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Abstract

This study was designed to evaluate the effects of dietary treatment, packaging, and irradiation singly or in combination on the oxidative stability of broiler chicken thigh meat. A total of 120 four-week-old chickens were divided into 12 pens (10 birds/pen), and 4 pens of broilers were randomly assigned to a control oxidized diet (5% oxidized oil) or an antioxidant-added diet [500 IU of vitamin E + 200 mg/kg of butylated hydroxyanisole (BHA)] and fed for 2 wk. After slaughter, thigh meats were separated, ground, packaged in either oxygen-permeable or oxygen-impermeable vacuum bags, and irradiated at 0 or 3 kGy. Lipid oxidation (TBA-reactive substances), protein oxidation (carbonyl), and color of the meat were measured at 1, 4, and 7 d of refrigerated storage. The lipid and protein oxidation of thigh meats from birds fed the diet supplemented with antioxidants (vitamin E + BHA) was significantly lower than the lipid and protein oxidation of birds fed the control diet, whereas the lipid and protein oxidation of broilers fed the oxidized oil diet was higher than that of birds fed the control diet. Vacuum packaging slowed, but irradiation accelerated, the lipid and protein oxidation of thigh meat during storage. Dietary antioxidants (vitamin E + BHA) and irradiation treatments showed a stronger effect on lipid oxidation than on protein oxidation. A significant correlation between lipid and protein oxidation in meat was found during storage. Dietary supplementation of vitamin E + BHA and the irradiation treatment increased the lightness and redness of thigh meat, respectively. It is suggested that appropriate use of dietary antioxidants in combination with packaging could be effective in minimizing oxidative changes in irradiated raw chicken thigh meat.

Keywords

antioxidant, irradiation, lipid oxidation, protein oxidation, oxidized diet

Disciplines

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Comments

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Effects of diet, packaging, and irradiation on protein oxidation, lipid oxidation, and color of raw broiler thigh meat during refrigerated storage

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ABSTRACT This study was designed to evaluate the effects of dietary treatment, packaging, and irradiation singly or in combination on the oxidative stability of broiler chicken thigh meat. A total of 120 four-week-old chickens were divided into 12 pens (10 birds/pen), and 4 pens of broilers were randomly assigned to a control oxidized diet (5% oxidized oil) or an antioxidant-added diet [500 IU of vitamin E + 200 mg/kg of butylated hydroxyanisole (BHA)] and fed for 2 wk. After slaughter, thigh meats were separated, ground, packaged in either oxygen-permeable or oxygen-impermeable vacuum bags, and irradiated at 0 or 3 kGy. Lipid oxidation (TBA-reactive substances), protein oxidation (carbonyl), and color of the meat were measured at 1, 4, and 7 d of refrigerated storage. The lipid and protein oxidation of thigh meats from birds fed the diet supplemented with antioxidants (vitamin E + BHA) was significantly lower than the lipid and protein oxidation of birds fed the control diet, whereas the lipid and protein oxidation of broilers fed the oxidized oil diet was higher than that of birds fed the control diet. Vacuum packaging slowed, but irradiation accelerated, the lipid and protein oxidation of thigh meat during storage. Dietary antioxidants (vitamin E + BHA) and irradiation treatments showed a stronger effect on lipid oxidation than on protein oxidation. A significant correlation between lipid and protein oxidation in meat was found during storage. Dietary supplementation of vitamin E + BHA and the irradiation treatment increased the lightness and redness of thigh meat, respectively. It is suggested that appropriate use of dietary antioxidants in combination with packaging could be effective in minimizing oxidative changes in irradiated raw chicken thigh meat.

