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Electron beam irradiated almond skin powder inhibition of lipid oxidation in cooked salted ground chicken breast

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ABSTRACT

Antioxidant ability of electron beam irradiated almond skin powder (ASP) in cooked refrigerated ground chicken breasts (GCB) was investigated. Phenolic compound identification, total phenols and metal binding ability was determined. GCB were treated with irradiated ASP at 10, 20 and 30 kGy and tested against a positive (with TBHQ) and negative control (GCB without ASP or TBHQ) for colour, conjugated dienes (CD), TBARS and hexanal content during one week of refrigerated storage. Irradiation had no effect on total phenols, but resulted in greater metal chelation. Addition of ASP decreased hunter L^* value in GCB. The 10 kGy ASP decreased lipid oxidation to the greatest extent (35.5–52.3%, 44.0–84.0%, and 74.9–87.4% decreases in CD, TBARS and hexanal formation, respectively) as compared to the negative control over one week of refrigerated storage. In general, cooked refrigerated GCB with ASP had lower oxidation products as compared to the negative control.

1. Introduction

Lipid oxidation is a self propagating chain reaction which attacks polyunsaturated fatty acids located within a meat system. It is one of the major factors in chemical deterioration of meats during storage due to the development of unpleasant flavours and off-odours, changes in texture and potential formation of toxic compounds (Niedernhofer, Daniels, Rouzer, Greene, & Marnett, 2003). Lipid composition of meats, processing, distribution and storage can affect the extent of lipid oxidation and rancidity in muscle foods. Increased susceptibility to lipid oxidation in chicken lipids as compared to beef or pork lipids is attributed to higher amounts of polyunsaturated fatty acids (Vasavada & Cornforth, 2006). Processes that disrupt the muscle membrane system, such as grinding, cooking, and deboning contribute to increased exposure of lipids to oxygen and the release of pro-oxidants, thus accelerating oxidative rancidity. Cooking meat accelerates oxidation due to the denatured state of the muscle proteins which results in the release of heme iron from the porphyrin ring (Chen, Pearson, Gray, Fooladi, & Ku, 1984).

Aldehydes such as hexanal and pentanal produced during lipid oxidation contribute to "warm-over flavour" (WOF) development which refers to the flavours and aromas that develop in cooked meats during storage and later reheating (Shahidi & Pegg, 1994). The extent of lipid oxidation can be evaluated by hexanal content, TBARS values and sensory panels (WOF) and all have been found to be highly correlated, where increases in secondary oxidation products have resulted in increased meat rancid flavour (Ahn, Gruen, & Fernando, 2002; Igene, Yamauchi, Pearson, Gray, & Aust, 1985; Vasavada & Cornforth, 2006). Analysis of secondary oxidation products like hexanal and malonaldehyde can thus be used to monitor possible effects of plant extracts on WOF development and their efficacy in retarding lipid oxidation.

Lipid oxidation in meat products can be controlled by various measures such as managing an animal's diet to lower the degree of lipid unsaturation, packaging changes to control the amount of oxygen, or the addition of antioxidants during processing. Synthetic antioxidants such as TBHQ (*tert*-butylhydroquinone), BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole) and PG (propyl gallate) at 100–200 ppm are generally recognized as safe by the US Food and Drug Administration for use in meat products (FDA, 2006). A shift away from the use of synthetic antioxidants has occurred due to health concerns about synthetic antioxidants, a preference for natural ingredient sources and the benefits of a diet containing elevated levels of antioxidants and other nutraceutical compounds.

Almonds (*Prunus amygladus*) consist of three main parts including the nut, skin and hull. The skins, which consist of about 4% of the almond, are removed by blanching and are traditionally ground into animal feed or discarded. Almonds contain high levels of flavonoids and phenolic acids antioxidants which are found exclusively or in high concentrations within the skins (Milbury, Chung-Yen, Dolnikowski, & Blumberg, 2006; Wijeratne, Amarowicz, & Shahidi, 2006). Antioxidants such as flavonoids which are found within the almond skins have the ability to quench free radicals as shown by

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inhibiting oxidation of low-density lipoprotein and cancer cell proliferation *in vivo* (Serafini, Laranjinha, Almeida, & Maiani, 2000). *In vitro* studies have likewise shown the effectiveness of phenolic antioxidative components of plant and plant extracts in retarding lipid oxidation in chicken (Jo et al., 2006; Vasavada & Cornforth, 2006).

A limiting factor in the use of natural antioxidants is the lower potency of natural antioxidants compared to the synthetic variants. Enhancing the antioxidant properties of natural antioxidants may increase their utilization. The effect of various forms of irradiation on antioxidant activity and the compounds responsible for this activity have been investigated in an assortment of food co-products (Kanatt, Ramesh, Radhakrishna, & Arun, 2005; Variyar, Limaye, & Sharma, 2004). Prasetyo, Chia, Hughey, and Were (2008) found that the application of increasing dosages of electron beam irradiated almond skin powder (ASP) in raw ground beef inhibited lipid oxidation during two weeks of refrigerated storage. Further study is needed to evaluate if ASP, a co-product of the almond industry, has the ability to work in different meat systems with different processing treatments. Therefore, the objective of this study was to evaluate the effect of irradiation on ASP and its effect on lipid oxidation in cooked skinless ground chicken breasts.

