Materials and Methods

Materials

Carmel variety almond skins from the 2006 harvest were provided by the Almond Board of California. Barium chloride dehydrate, (II) sulfate, hydrochloric acid, HPLC grade chloroform, HPLC grade water, sodium carbonate, quercetin, Folin–Ciocalteu’s phenol reagent, methanol, isooctane, hexane, petroleum ether, trichloroacetic acid (TCA), and isopropanol were purchased from Fisher Scientific (Fair Lawn, N.J., U.S.A.). Ammonium thioxyanate and 2-thiobarbituric acid (TBA) were purchased from Spectrum Chemicals (Fair Lawn). Antifoam A and hexanal were purchased from Sigma Aldrich (St. Louis, Mo., U.S.A.). A Supelcowax-10 column was purchased from Supelco (St. Louis, Mo., U.S.A.), and top round beef was purchased from a local market (Costco, Tustin, Calif., U.S.A.).

Preparation of meat sample

Top round beef was purchased from a local market (Costco, Tustin, Calif., U.S.A.). The excess fat was trimmed and beef was cut into cubes, and ground using a Kitchen Aid Professional 5 food grinder with a 3-mm fine grinding plate (St Joseph, Mich., U.S.A.). Five kilograms of meat were divided into the following 6 treatments: incorporation of 0.5% (w/w) defatted ASP at 0, 10, 20, 30 kGy, a positive control with 0.01% (w/w) BHT, and a negative control without any ASP or BHT. Duplicates were prepared for each sample. Samples were separated for various analyses (color, moisture, and fat, lipid extraction for PV and CD, TBARS, and GC) and stored in amber glass jars at 4 °C for a maximum of 14 d.

Moisture and total fat determination of ground beef

The moisture content in ground beef was measured as outlined in AOAC method 991.02. Ground beef (2 g) was added to pre-weighed aluminum pans, and was dried in a vacuum oven (95 to 100 °C under ≤ 10 mmHg pressure) for 24 h. The sample was weighed before and after drying and % moisture was determined based on the weight loss. Fat was extracted using a Soxhlet method (AOAC method 991.08).

Lipid extraction for peroxide value and conjugated dienes

The method by Nilsson and others was used to extract lipids from ground beef (Undeland and others 1998). Twenty grams of each sample treatment was placed in a 250 mL Erlenmeyer flask. Isopropanol (32 mL) was added to the sample prior to homogenizing with a Brinkmann homogenizer (Polytron PT 10/35, Kinematica AG, Switzerland) at a speed of 5 for 30 s. Hexane (64 mL) was added and mixture was homogenized (speed of 5 for 30 s). These steps were done in an ice bath. All samples were centrifuged at 14000 rpm for 15 min at 4 °C, and the supernatant (n-hexane phase) was collected in a beaker. Evaporation of the n-hexane was done in a hood overnight, leaving the extracted beef lipids for PV and CD analyses.

Peroxide value determination

The IDF method 74A:1991 was used to determine the peroxide value. Extracted beef lipid (0.05 g) was mixed with 9.8 mL chloroform–methanol (7:3 v/v) and vortexed for 2 to 4 s. Fifty microliters of 1% ferrous iron solution were added and vortexed for 2 to 4 s. The samples were incubated for 5 min at room temperature and absorbance at 500 nm was measured using DU800 UV/Visible spectrophotometer (Beckman Coulter, Calif., U.S.A.). This entire procedure was conducted in subdued light. The peroxide values were expressed as mEq of peroxides/kg of sample which were calculated using the formula below:

\[
\text{Peroxide value} = \frac{(A_b - A_s) \times m}{(55.84 \times m) \times (0.015 \times 2)}
\]

where \(A_s\) = absorbance of sample; \(A_b\) = Absorbance of the blank; m = slope of the curve; \(mO\) = mass in grams of the sample; and 55.84 = atomic weight of iron (Shantha and Decker 1994).