Key words: antioxidant, irradiation, lipid oxidation, protein oxidation, oxidized diet

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INTRODUCTION

Oxidative deterioration is a major quality loss in muscle foods. The adverse effects of oxidation are involved not only in economic loss, but are also in related off-flavor development, discoloration, nutrient loss, and health risks (Jensen et al., 1997a). Muscle tissues have endogenous antioxidant mechanisms to control the oxidative process in vivo. These antioxidants, which are classified as preventive antioxidants, continue to function but diminish in activity as the time postmortem increases. The preventive antioxidants include antioxidant enzymes, such as catalase, glutathione peroxidase, and superoxide dismutase (Xiong, 2000). Muscle also contains nonenzymic components, such as α-tocopherol (vitamin E), ubiquinone, β-carotene, ascorbate (vitamin C), and other reducing components (Griffiths, 2002). Several intrinsic and extrinsic factors, including the content and composition of unsaturated fatty acids and the concentration and activity of antioxidant substances in meat muscle, can affect the oxidative stability of meat. In addition, postslaughter processing and treatments, including storage temperature, restructuring, nonmeat ingredients, packaging, and irradiation, can influence the oxidative status of meat (Ahn et al., 1998; Nam et al., 2006).

Chicken meat contains relatively high amounts of unsaturated fatty acids, which increases the concerns regarding oxidative deterioration. Many technologies had been developed to prevent or minimize oxidative changes in chicken meat, and the use of dietary strategies to improve the oxidative stability of chicken meat has been studied extensively. Among them, dietary supplementation of vitamin E is well accepted as an effective method of reducing lipid oxidation and extending the shelf life of meat (Ann and Patrick, 1996). Oxygen

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is the most common and essential component for the progress of lipid oxidation (Ahn et al., 1992). Restructuring and grinding processes can increase the exposure of lipid to air. Many studies have shown that vacuum packaging (Ahn et al., 1993) or oxygen-depleted modified atmosphere packaging (Phillips, 1996) could decrease lipid oxidation in meat during storage. Lund et al. (2007) reported that the combination of dietary vitamin E supplementation and vacuum packaging decreased the lipid oxidation of meat during refrigerated storage and retail display.

To meet the high energy demand of fast-growing broilers, the addition of oils or fats to broiler chicken diets is common. However, this can possibly increase the susceptibility of such diets to lipid oxidation, which may eventually influence the oxidation and storage stability of the chicken meat, because feeding diets containing high levels of oxidized fats increases oxidative stress in the body of the bird (Buckley et al., 1995; Galvin et al., 1997; Racanicci et al., 2008; Zouari et al., 2010).

With the approval of irradiation to improve the safety of poultry meat, concerns have been raised about negative quality changes (color, odor, tenderness, lipid oxidation) resulting from irradiation. Some technologies have been used to minimize the negative effects of irradiation on poultry meat quality, including adding antioxidant substances to poultry diet, modifying the packaging method, and adding antioxidants to meat during processing (Ahn et al., 1998; Nam and Ahn, 2003; Nam et al., 2006).

Wolff et al. (1986) reported that lipid oxidation produced reactive oxygen species, which could modify many intracellular and membrane proteins in muscle. During meat processing and storage, oxidative modification of amino acid residues and the polypeptide backbone could result in physical and chemical changes, including changes to conformational stability, solubility, and nutritional quality (Xiong, 2000). The formation of carbonyls is one of the most prominent changes in oxidized muscle proteins (Lund et al., 2008). In fact, proteins in whole muscle are susceptible to oxidative changes during processing and storage because of the depletion of endogenous antioxidants (Xiong, 2000). However, research about the effects of dietary treatments, packaging, and irradiation on protein oxidation in meat is limited. The purpose of this study was to evaluate the effects of addition of antioxidants or oxidized oil to the diets fed, irradiation, and different types of packaging, singly or combination, on the oxidative stability of broiler chicken thigh meat.

MATERIALS AND METHODS

Birds and Diets

A total of 120 one-day-old commercial broiler chicks were fed a standard broiler corn-soybean diet (Table 1) for 28 d. On d 29, 10 broilers were assigned to each of 12 floor pens. Four floor pens were randomly allotted to 1 of 3 experimental diets: a control diet, an oxidized diet, or an antioxidant-fortified diet. The control diet was prepared with a fresh animal-vegetable (**AV**) fat blend (Feed Energy Co., Des Moines, IA) with 25 IU of vitamin E; the oxidized diet was prepared after oxidizing the same AV fat by exposing it to room temperature for a long time until it attained a peroxide value of 100; and the antioxidant-fortified diet was prepared with the fresh AV fat supplemented with butylated hydroxyanisole (200 mg/kg) and vitamin E (500 IU) per kilogram of feed. Each of the diets was fed to the broilers for 2 wk, and bird had free access to water and the diet. Work with poultry was performed in accordance with ethical guidelines of Iowa State University and was approved by the Assurance Committee for the Use of Animals in Research.