2. Materials and methods

2.1. Materials

Non-pareil almond skins from the 2006 harvest were obtained from the Almond Board of California (Modesto, CA). Almond skins were prepared as previously reported by Prasetyo et al. (2008). Briefly, skins were sorted to eliminate residual almond meal, frozen at −80 °C (Revco, Vernon Hills, IL) and lyophilized (Dura-Dry II MP, Stoneridge, NY) for 24 h. Lyophilized almond skins were pulverized in the presence of dry ice using a mortar and pestle and sieved (400-mesh). The almond skin powder (ASP) was then defatted (1/10, w/v), sonicated (Fisher Scientific FS60H, Pittsburgh, PA), filtered and dried under a laminar hood. Samples were packaged into quart size Ziploc™ bags and irradiated at 10, 20, and 30 kGy at ambient temperature (NEObeam Facility at Kent State University, OH). Irradiated samples were shipped back to Chapman University and stored frozen at −80° C until experiments were performed.

2.2. Chemicals

Hexanes, isopropanol, trichloroacetic acid (TCA), methanol, ferrozine iron reagent and HPLC grade water were purchased from Fisher Scientific (Tustin, CA). 2-thiobarbituric acid (TBA) was purchased from Spectrum Chemicals (Gardena, CA). Reagent 1,1-3,3-tetraethoxypropane (TEP) was purchased from Aldrich Chemical (Milwaukee, WI). Folin–Ciocalteu phenol reagent, hexanal (98%), pentanal (98%), nonanal (95%) and quercetin were purchased from Sigma Aldrich (St. Louis, MO). Ferrous sulphate was purchased from JT Baker (Phillpsburg, NJ). Tenox20A (*tert*-butylhydroquinone, TBHQ) was obtained from Eastman Chemical Company (Kingsport, TN). Phenolic authentic standards were obtained from Sigma Aldrich Chemicals (St.Louis, MO) and ABCR Gmbh & Co. (Postbank Karlsruche, Konto, Germany). Dry ice was obtained from a local grocery store, Albertsons (Orange, CA). All other chemicals were of reagent grade.

2.3. Total phenols and metal binding capacity of almond skin powder

Total phenols of ASP that passed through a 400-mesh sieve were determined as previously reported by Prasetyo et al. (2008).

A modified method by Dinis, Maderia, and Almeida (1994) was used to estimate the ability of ASP to bind ferrous ions. Samples were prepared at 0.25%, 0.5% and 0.75% (w/v) ASP in methanol/HPLC grade water/HCl (80/19/1, v/v) and centrifuged at 500 g for 5 min. One millilitre of the supernatant was then mixed with 3 ml of 2 mM $FeSO_4 \cdot H_2O$ prior to the addition of 6 ml of 5 mM of the iron indicator ferrozine. Samples were incubated at room temperature for 10 min and absorbance was read at 562 nm against a blank of HPLC grade water. The % metal binding capacity inhibition was recorded

$$\text{Inhibition } (\%) = \frac{A_{562}\ \text{Control} - A_{562}\ \text{Treatment}}{A_{562}\ \text{Control}} * 100$$

2.4. Identification of phenolic compounds by HPLC

An ASP extract (31.5 g ASP in 450 ml of 1000/1, v/v, methanol/HCl) was sonicated and then filtered through a Whatman No. 1 filter twice to obtain a clear extract (Garrido, Monagas, Gomez-Cordoves, & Bartolome, 2007). The filtrate was then lyophilized using a Dura-Dry II MP Freeze Drier (Stoneridge, NY) for 48 h. Dried almond skin phenolic extracts were dissolved in 60/40 (v/v) HPLC water/methanol (4000 ppm) for characterization using an Agilent Series 1100 HPLC system equipped with Chemstation software (Agilent Technologies, Inc. Santa Clara, CA), a diode array detector (DAD) and a fluorescence lamp detector (FLD). A Synergi C_{18} MAX-RP 80A LC column (Phenomenex, Torrance, CA) with dimensions of 250 mm × 4.6 mm was used to characterize the phenolic compounds present. Wavelengths of 250, 260, 270, 280, 320, 360 and 365 nm were used for DAD. For FLD detection, excitation λ at 280 nm and emission λ at 310 nm were used (Garrido et al., 2007). A mobile phase composed of a gradient from 30% to 99% methanol run at a flow rate of 0.2 ml/min was used. Individual phenolic compounds were identified by comparing the retention time of sample chromatographic peaks with those of phenolic standards using the same HPLC operating conditions.

2.5. Preparation of chicken meat samples

Chicken breast preparation, total fat and moisture content determination were based on methods outlined by Prasetyo et al. (2008). Briefly, 0.5% (w/w) sodium chloride was added to ground chicken breasts (GCB) and mixed prior to the addition of 0.02% TBHQ (Tenox20A), 0.5% (w/w) of non-irradiated almond skin powder (ASP) and 0.5% (w/w) of irradiated ASP at 10, 20 and 30 kGy. A negative control with neither ASP nor TBHQ was also prepared. Propylene glycol (0.02%) was added to both the negative control and GCB with ASP to account for the propylene glycol in the Tenox20A. Next each treatment was placed into polypropylene bags, spread to a thickness of 1 cm and vacuum packed (Multivac, Kansas City, MI). All treatments were then heated in an electric water bath to a final internal temperature of 80 °C (Juntachote, Berghofer, Siebenhandl, & Bauer, 2007). Treatments were then divided into individual Ziploc bags and refrigerated at 4 °C for 8 days. Each sample was analyzed every other day for conjugated dienes, TBARS values and pentanal, hexanal and nonanal content.

2.6. Colour measurement

The Hunter L^* value for lightness, a^* for redness and b^* for yellowness of each sample was measured using a Hunter Lab Colorimeter (D25M Optical Sensor, Reston, VA) on initial day of analysis once the cooked meat reached room temperature, and periodically thereafter. A total of three points were read by rotating a 10 g sample of 1 cm thickness, 120° clockwise for each measurement.