UV detection of conjugated dienes

Conjugated dienes were determined using a modified method adopted from Juntachote and others (2006). Extracted beef lipids (0.015 g) were massed into a 25 mL volumetric flask and brought to volume with isooctane and mixed. Absorbance was read at 234 nm with isooctane used as the blank. The CD concentration was calculated using a molar extinction coefficient of 25200/M/cm and the results were expressed as \(\mu\)mol/mg meat lipid sample (Juntachote and others 2006).
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Thiobarbituric acid reactive species (TBARS) values determination

Beef sample (5 g) was mixed with 12.5 mL of 20% TCA solution and homogenized for 30 s set at high speed (Stomacher 400, Seward, U.K.). The homogenate was filtered using a Whatman nr 1 filter paper. Two milliliters of the filtrate were transferred into a test tube; 2 mL of TBA solution (0.02 M) were then added and incubated for 15 h at room temperature in the dark. The absorbance was measured at 532 nm. TBAR values were recorded as nmol malondialdehyde/g sample (Yin and Cheng 2003).

Hexanal determination by gas chromatography (GC)

The volatile oxidation products bound to the beef protein were extracted by distillation (Ross and Smith 2006). Beef sample (5 g) was placed in a round bottom flask and mixed with 25 mL of distilled water and 5 drops of antifoam A. The flask was attached to a Bidwell–Sterling moisture trap connected to a condenser. The flask was then heated on individually controlled soxhlet apparatus heaters for 15 to 20 min until 5 mL or more of the distillate was collected. The distillate (5 mL) was then transferred into a test tube (18 mm × 150 mm), and stored in an ice bath until analysis. Purge and trap (OI Analytical 4560, College Station, Tex., U.S.A.) was used by attaching the test tube to purge at 70 °C for 15 min. Hexanal was trapped in a Tenax trap (Trap nr 7, 0.125” O.D. × 0.105” I.D.) and quantified using a GC (Varian 3800, Walnut Creek, Calif., U.S.A.) with an FID detector set at 250 °C. Helium was used as a carrier gas at a flow rate of 20 mL/min. A Supelcowax-10 column (Supelco) was used with the following temperature program: 50 °C was held for 2 min and then increased to 225 °C at a rate of 20 °C/min and held for 5 min. External calibration was used to quantify hexanal concentrations.

Statistical analysis

There were a total of 6 treatments (negative control, 0.01% BHT, 0.5% of 0 kGy, 10 kGy, 20 kGy, and 30 kGy irradiated ASP) and 6 storage time points. Meat preparation for each treatment was performed in duplicates and 2 readings for each sample were taken. The data were analyzed using the general linear model procedure to determine differences among treatments, with the significance level determined at 0.05 using Duncan’s test (SAS software V9.1.2, SAS Inst. Inc., Cary, N.C., USA). Pearson correlation coefficients were determined between TBARS and hexanal.

Results and Discussion

Moisture and fat content of ground top round beef samples

The moisture and fat content were 73.15 ± 0.30% and 2.79 ± 0.02% in beef sample without ASP and 72.27 ± 0.56% and 2.78 ± 0.03% in samples containing almond skin, respectively. The moisture content of samples with ASP was slightly lower due to a lesser beef amount, which was replaced by the lyophilized defatted ASP. The fat content of raw top round beef determined in this study was similar to that observed by previous investigators who obtained a value of approximately 2.6% (Yin and Cheng 2003).

Total soluble phenolics in almond skin powder (ASP)

The phenolic content in 20 and 30 kGy irradiated ASP was higher compared to 10 kGy and the nonirradiated ASP (Table 1). The increase in total phenolics with the 30 kGy ASP may have been due to possible liberation and activation of low molecular weight phenolic compounds. Irradiation has been shown to increase the aglycone content in various plant-derived antioxidants (Variyar and others 1998, 2004; Lee and others 2003; Murcia and others 2004). It is unclear why the 10 kGy ASP sample exhibited higher total soluble phenolics compared to the nonirradiated sample.