Sample Preparation

At the end of the feeding trial, the birds were slaughtered according to USDA (1982) guidelines. Broilers were chilled in ice water for 2 h and drained in a cold room, and thigh muscles were separated from the carcasses at 24 h after slaughter. The thigh meats of birds from each pen were pooled and ground twice through a 3-mm plate, and patties (approximately 100 g) were prepared. Patties of the pooled, ground thigh meat of birds from each pen were used as a replication. Half of the patties from birds in each treatment were packaged in vacuum bags (Koch, Kansas City, MO; nylon-polyethylene, 9.3 mL of $O_2/m^2/24$ h at 0°C) and the other half were packaged in oxygen-permeable bags (Associated Bag Company, Milwaukee, WI; polyethylene, 2,300 $mL/m^2/24$ h, 4 × 6, 2 mil). They were then transported to Sadex Corporation in Sioux City, Iowa, and irradiated at 3.0 kGy in a Linear Accelerator Facility (Titan Corp., San Diego, CA) with a 10-MeV and 10-kW power level. The average dose rate was 35.7 kGy/min and the temperature was 0.5°C. Alanine dosimeters were placed on the top and bottom surfaces of a sample and were read with a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, MA) to check the absorbed dose. The dose range absorbed by meat samples was 3.0 to 3.4 kGy (maximum to minimum ratio of 1.13). The nonirradiated control (0 kGy) samples were exposed to 0.5°C, whereas the other samples were irradiated. After irradiation, the irradiated and nonirradiated meat samples were immediately placed in an ice chest filled with ice, transported to Iowa State University, and stored in a cold (4°C) room for 7 d. Color, lipid oxidation, and protein oxidation were determined at 1, 4, and 7 d after irradiation.

Lipid and Protein Oxidation

Lipid oxidation was determined by a TBA-reactive substances (**TBARS**) method (Ahn et al., 1998). Pro-

Table 1. Percentage composition of diets fed to broiler

Item	Starter (1 to 2 wk)	Grower (3 to 4 wk)	Finisher[1] (5 to 6 wk)
Ingredient			
Corn	56.826	63.954	64.131
Soybean meal	35.98	29.111	27.27
Animal-vegetable fat	2.473	2.822	5
Dicalcium phosphate	1.746	1.26	1.282
Calcium carbonate	1.275	1.37	1.082
Iowa vitamin and mineral premix[2]	0.625	0.5	0.5
Sodium chloride	0.462	0.462	0.463
Methionine, 99%	0.266	0.258	0.184
Biolysine, 50.7%	0.237	0.21	0.045
Threonine, 99%	0.073	—	0.041
Choline chloride, 60%	0.037	0.053	0.002
Calculated analysis			
ME (kcal/kg)	3,005	3,100	3,226.2
Protein	22.48	19.71	18.7
TSAA	0.98	0.9	0.8
Methionine	0.61	0.57	0.48
Lysine	1.34	1.14	1
Arginine	1.45	1.24	1.18
Glycine + serine	2.02	1.76	1.68
Histidine	0.59	0.52	0.49
Isoleucine	0.92	0.79	0.76
Leucine	1.9	1.72	1.65
Phenylalanine + tyrosine	1.35	1.17	1.12
Threonine	0.91	0.73	0.74
Valine	1.01	0.89	0.85
Calcium	1	0.95	0.85
Available phosphate	0.45	0.35	0.35
Total phosphate	0.73	0.61	0.6
Sodium	0.2	0.2	0.2

[1]Control diet: 5% fresh animal-vegetable fat (Feed Energy Co., Des Moines, IA) and Iowa vitamin and mineral premix (Iowa State University, Ames); oxidized diet: 5% oxidized animal-vegetable fat (peroxide value of 100); antioxidant diet: 5% fresh animal-vegetable fat and 500 IU + 200 mg/kg of butylated hydroxyanisole per kilogram of diet.