2.7. Analysis of conjugated dienes

Conjugated dienes were determined using a modified method adopted from Juntachote et al. (2007). Two grams of cooked GCB were homogenized with 20 ml of distilled water for 30 s. Two millilitres of the slurry were homogenized with 20 ml of hexane/isopopanal (3/1 v/v) for 1 min, centrifuged at 2500g for 5 min and the lipid/hexane supernatant was read at 233 nm using a Beckman DU800 UV/Visible Spectrophotometer (Beckman Coulter, Fullerton, CA) against a blank of hexane. Conjugated dienes concentration was expressed as μmol per g meat sample using a molar extinction coefficient of 25,200 M^{-1} cm^{-1}.

2.8. Analysis of thiobarbituric acid reactive substances (TBARS)

Five grams of GCB and 12.5 ml of 20% (w/v) trichloroacetic acid (TCA) solution were homogenized at high speed in a Stomacher 400 (Seward, London, UK) for 30 s. The homogenate was filtered using Whatman No. 1 filter paper and 5 ml of the filtrate were mixed with 5 ml of TBA solution (0.02 M) and incubated for 15 h at room temperature in the dark. Absorbance was read at 532 and 600 nm against a blank of 5 ml distilled water and 5 ml TBA-reagent. The absorbance difference A_{532}–A_{600} was calculated with 600 nm accounting for any potential turbidity (Juncher et al., 2001). A standard curve was obtained using a 25 μM TEP (1,1,3,3-tetraethoxypropane) standard stock solution to construct 0–5 nmol MDA equivalents/ml. Results were recorded as μg of MDA per kilogram of meat (Juntachote et al., 2007).

2.9. Analysis of dominant aldehyde content

Volatile compounds released during oxidation were extracted via distillation. Five grams of GCB and 25 ml of distilled water were placed into a 50 ml round bottom flask which was then attached to a Bidwell–Sterling moisture trap connected to a condenser. The flask was then heated on an electrothermal individually controlled soxhlet apparatus heater for 15–20 min until approximately 5 ml of distillate was collected. Distillate (5 ml) was then transferred into a test tube (18 mm × 150 mm) and 4-heptanone [0.5 ppb (v/v)] was added and used as an internal standard (Hwang, Bowers, & Kropf, 1990). Volatiles were purged at 70 °C for 15 min, trapped at 75 °C using a Tenax trap (Trap #7, 0.125″ O.D. × 0.105″ I.D.) and desorbed for 1 min at 220 °C using a purge and trap (OI Analytical 4560, College Station, TX). A splitless inlet was used to inject volatiles into a HP-1 fused silica capillary column, 30 m × 0.32 mm × 0.25 μm film thickness (Agilent Technologies, Inc. Santa Clara, CA) equipped with a flame ionizing detector (FID) using ramped oven temperature conditions (30 °C for 2 min, increased to 40 °C at 2 °C/min, increased to 50 °C at 5 °C/min, increased to 100 °C at 10 °C/min, increased to 140 °C at 20 °C/min, increased to 200 °C at 30 °C/min, and held for 4.5 min). Helium was used as the carrier gas and column flow was 2.2 ml/min. The area of each peak was integrated using ChemStation software (Agilent Technologies, Inc. Santa Clara, CA) and concentrations were determined based on ratio relationships between 4-heptanone, an internal standard, and the sample.

2.10. Statistical analysis

Data collected for each experiment were an average of duplicate readings of two different meat samples for each treatment carried out on each day of analysis (every other day for 8 days of cooked refrigerated storage) for all six treatments [negative control (GCB without TBHQ or almond skin powder/ASP), positive control (GCB with TBHQ) and GCB with 0.5% of 0, 10, 20 and 30 irradiated ASP)]. Statistical analysis using SAS 9.1.3 software was done using a Duncan's Test and levels of significance were determined at $p \leqslant 0.05$ (SAS, 2003). Correlations between secondary oxidation products were also determined.

3. Results and discussion

The average moisture content on a dry weight basis and total fat (%) of GCB used for the experiments was 79.91 ± 0.24 and 2.26 ± 0.11%, respectively, for GCB without ASP and 77.98 ± 0.21 and 2.62 ± 0.07%, respectively, for GCB with ASP. The slightly lower moisture content in GCB with ASP could be attributed to the displacement of water weight per dry weight of ASP.

3.1. Phenolic content

The total phenolic content was 11.49, 11.70, 11.22 and 11.38 mg quercetin equivalents per gram of Nonpareil ASP for 0, 10, 20 and 30 kGy irradiation doses, respectively. One of the objectives of this study was to investigate the potential of irradiation to increase the phenolic content of ASP and in turn increase its ability to retard lipid oxidation in cooked GCB. It was hypothesized that with increasing irradiation, increases in total phenolic content would result due to the cleavage and liberation of polyphenols as seen with far-infrared irradiation of rice hulls and deffated sesame extracts (Lee, Jeong, Kim, Nam, & Ahn, 2005; Lee et al., 2003). Irradiation had no effect on total phenols but may play a role in changing the phenolic structures. Flavonoid glycosides have been shown to exhibit decreased radical scavenging activity as compared to their aglycons (Ioku, Tsushida, Takei, Nakatani, & Terao, 1995). Irradiation is one means of changing the phenolic structure as high correlation between increasing gamma irradiation and increases in aglycone content ($r^2 = 0.98443$) and decreases in glucoside content ($r^2 = -0.9678$) have previously been observed (Variyar et al., 2004).