Effect of almond skin powder on peroxide value and conjugated dienes of refrigerated raw ground beef

Peroxide values were affected by storage time and the presence or absence of ASP (Figure 1). Peroxide values increased for all samples until day 6 when they started to decline for all treatments. After day 6, the rate of hydroperoxide formation may have been lower than the rate at which they were decomposing resulting in decreased PV after day 6 (Georgantelis and others 2007). Generally, samples containing ASP irradiated at 30 kGy exhibited lower peroxide values than samples with ASP irradiated at a lower dose or the control samples with no ASP. The PV values generally decreased with increase in dose, with the greatest difference observed on day 2, in which the PV values for the 30 kGy sample were 85% lower than the negative control and 80% lower than the positive control. The smallest differences were observed on days 4 and 6, with a percent reduction of 13% and 16% compared to the negative control and a 12% and 9% reduction compared to the positive control, respectively. On days 8 to 12, PV values were 32% to 51% lower than the negative control and 20% to 26% lower than the positive control. Significantly lower peroxide values on days 2 and 4 are similar to results previously found in which γ-irradiation resulted in decreased PV in a soybean oil emulsion (Harrison and Were 2007). Samples containing ASP irradiated at 20 and 30 kGy were the most effective in reducing the peroxide values, pointing to a greater suppression of hydroperoxide formation by 20 and 30 kGy ASP. The antioxidant effect of ASP could be attributed to structural changes in the phenolic compounds. High irradiation doses of greater than 20 kGy could enhance the antioxidant capacity by releasing aglycones from their structures.

Table 1 – Effect of electron beam irradiation on the phenolic content of almond skin powder.

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>Quercetin equivalent (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>416 ± 22d</td>
</tr>
<tr>
<td>10</td>
<td>386 ± 4*</td>
</tr>
<tr>
<td>20</td>
<td>439 ± 2*</td>
</tr>
<tr>
<td>30</td>
<td>514 ± 16*</td>
</tr>
</tbody>
</table>

N = 2. Means with different superscript letters are significantly different (P < 0.05).

Figure 1 – Effect of 0.5% (w/w) irradiated and nonirradiated almond skin powder on peroxide value (mEq peroxides/kg) of refrigerated top round ground beef lipids. Values were an average of duplicate determinations.
sugar moieties. This redistribution of phenolic compounds could result in enhanced antihydroperoxide formation of the aglycone forms (Jeong and others 2004; Variyar and others 2004; Lee and others 2006).

The conjugated dienes (CD) of the samples increased significantly after day 4 (Figure 2). During lipid peroxidation, instantly after peroxides are formed, the nonconjugated double bonds (C=CH–CH–C=O) which are present naturally in unsaturated lipids are converted to conjugated double bonds (C=CH–CH=CH) with strong absorption measured at 232 to 234 nm (Kulas and Ackman 2001). Conjugated dienes are formed at the early stages of lipid oxidation under conditions in which hydroperoxides go through little or no decomposition, which at later stages of oxidation the hydroperoxides decompose into secondary products that may also absorb at 234 nm (Kulas and Ackman 2001). During the 2 wk analysis, there was little or no difference in CD between samples with ASP and those without ASP on each day of analysis. Time was a greater determinant of CD increase compared to treatment, with samples after day 4 exhibiting much higher CD values compared to those on day 2 (Figure 2).

**Thiobarbituric acid reactive substance values and hexanal content**

The samples containing ASP started off with similar malondialdehyde values on day 2 (Figure 3A). The TBARS values increased with storage time for all treatments with the samples containing no ASP exhibiting higher increases in TBAR values with time. The decrease in TBARS on the various days was greater for samples with irradiated ASP at 20 and 30 kGy compared to the control sample with no ASP or samples with nonirradiated ASP (0 kGy). The samples with ASP irradiated at 0, 10, 20, and 30 kGy had 40, 41, 57, and 78% lower TBARS compared to the negative control on day 4, respectively. Similarly on day 11, the decrease was lower for the samples with 20 and 30 kGy irradiated ASP. The decrease in TBARS was 55%, 53%, 73%, and 82% for the samples with 0, 10, 20, and 30 kGy ASP compared to negative control samples on day 11.