[2]Iowa vitamin and mineral premix supplies per kilogram of diet: retinyl acetate, 8,065 IU; cholecalciferol, 1,580 IU; 25-hydroxy-cholecalciterol, 31.5 μg, DL-α-tocopheryl acetate, 25 IU; vitamin B_{12}, 16 μg; menadione, 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline chloride, 509 mg; folic acid, 1.62 mg; biotin, 0.27 mg; Mn, 80 mg; Zn, 90 mg; Fe, 60 mg; Cu, 12 mg; Se, 0.147 mg; sodium chloride, 2.247 g.

tein oxidation was determined by the method of Lund et al. (2008), with minor modifications. One gram of muscle was homogenized with a Brinkman Polytron instrument (Type PT 10/35, Brinkman Instrument Inc., Westbury, NY) in 10 mL of pyrophosphate buffer (2.0 m*M* $Na_4P_2O_7$, 10 m*M* Trizma-maleate (Sigma, St. Louis, MO), 100 m*M* KCl, 2.0 m*M* $MgCl_2$, and 2.0 m*M* ethylene glycol tetraacetic acid, pH 7.4). Two equal amounts of meat homogenate (2 mL) were taken from a sample, precipitated with 2 mL of 20% trichloroacetic acid, and centrifuged at 12,000 × *g* for 5 min at room temperature. After centrifugation, the pellet from 1 sample was treated with 2 mL of 10 m*M* 2,4-dinitrophenylhydrazine dissolved in 2 *M* HCl, and the pellet from the other was incubated with 2 *M* HCl as a blank. During 30 min of incubation in the dark, samples were vortex-mixed for 10 s every 3 min. The proteins were further precipitated with 2 mL of 20% trichloroacetic acid and centrifuged 12,000 × *g* for 5 min. The 2,4-dinitrophenylhydrazine was removed by washing 3 times with 4 mL of 10 m*M* HCl in 1:1 (vol/vol) ethanol:ethyl acetate, followed by centrifuging at 12,000 × *g* for 5 min after each washing. The pellets were finally solubilized in 2 mL of 6.0 m*M* guanidine hydrochloride dissolved in 20 m*M* potassium dihydrogen phosphate (pH = 2.3). The samples were kept at 5°C overnight. The next day, the samples were centrifuged to remove insoluble material. The absorbance of supernatant was read at 370 nm. The absorbance values for blank samples were subtracted from their corresponding sample values. Protein concentration was determined using a Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). Briefly, protein determination was measured using a spectrophotometer at 595 nm.

Protein concentration was expressed as milligrams per milliliter. The carbonyl content was calculated as nanomoles per milligram of protein, using an absorption coefficient of 22,000 as described by Levine et al. (1994).

Color Measurement

Color values of meat were determined on the sample surface by using a LabScan colorimeter (HunterLab Associates Inc., Reston, VA) that was calibrated against black and white reference tiles covered with the same packaging bags. The CIE L* (lightness), a* (redness), and b* (yellowness) values were obtained by an illuminant A (light source). Two random readings were averaged from the top and bottom locations on a sample surface for statistical analysis.

Table 2. Effects of dietary treatment, packaging, and irradiation on lipid oxidation of chicken thigh patties during refrigerated storage

Storage time (d)	TBA-reactive substances (mg of malondialdehyde/kg of meat)				
	Control diet	Oxidized diet	Antioxidant diet	SEM	*P*-value
1	0.11[b]	0.16[a]	0.08[c]	0.0096	<0.05
4	0.92[b]	1.44[a]	0.27[c]	0.068	<0.001
7	1.92[b]	2.16[a]	0.92[c]	0.12	<0.001
	O_2-permeable packaging	Vacuum packaging			
1	0.14[a]	0.09[b]		0.0076	<0.001
4	1.60[a]	0.17[b]		0.056	<0.001
7	2.96[a]	0.35[b]		0.10	<0.001
	0 kGy of irradiation	3 kGy of irradiation			
1	0.06[b]	0.18[a]		0.0076	<0.001
4	0.20[b]	1.56[a]		0.056	<0.001
7	0.31[b]	3.00[a]		0.10	<0.001

[a–c]Means in the same row with different letters differ significantly.

Statistical Analysis

The experiment was a factorial design with 3 diets, 2 types of packaging, 2 levels of irradiation, and 4 replications. Data were analyzed by the generalized linear model procedure using SAS software version 9.1 (SAS Institute, 1995). Mean values and SEM are reported.