Electron beam irradiation at 10, 20 and 30 kGy did not significantly change the total phenol concentration of the ASP similar to the findings seen by Mishra, Gautam, and Sharma (2006) and Lee, Jo, Sohn, Kim, and Byun (2006) who found that gamma irradiation dosages ranging from 1 to 10 kGy and irradiation at 20 kGy, respectively, did not significantly affect the total phenol concentration of tea leaves or green tea leaf by-product powders. These results with Nonpareil variety ASP differ from those by Prasetyo et al. (2008) who found that increasing electron beam irradiation at 20 and 30 kGy increased total phenols of Carmel variety ASP. While electron beam irradiation resulted in no significant differences in total phenols, changes to the distribution of phenolic compounds could have resulted. Kanatt et al. (2005), for instance, found a 29% decrease in total phenols as a result of 2.5 kGy λ-irradiation, however, there was no effect on the major phenolic acid in potato peel extract (PPE), chlorogenic acid, resulting in the continued ability of PPE to retard lipid oxidation in lamb meat. The discrepancy in above findings could be the result of differences in types of phenols present in different plant co-products, type of irradiation and actual dosage absorbed which could contribute to the different effects of irradiation on total phenol concentrations.

3.2. Identification of phenolic compounds

Major peaks were identified by comparison with authentic standards and included two flavan-3-ols, catechin and epicatechin; six flavonols, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, kaempferol, isorhamnetin, quercetin; two flavanones, naringenin-7-*O*-glucoside and naringenin; and two phenolic acids, protocatechuic acid and *p*-hydroxybenzoic acid.

3.3. Percent metal chelation

The ability of 0.25–0.75% non-irradiated and electron beam irradiated ASP to compete with ferrozine for ferrous ions was evaluated. The percent ferrous ion chelation for the 0.25% (w/v) ASP was 2.07 ± 0.18, 9.25 ± 0.13, 4.84 ± 0.11, and 4.10 ± 0.26 for 0, 10, 20 and 30 kGy ASP, respectively. The percent ferrous ion chelation for the 0.5% (w/v) ASP was 2.35 ± 0.21, 9.83 ± 0.21, 8.15 ± 0.16 and 5.68 ± 0.18 for the 0, 10, 20 and 30 kGy ASP, respectively, while percent ferrous ion chelation for the 0.75% (w/v) ASP was 5.75 ± 0.29, 12.25 ± 0.11, and 3.89 ± 0.24 for the 0, 10, 20 and 30 kGy ASP, respectively. Percent iron chelation was dependent on the concentration of ASP and the irradiation dose. Higher ASP concentrations resulted in greater percent chelation, and irradiation likewise enhanced percent chelation. Similar concentration-dependent chelating activity on Fe^{2+} was also found in potato peel extracts (Kanatt et al., 2005).

Metal chelation of flavanoids occurs via the formation of complexes between metals and the hydroxyl groups on flavanoids. The binding site for iron on flavanoids is the catechol (*ortho* dihydroxy) group on the flavanoid A and B rings (Khokhar & Apenten, 2003; Souza & Giovani, 2004). Compounds in ASP that possess these structural features included catechin, epicatechin and quercetin (Fig. 1). Denaturation of myoglobin and hemoglobin during cooking releases iron that can accelerate lipid oxidation. Minerals such as sodium chloride and iron present during meat processing can act as initiators of lipid oxidation. The ability of phenolic compounds to bind Fe^{2+} and change the redox potential for converting the ferrous ion to the ferric state is one way to inhibit oxidative deterioration (Khokhar & Apenten, 2003). The ability of flavonoids to complex with metals ions does not negatively impact its radical scavenging antioxidant ability. Souza and Giovani (2004) found that flavonoids complexed with metal ions had increased antioxidant activities as compared to free flavonoids.

Electron beam irradiation (10–30 kGy) generally increased Fe^{2+} chelation at all concentrations tested. Possible rearrangement of the phenolic compounds could have occurred with irradiation that may have resulted in greater aglycone concentration of the catechol containing flavanoids in Nonpareil ASP. Khokhar and Apenten (2003) showed that the glucoside rutin had lower Fe-binding ability compared to the aglycone quercetin due to the substitution of a sugar at the C3 position which is one of the sites involved in metal complexation.

3.4. Colour measurement of cooked ground chicken breasts during refrigerated storage

No significant changes in both Hunter a^* and b^* values were detected with ASP addition (data not shown). The addition of ASP in GCB decreased Hunter L^* values by an average of 7.4% as compared to the negative control due to the brown colour of the ASP (Fig. 2). During the 8 days of refrigerated storage, no significant changes in colour as a result of storage time were observed. Similar results were seen by Jo et al. (2006) who reported no colour change during 4 days of storage of cooked chicken. Lower L^* values caused by addition of ASP affected visual appearance and could affect overall acceptability of the chicken product (Fig. 2). Alterations to the ASP that could improve consumer acceptability include decreasing particle size of the powder, decolourizing ASP via bleaching or using