As the TBARS increased so did the hexanal levels. Pearson correlation coefficients for hexanal and TBARS on the different days of analyses ranged from 0.74 to 0.93. The hexanal levels of all samples increased with storage time, although samples treated with BHT and ASP increased at a slower rate than the negative control (Figure 3B). Hexanal values for the negative control sample ranged from 34 to 524 ppb while those with added ASP ranged from 17 to 115 ppb over the 12-d duration of the study. After 1 d of storage, the hexanal level of the meat with no ASP was 33.92 ppb, while that of the samples with ASP ranged from 17.39 to 21.48 ppb. The hexanal levels remained relatively unchanged for the first 4 d. After day 4, the samples containing BHT had a lower hexanal concentration when compared to the control samples, and those with ASP had even lower hexanal values compared to the control sample or the sample with BHT. There was no difference between the effect of irradiated and nonirradiated ASP on the formation of hexanal over time as both effectively suppressed the hexanal content formation (Figure 3B). The increase in phenolic content with irradiation may not have been high enough to significantly affect the hexanal content in a complex food system like meat.

**Effect of almond skin powder on color of refrigerated top round raw ground beef**

The changes in the red color and lightness in the ground beef are shown in Table 2. The bright red color of raw red meat is synonymous with freshness for many consumers. The beef samples...
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Table 2—The effect of almond skin powder on the lightness (L value) and Hunter red color values (a value) of refrigerated top round ground beef samples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 11</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>30.10 ± 0.35*</td>
<td>30.8 ± 0.15*</td>
<td>32.1 ± 0.30</td>
<td>31.6 ± 0.76*</td>
<td>22.33 ± 0.72*</td>
<td>18.87 ± 0.17*</td>
<td>3.44 ± 0.11*</td>
<td>3.28 ± 0.15*</td>
</tr>
<tr>
<td>(BHT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>32.00 ± 0.34*</td>
<td>31.57 ± 0.40*</td>
<td>32.1 ± 1.35*</td>
<td>31.86 ± 0.76*</td>
<td>21.83 ± 0.86*</td>
<td>18.87 ± 0.15*</td>
<td>3.40 ± 0.10*</td>
<td>3.28 ± 0.21*</td>
</tr>
</tbody>
</table>

N ≥ 2. For each type of meat, within each storage time, means from different treatments with different superscript letters are significantly different (P ≤ 0.05).

containing ASP had a slightly lower red color (lower a values) on days 1 and 4 when compared to the ground beef samples without ASP. The incorporation of ASP thus lowered the a values of the beef samples (Table 2). On day 8, a values for all treatments decreased by at least 74% when compared to a values on day 1. The substantial decrease in a values after day 8 could be due to oxidation of oxy-myoglobin to met-myoglobin which results in a color change from bright red to brown (Yin and Cheng 2003). Brown color in meat affects sales and is estimated to result in an annual economic loss of greater than a billion dollars at the retail level (Connolly and others 2002). The a values on days 8 and 11 were higher for the samples with ASP compared to those without ASP. The antioxidant properties of ASP may have similarly reduced the formation of met-myoglobin. Lipid oxidation and color change in meat are normally inhibited by addition of synthetic antioxidants, for example, BHA and BHT (Moore and others 2003). In the current study, the control ground beef and that with BHT had higher a values than samples with ASP. The redness of the meat with BHT over time was, however, not significantly higher than that of the control meat. Similar findings of a lack of higher Hunter a values with BHA and BHT compared to control raw and cooked ground beef have been observed by previous investigators (Hettriaichchly and others 1996; Ahn and others 2007). BHT is a less effective antioxidant compared to BHA due to the presence of 2 tert-butyl groups in BHT that causes steric hindrance. This steric hindrance may partially have accounted for the lack of significantly higher a values between the control and meat sample with BHT. There was little change in L values between samples with ASP and those containing no ASP.

Conclusions

The greatest degree of lipid oxidation over time occurred in the negative control minced meat sample with no ASP or BHT. The almond skin powder effectively retarded lipid oxidation in refrigerated raw ground beef as indicated by PV, TBARS, and hexanal content over 12 d of analysis. The effect of electron-beam irradiation of ASP on retardation of lipid oxidation was only significant for PV and TBARS at doses greater than 20 kGy. This is the 1st study investigating the effect of ASP in ground beef and the potential effect of e-beam irradiation on enhancing the antioxidant properties of ASP in a meat system. E-beam irradiation at doses greater than 20 kGy could potentially enhance the antioxidant activity of ASP and similar agricultural by-products.

Acknowledgments

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