RESULTS AND DISCUSSION

Lipid Oxidation

Two weeks of dietary vitamin E supplementation (500 IU/kg) decreased lipid oxidation in thigh muscle up to 7 d of refrigerated storage ($P < 0.001$; Table 2). These data agree with recent reports by Zouari et al. (2010) and Coetzee and Hoffman (2001). Previous studies demonstrated that the accumulation of vitamin E in chicken muscle was highly dependent on the dose of vitamin E and the duration of feeding trials (Jensen et al., 1998). Generally, the greater the amount of vitamin E in the diet and the longer the supplementation, the greater the tissue concentration of α-tocopherol deposited (Arnold et al., 1993). Jakobsen et al. (1995) observed that chicken tissues did not achieve saturation when the dietary vitamin E level was 500 mg/kg of feed. A similar result was also found in pigs when 700 mg of α-tocopheryl acetate/kg of diet was used (Jensen et al., 1997). With respect to beef, Liu et al. (1995) proposed that α-tocopherol concentration in muscle tissues could be elevated from 1.4 to 3.5 mg/g by feeding 1,300 IU/d for 44 d. Arnold et al. (1993) observed that saturation of α-tocopherol in cattle tissues occurred at 1,840 IU/d of vitamin E supplementation for 3 mo. Some researchers indicated that it was possible to increase the concentration of muscle vitamin E during a short (5-d) dietary supplementation (Marusich et al., 1975). However, Sheehy et al. (1991) reported that chicken muscles are relatively slow in taking up vitamin E compared with other tissues, and they questioned whether short-term feeding of vitamin E to chickens would be able to deposit enough α-tocopherol to specific locations in the muscle membranes to attain the optimal resistance to lipid oxidation.

It is believed that lipid oxidation of muscle foods is initiated by highly unsaturated phospholipids in subcellular membranes (Frankel, 1980). Vitamin E is much more competitive for reaction with peroxyl radicals than are polyunsaturated fatty acids (**PUFA**). Therefore, even a small amount of vitamin E was able to have a significant protective effect on polyunsaturated fat. In this study, 2 wk of feeding antioxidants was effective against lipid oxidation during 7 d of refrigerated storage of raw chicken thigh meat. Consumption of oxidized oil can increase oxidative stress in live birds, which causes the chickens to utilize antioxidants such as vitamin E to protect themselves. In addition, the oxidized oils that the birds have consumed can directly destroy or denature vitamins and increase the susceptibility of the gastrointestinal tract or other tissues to lipid oxidation (Sheehy et al., 1994). Therefore, consumption of oxidized oil can result in a decreased accumulation of antioxidants, such as vitamin E, in the muscle of broiler chickens (Liu and Huang, 1996). The decreased levels of vitamin E and other antioxidants eventually could reduce the antioxidant ability of meat samples during postmortem storage.

Adding fat or oil to poultry diets not only supplies energy, but also provides other benefits, such as increasing the absorption of fat-soluble vitamins, increasing peristaltic movements of the intestine, and improving the palatability of the diets (Baião and Lara, 2005). However, vegetable oils such as corn, canola, and sunflower oils are highly susceptible to lipid oxidation because of their high content of PUFA and will affect the oxidative stress of livestock if they become oxidized. Several authors have reported that dietary supplementation of oxidized oil or fat had a negative effect on the

performance of pigs (Dibner et al., 1996) and chickens (Engberg et al., 1996; Wang et al., 1997). Additionally, it adversely affected the oxidative stability of pork (Buckley et al., 1995) and chicken meat (Racanicci et al., 2008) during the postmortem storage period. Mercier et al. (1998) recommended increasing the vitamin E content when diets containing oxidized oils are fed.