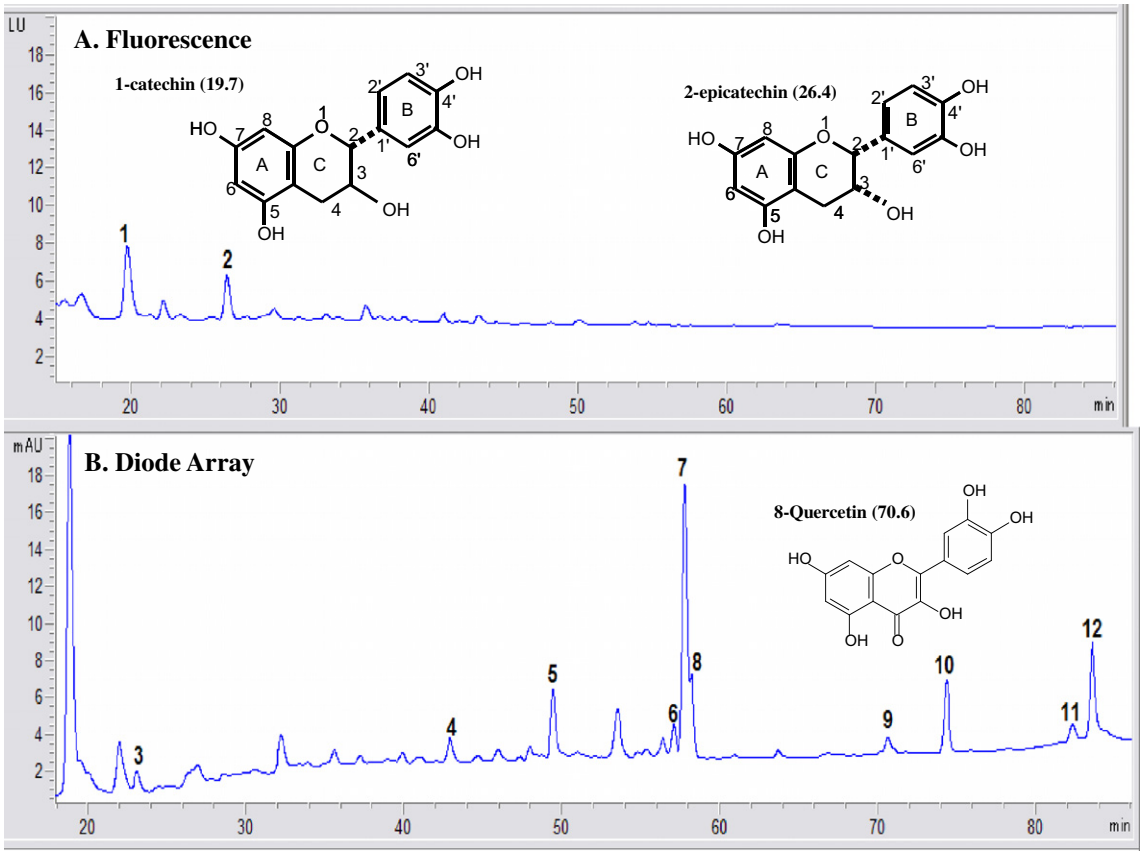

Fig. 1. HPLC profile of almond skin. 1 – catechin (19.7); 2 – epicatechin (26.4); 3 – procatechuic acid (23.9); 4 – dihydroquercetin (44.6); 5 – naringenin-7-*O*-glucoside (49.4); 6 – kaempferol-3-*O*-rutinoside (57.0); 7 – isorhamnetin-3-*O*-rutinoside (57.7); 8- isorhamnetin-3-*O*-glucoside (58.1); 9 – quercetin (70.6); 10 – naringenin (74.4); 11 – kaempferol (82.3); 12 – isorhamnetin (83.5). Numbers within parentheses indicate retention time in min.

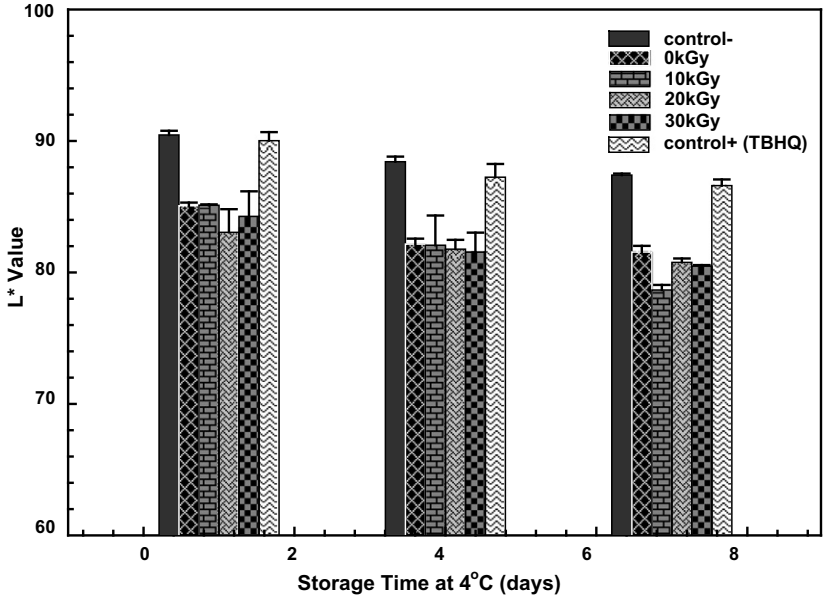

Fig. 2. Effect of almond skin powder on Hunter L^* value in cooked ground chicken breasts for one week of refrigerated storage at 4 °C.

ASP in dark meat e.g. chicken thigh meat where effect on Hunter L^* values would be less noticeable.

3.5. Conjugated dienes of cooked ground chicken breasts during refrigerated storage

Conjugated diene (CD) formation occurs during the early stages of lipid oxidation and serves as a marker of the oxidation process (Juntachote et al., 2007). Primary lipid oxidation was measured in terms of the amount of CD produced during storage and by the treatment (Table 1). All cooked GCB treated with ASP had significantly lower CD as compared to the negative control after one day of refrigerated storage ($p < 0.05$). Initially (day 1), only 10 kGy ASP significantly lowered the formation of CD as compared to the negative control. The increase in total conjugated diene concentration for most samples peaked on day five followed by a decrease thereafter. The results are in agreement with those of Juntachote, Berghofer, Siebenhandl, and Bauer (2006) who reported an increase in CD until the third day, followed by a decrease thereafter for cooked pork treated with holy basil and Galangal. This phenomenon of decreased CD is due to conjugated diene hydroperoxides decomposing into secondary oxidation products over time. The resulting decrease in CD from day 5 to 7 for GCB incorporated with 0, 10 and 30 kGy ASP (Table 1) was accompanied by a 1.7 fold increase in hexanal content and 1.3 fold increase in TBARS values (Tables 2 and 3). Similar trends of decreased CD accompanied by increased TBARS were observed by Juntachote et al. (2006) in cooked pork. Overall, all GCB samples treated with TBHQ and ASP had significantly lower conjugated dienes as compared to the negative control throughout one week of refrigerated storage (Table 1).

Irradiation dosage had no effect on the concentration of phenolics in ASP, however, ASP irradiated at 10 kGy had significantly lower CD throughout the seven days of storage as compared to all other treatments (Table 1). The 10 kGy irradiated ASP also showed increased metal chelating capacity which could have partially contributed to decreased lipid oxidation. In the present study, the ASP irradiated at dosages of 20 and 30 kGy did not significantly retard the formation of CD as compared to the non-irradiated ASP (0 kGy) and TBHQ (positive control) in GCB even though significant differences in percent iron chelation were found among 0, 10, 20 and 30 kGy (2.35%, 9.83%, 8.15% and 5.68%, respectively). Metals act as initiators of lipid oxidation, while free radicals propagate the reaction. Antioxidants work by chelating metals and acting as free radical scavengers and the antioxidant capacity of ASP could result from a combined effect of both mechanisms.

3.6. TBARS values of cooked ground chicken breasts during refrigerated storage

The effect of natural and synthetic antioxidants on TBARS values in cooked GCB over 7 days of refrigerated storage is shown in Table 2. On initial day of analysis, all GCB treated with ASP and TBHQ were significantly lower than the negative control, an

Table 1
Conjugated diene (μmol/g meat) of cooked ground chicken breast lipid treated with irradiated and non-irradiated almond skin powder for one week of refrigerated storage at 4 °C

Treatment	Conjugated diene (μmol/g meat) during refrigerated storage (days)			
	1	3	5	7
Control−	4.13 ± 0.36^{a}	4.88 ± 0.35^{a}	8.42 ± 0.14^{a}	7.57 ± 0.44^{a}
Control+	3.20 ± 0.65^{ab}	3.72 ± 0.18^{b}	5.61 ± 0.11^{c}	6.34 ± 0.73^{ab}
0 kGy	3.76 ± 0.04^{a}	3.49 ± 0.12^{b}	6.38 ± 0.13^{b}	4.85 ± 0.55^{bc}
10 kGy	2.66 ± 0.05^{b}	2.77 ± 0.27^{c}	4.78 ± 0.16^{d}	3.62 ± 0.49^{d}
20 kGy	3.41 ± 0.29^{ab}	3.48 ± 0.09^{b}	5.37 ± 0.07^{c}	6.28 ± 0.64^{ab}
30 kGy	4.07 ± 0.17^{a}	3.92 ± 0.13^{b}	6.24 ± 0.19^{b}	5.50 ± 0.080^{b}

Control− refers to meat without almond skin powder or TBHQ. Control+ refers to meat incorporated with 200 ppm TBHQ. Means within columns with different superscript letters are significantly different ($p \leqslant 0.05$). Numbers after ± indicate standard deviations ($n = 2$).

Table 2
TBARS values (μg MDA/kg meat) of cooked ground chicken breast treated with irradiated and non-irradiated almond skin powder for one week of refrigerated storage at 4°C

Treatment	TBARS value (μgMDA/kg meat) during refrigerated storage (days)			
	1	3	5	7
Control−	108.58 ± 0.87[a]	481.73 ± 67.20[a]	746.83 ± 26.02[a]	1042.46 ± 23.42[a]
Control+	76.41 ± 6.18[b]	377.66 ± 2.35[b]	662.64 ± 50.86[b]	960.12 ± 24.72[b]
0 kGy	70.09 ± 1.50[bc]	167.26 ± 11.40[c]	195.43 ± 1.65[cd]	299.39 ± 21.08[c]
10 kGy	60.76 ± 1.48[dc]	92.46 ± 3.30[d]	124.70 ± 2.88[e]	166.74 ± 9.90[e]
20 kGy	68.60 ± 0.37[bc]	131.80 ± 12.05[c]	205.44 ± 12.96[c]	213.24 ± 1.41[d]
30 kGy	53.98 ± 4.32[d]	144.88 ± 8.53[c]	177.50 ± 7.23[cd]	188.45 ± 4.25[d]

Control− refers to meat without ASP or TBHQ. Control+ refers to meat incorporated with 200 ppm TBHQ. Means within columns with different superscript letters are significantly different ($p \leqslant 0.05$). Numbers after ± indicate standard deviations ($n = 2$).