Jensen et al. (1997b) established that chicken thigh muscle could accumulate a higher amount of vitamin E than breast meat, but that thigh meat was oxidized faster than breast meat because of its higher fat content. Sülzle et al. (2004) found that the amounts of lipid peroxidation products in oxidized fat varied greatly depending on the methods of thermal treatment. They documented that fats heated at a relatively low temperature for a long period produced greater concentrations of primary lipid peroxidation products than those heated at a high temperature for a shorter period. As a result, fats oxidized at low-temperature conditions for a long time had a more severe effect on the lipid metabolism of rats consuming these kinds of oxidized fats (Sülzle et al., 2004). When broiler chickens were fed the rations for 2 wk containing the 5% oxidized AV oil blend that was exposed to room temperature for a long time, it did not affect broiler performance (data not shown). However, the increase in TBARS in the meat of chickens fed the diet with oxidized oil was significantly greater than that of the control diet after 7 d of storage at 4°C (Table 2). Dietary consumption of oxidized lipids can increase the absorption of lipid hydroperoxides, secondary oxidation products (dienes and trienes), or both in the intestinal lumen. Therefore, lipid hydroperoxides and secondary oxidation products would be present in the plasma, lymph, and muscle (Ursini and Sevanian, 2002), which would lead to increased α-tocopherol turnover in bird tissues. Thus, the increased level of lipid oxidation in meat from chickens fed the oxidized diets could be related to the decreased α-tocopherol content in chicken muscle (Sheehy et al., 1994).

Oxygen can form reactive oxygen species and free radicals that can initiate lipid oxidation. Various extrinsic factors, such as deboning, grinding, mixing, tumbling, and restructuring, can increase the possibility of meat exposure to oxygen. These processes would disrupt the integrity of the muscle membranes that facilitate the interactions between pro-oxidants and unsaturated fatty acids (Morrissey et al., 1994). The packaging environment could also affect the extent of lipid oxidation during meat storage. It is well established that vacuum packaging is an excellent method for lowering lipid oxidation and extending the shelf life during meat processing and storage (Ahn et al., 1992, 1993; Veberg et al., 2006). Compared with oxygen-permeable packaging, thigh meats packaged in vacuum bags significantly reduced lipid oxidation at 1, 4, and 7 d of refrigerated storage (Table 2). Especially at d 4 and 7, the TBARS values of vacuum-packaged chicken meat were very low compared with those in oxygen-permeable packaging (0.17 vs. 1.60 and 0.35 vs. 2.96, respectively; Table 2). As the storage time increased and intrinsic antioxidants diminished, vacuum packaging had a more pronounced role in reducing lipid oxidation than did oxygen-permeable packaging. These results agree with the observation of Conchillo et al. (2003).

Fats are highly susceptible to ionizing radiation and can undergo many auto-oxidizing and hydrolytic reactions (Hammer and Wills 1979). These reactions give rise to undesirable organoleptic changes and nutritional losses, which hinder commercial utilization of irradiation in foods. The result showed that 3 kGy of irradiation significantly increased lipid oxidation in chicken thigh meat during refrigerated storage for 7 d (Table 2). These results further confirmed the work of Du et al. (2000), who reported that irradiation increased the level of lipid oxidation in chicken meat. Chicken muscle contains approximately 75% water, and irradiation can produce free radicals such as $^{\bullet}OH$, $^{\bullet}H$, and H_3O^+ by the radiolysis of water (Brewer, 2004). These radicals can modify unsaturated fatty acids or triglycerides and then initiate lipid oxidation (Frankel, 1980). Moreover, the formation of peroxyl compounds in irradiated meat may negatively affect some sensitive components such as vitamins. Lakritz et al. (1995) reported that irradiation resulted in a loss of α-tocopherol in meat. Ahn et al. (1997) indicated that significant amounts of vitamin E in turkey leg (60%) and breast muscle (25%) were destroyed by irradiation. Therefore, the increased lipid oxidation in irradiated meat, to some degree, is due to a decreased level of α-tocopherol. This study demonstrated that the interactions of diet × irradiation ($P = 0.0157$), packaging × irradiation ($P = 0.0001$), and diet × packaging × irradiation ($P = 0.0007$) had a significant effect on TBARS. The results agreed with a previous study showing that high levels of dietary α-tocopherol (200 to 600 IU/kg) significantly decreased lipid oxidation in irradiated turkey leg meat (Ahn et al., 1997). Vacuum packaging effectively prevented lipid oxidation by irradiation (Table 2). This result is consistent with other studies reporting that irradiated meat stored in oxygen-permeable bags had much faster oxidation rates than those stored in vacuum-packaged bags (Ahn et al., 1997). Mahrour et al. (2003) concluded that the combination of antioxidant and vacuum packaging might be a better alternative to control lipid oxidation in irradiated chicken meat. This suggests that an appropriate use of dietary supplementation of antioxidants in combination with vacuum packaging could be effective in minimizing the adverse oxidative effects resulting from irradiation.

Protein Oxidation

Dietary vitamin E or oxidized oil, different packaging methods, and irradiation significantly influenced protein oxidation of thigh meat during 7 d of refriger-

Table 3. Effects of dietary treatment, packaging, and irradiation on protein oxidation of chicken thigh patties during refrigerated storage

Storage time (d)	Carbonyl (nmol/mg of protein)			SEM	*P*-value
	Control diet	Oxidized diet	Antioxidant diet		
1	0.43[b]	0.51[a]	0.30[c]	0.011	<0.001
4	0.54[b]	0.62[a]	0.43[c]	0.007	<0.001
7	0.73[ab]	0.76[a]	0.66[b]	0.028	<0.05
	O_2-permeable packaging	Vacuum packaging			
1	0.46[a]	0.37[b]		0.0086	<0.001
4	0.62[a]	0.44[b]		0.0061	<0.001
7	0.81[a]	0.63[b]		0.023	<0.001
	0 kGy of irradiation	3 kGy of irradiation			
1	0.31[b]	0.52[a]		0.0086	<0.001
4	0.45[b]	0.62[a]		0.0062	<0.001
7	0.56[b]	0.84[a]		0.022	<0.001

[a–c]Means in the same row with different letters differ significantly.

ated storage (Table 3). Protein oxidation occurring in muscle foods is linked to many factors, including high concentrations of oxidizable lipids, heme pigments, transition metal ions, and oxidative enzymes (Xiong, 2000). Protein oxidation is also a free radical chain reaction that is similar to lipid oxidation. The oxidative reactions occurring in muscle can result in the generation of carbonyls (aldehydes and ketones), protein polymers, and peptide scissions (Ooizumi and Xiong, 2004). Among them, formation of carbonyls is one of the most prominent changes in oxidized proteins, and the content of carbonyl is widely used as a marker of protein damage (Lund et al., 2008). The protein damage in postmortem muscle can lead to functional changes of proteins, including gel-forming ability, meat-binding ability, emulsification capacity, solubility, viscosity, and water-holding capacity (Xiong, 2000), which can significantly affect the quality of meat and meat products.

Previous studies reported strong interactions between protein and lipid oxidation in muscle foods, and oxidative reactions could easily be transferred from lipids to proteins (Schaich and Pryor, 1980). The primary (hydroperoxides) and secondary lipid oxidation products (aldehydes and ketones) can react with proteins and further induce protein oxidation (Kikugawa et al., 1991). Briefly, lipid oxidation can form free radicals such as peroxyl radicals ($ROO^{\bullet}$), which can initiate protein oxidation. The $ROO^{\bullet}$ can abstract hydrogen atoms from protein molecules (PH; reaction 1). Protein radicals ($P^{\bullet}$) are formed, and they can then generate a protein net (P–P) via cross-linking (reaction 2):