Table 3
Aldehyde content (ppm) of cooked ground chicken breast treated with irradiated and non-irradiated almond skin powder for one week of refrigerated storage at 4 °C

Treatment	Hexanal content (ppm) during refrigerated storage (days)			
	2	4	6	8
Control−	8.63 ± 0.71[a]	29.27 ± 0.22[b]	52.65 ± 0.68[a]	104.92 ± 0.33[b]
Control+	6.25 ± 0.27[b]	34.88 ± 0.29[a]	52.50 ± 0.31[a]	118.76 ± 0.53[a]
0 kGy	2.86 ± 0.04[cd]	12.44 ± 0.21[c]	10.40 ± 0.08[c]	18.97 ± 0.28[d]
10 kGy	2.17 ± 0.30[d]	7.12 ± 0.06[f]	7.02 ± 0.43[d]	13.19 ± 0.38[f]
20 kGy	3.38 ± 0.11[c]	8.71 ± 0.33[e]	14.46 ± 0.50[b]	21.54 ± 0.24[c]
30 kGy	3.47 ± 0.29[c]	11.27 ± 0.32[d]	9.65 ± 0.45[c]	14.91 ± 0.14[e]
	Pentanal content (ppm) during refrigerated storage (days)			
Control−	1.11 ± 0.08[a]	3.17 ± 0.04[b]	5.79 ± 0.10[a]	9.51 ± 0.15[b]
Control+	0.83 ± 0.03[b]	3.97 ± 0.05[a]	5.32 ± 0.04[b]	13.40 ± 0.21[a]
0 kGy	0.49 ± 0.12[c]	1.35 ± 0.03[c]	1.36 ± 0.03[c]	1.85 ± 0.06[c]
10 kGy	0.23 ± 0.09[d]	0.71 ± 0.01[e]	0.69 ± 0.04[d]	1.29 ± 0.09[d]
20 kGy	0.43 ± 0.01[c]	0.87 ± 0.04[e]	1.33 ± 0.02[c]	1.86 ± 0.09[c]
30 kGy	0.43 ± 0.02[c]	1.06 ± 0.02[d]	0.82 ± 0.03[d]	1.74 ± 0.06[c]
	Nonanal content (ppm) during refrigerated storage (days)			
Control−	0.44 ± 0.00[a]	1.30 ± 0.07[a]	2.76 ± 0.06[a]	3.50 ± 0.18[b]
Control+	0.31 ± 0.05[b]	1.25 ± 0.03[a]	1.70 ± 0.07[a]	4.12 ± 0.17[a]
0 kGy	0.31 ± 0.04[b]	0.92 ± 0.02[b]	0.85 ± 0.02[b]	1.34 ± 0.11[c]
10 kGy	0.31 ± 0.02[b]	0.59 ± 0.04[d]	0.53 ± 0.07[d]	0.74 ± 0.01[e]
20 kGy	0.28 ± 0.03[b]	0.75 ± 0.04[c]	0.65 ± 0.13[c]	0.96 ± 0.03[d]
30 kGy	0.19 ± 0.04[c]	0.93 ± 0.06[b]	0.54 ± 0.04[b]	1.47 ± 0.05[c]

Control− refers to meat without ASP or TBHQ. Control+ refers to meat incorporated with 200 ppm TBHQ. Means within columns of different aldehydes with different superscript letters are significantly different ($p \leqslant 0.05$). Numbers after ± indicate standard deviations ($n = 2$).

indication that the antioxidants had a good carry through effect. Similar results were observed by Jo et al. (2006) and Juntachote et al. (2007). Lipid oxidation was considerably accelerated as storage time increased due to the denatured state of the muscles upon heating and exposure to oxygen. TBARS measure the formation of secondary lipid oxidation products which contribute to WOF in oxidized meat. The development of WOF in chicken has been shown to be closely related to the oxidation of the phospholipid fraction, with non-heme iron being the active catalyst in lipid oxidation (Igene & Pearson, 1979; Sato, Hegarty, & Herring, 1973). Chen et al. (1984) showed that during cooking, iron was released from the heme pigment and that the resultant increase in iron was responsible for lipid oxidation.

Seven days of refrigerated storage had a great impact on the development of lipid oxidation in cooked GCB resulting in large increases in TBARS values. The incorporation of ASP and TBHQ significantly lowered TBARS values ($p < 0.05$) as compared to the negative control during the entire storage period, indicating a high defense against lipid oxidation. The ability of irradiated and non-irradiated ASP to decrease lipid oxidation is most likely due to their high levels of phenolic compounds, which are mainly concentrated within the skins (Milbury et al., 2006).

The 10 kGy irradiated ASP was the most effective at reducing the formation of secondary oxidation products, e.g. malonaldehyde throughout the seven days of refrigerated storage, ranging from 44.0% on day one to 84.0% on day seven as compared to the negative control (Table 2). Maximum metal chelation was observed in the 10 kGy irradiated ASP which could partially explain the increased ability to inhibit lipid oxidation in cooked GCB. Samples treated with 20 and 30 kGy ASP had significantly lower TBARS values as compared to the both the positive and negative control but had significantly higher TBARS values than 10 kGy ASP for all seven days of refrigerated storage. The addition of ASP and the effect of irradiation dosage influenced the TBARS values of cooked GCB. The present study along with those of previous investigators attests to the effectiveness of natural antioxidants in retarding lipid oxidation in cooked chicken (Jo et al., 2006; Rababah et al., 2004).

3.7. Volatile aldehyde content of cooked ground chicken breasts during refrigerated storage

The development of volatile aldehydes in cooked GCB is attributed to the oxidation of unsaturated lipids. Beginning on day 2, the concentration of all volatile aldehydes was significantly lower after cooking as compared to the negative control ($p < 0.05$). In general, the concentration of volatile aldehydes increased with storage time. All samples treated with ASP had reduced volatile formation when compared to both the positive (GCB with TBHQ) and negative control for all eight days of refrigerated storage (Table 3). Hexanal content for both the negative and positive control were at least 2 fold higher for all 8 days of refrigerated storage compared to GCB with irradiated or non-irradiated ASP. Hexanal content results followed a similar trend to TBARS values in that cooked GCB treated with 10 kGy ASP resulted in the lowest formation (74.9–87.4% reduction) of secondary oxidation products for all eight days of refrigerated storage. The 10 kGy ASP also resulted in significant decreases in pentanal and nonanal concentration for all eight days of refrigerated storage as compared to both the positive and negative control (Table 3).

Correlation coefficients between TBARS values and pentanal were $r^2 = 0.8999$ ($p < 0.05$), TBARS values and hexanal, $r^2 = 0.9306$ ($p < 0.05$) and TBARS values and nonanal were $r^2 = 0.9106$ ($p < 0.05$) suggesting that changes in TBARS content followed the same trend as all the aldehydic volatiles produced during lipid oxidation (Fig. 3). Hexanal was the most correlated volatile aldehyde with TBARS values for all eight days of refrigerated storage (Fig. 3, $r^2 = 0.9306$). Oxidation of unsaturated fatty acids, e.g. linoleic acid produces hexanal and other aldehydes in food systems. Due to the fact that both hexanal content and TBARS values measure secondary lipid oxidation products, the two tests have been found to be qualitatively analogous in evaluating lipid oxidation (Jo et al., 2006; Juntachote et al., 2006; Juntachote et al., 2007; Shahidi & Pegg, 1994). Aldehydes are major contributors to the formation of WOF and increased aldehyde formation has been found to be correlated with increased WOF (Ahn

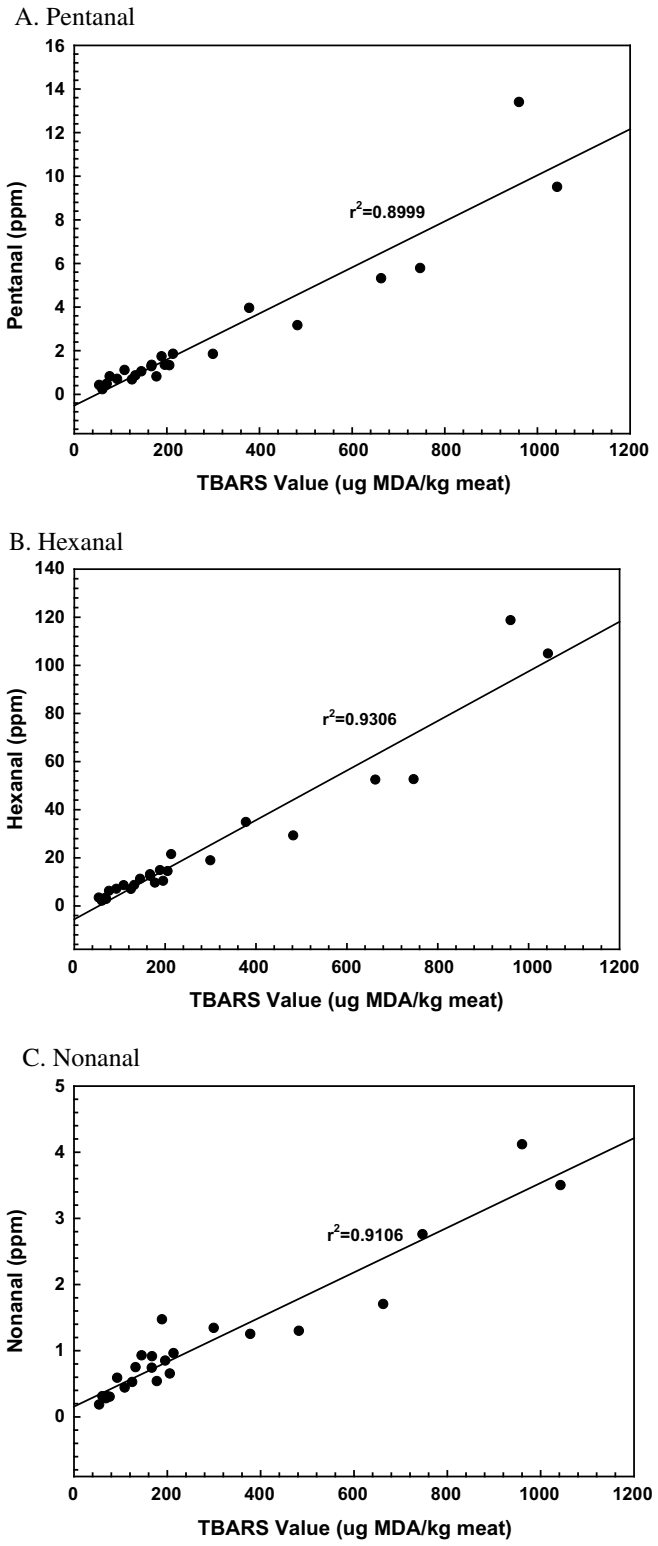

Fig. 3. Correlation between volatile aldehydes and TBARS in cooked ground chicken breasts for one week of refrigerated storage at 4 °C.

et al., 2002; Hwang et al., 1990; Igene et al., 1985; Vasavada & Cornforth, 2006). Synthetic antioxidants are added to inhibit WOF created by the aldehydes formed. The inhibition of pentanal, hexanal, nonanal and TBARS values with ASP suggests that it could potentially be used to retard lipid oxidation and WOF development in cooked chicken.

4. Conclusions

Significant differences in metal chelation and lipid oxidation were observed between irradiation doses. The lowest degree of lipid oxidation was observed with ASP irradiated at 10 kGy as evidenced by decreased CD, TBARS and hexanal content concentrations, and maximum metal chelation. These results show that shelf life extension of chicken could be achieved with the addition of both irradiated and non-irradiated ASP, and the selection of specific doses of electron beam irradiation can enhance the antioxidant capacity of the almond skins. The ASP was stable under cooking conditions, and proved to be as good as and a more effective antioxidant than TBHQ, in cooked GCB.

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