$$ROO^{\bullet} + PH = P^{\bullet} + ROOH \text{ (reaction 1), and}$$

$$P^{\bullet} + P^{\bullet} = P\text{–}P \text{ (protein net; reaction 2).}$$

Therefore, the factors that affect lipid oxidation could contribute to protein oxidation as well. Many studies have indicated that protein oxidation (detected by carbonyl content) is coupled with lipid oxidation (detected by TBARS) in beef (Mercier et al., 1995), fish (Srinivasan and Hultin, 1995), turkey (Mercier et al., 1998), and chicken (Coetzee and Hoffman, 2001). The chicken meat with greater lipid oxidation also showed greater protein oxidation (Tables 2 and 3). The correlation between lipid and protein oxidation was statistically significant ($P < 0.001$). Feeding high levels of vitamin E (500 IU/kg of feed) to broilers significantly ($P < 0.001$) reduced protein oxidation (detected by carbonyl) in thigh patties on d 1 and 4 of refrigerated storage (Table 3). This may be because the addition of vitamin E in the diet led to the accumulation of α-tocopherol in chicken muscle, which could prevent oxidative damage in muscle proteins. The thigh meat patties contained a relatively higher concentration of PUFA and were subjected to grinding during preparation, which increased oxidative reactions because of the incorporation of oxygen and the contact of oxidative catalysts with lipids (Xiong, 2000). However, during 1, 4, and 7 d of storage, meats from the vitamin E treatment had 30, 70, and 53% less lipid oxidation (Table 2) and had 30, 30, and 10% less protein oxidation than the control on the respective storage days (Table 3). This suggested that as the storage time progressed, vitamin E had a more pronounced role in lipid oxidation than did protein oxidation. The results agreed with previous reports demonstrating that the effects of vitamin E on protein oxidation were less marked than the lipid oxidation in turkey (Mercier et. al., 1998), beef (Marianne et al., 2007), and lamb (Petron et al., 2007).

Broilers fed oxidized diets (5% oxidized oil) produced higher amounts of carbonyls than did broilers fed the vitamin E and control diets at 1 and 4 d storage. However, the difference between the control and oxidized groups at d 7 was not significant (Table 3). To some degree, protein oxidation was initiated as lipid oxidation

Table 4. The interactive effects of diet, packaging, and irradiation on lipid oxidation and protein oxidation of chicken thigh patties

Source	Storage time (d)	*P*-value (TBA-reactive substances)	*P*-value (carbonyl)
Diet × packaging	1	<0.001	0.074
	4	<0.001	<0.001
	7	<0.001	0.56
Diet × irradiation	1	0.016	0.13
	4	<0.001	0.009
	7	<0.001	0.18
Packaging × irradiation	1	<0.001	<0.001
	4	<0.001	<0.001
	7	<0.001	0.27
Diet × packaging × irradiation	1	<0.001	0.56
	4	<0.001	0.077
	7	<0.001	0.15

increased. Vacuum packaging protected chicken meats from protein oxidation, to some extent, because of decreased lipid oxidation during storage.

Irradiation at 3 kGy significantly increased protein oxidation in chicken thigh meat during the 7 d of refrigerated storage (Table 3). Irradiation can produce hydroxyl radicals by splitting water molecules in chicken muscle (Giroux and Lacroix, 1998). These radicals react with the peptide chain or migrate to the side chain of amino acids such as cysteine, cystine, methionine, tyrosine, phenylalanine, histidine, tryptophan, and lysine, which are susceptible to irradiation (Grolichová et al., 2004). Irradiation can also break the organized structure of proteins by splitting hydrogen and –S–S– bridges. Moreover, the secondary and tertiary structures of proteins can be destroyed because of reduction of the –S–S– bond or oxidation of the –SH group. In some cases, the configuration of proteins can be changed because of the formation of new bonds (Ressouany et al., 1998). These chemical changes depend not only on the structure and state of proteins (such as whether they are liquid or frozen), but also on the irradiation conditions, such as the dose, dose rate, temperature, and presence of oxygen (Giroux and Lacroix, 1998). The interaction between irradiation and proteins can produce carbonyl groups, ammonia, free amino acids, hydrogen peroxide, and organic peroxides (Brault et al., 1997) and can influence the tenderness (Rowe et al., 2004b), water-holding capacity (Huff-Lonergan and Lonergan, 2005), and flavor and sensory attributes of meat products (Ahn et al., 2000). At 1, 4, and 7 d of refrigerated storage, irradiation increased carbonyl values of chicken patties by 1.68-, 1.38-, and 1.5-fold, respectively, over those of nonirradiated meat (Table 3). However, irradiation increased TBARS values by 3.2-, 7.8-, and 10-fold at the respective storage times over those of nonirradiated meat (Table 2). Irradiation significantly increased both lipid and protein oxidation in chicken meat during refrigerated storage, but its effects on lipid oxidation were much greater than the effects on protein oxidation. There was a significant interaction effect for packaging × irradiation on carbonyl content at d 1 and 4 of storage ($P < 0.001$). However this significant interaction was not found after 7 d of storage (Table 4). Carbonyl content was found to be higher in irradiated meat samples in oxygen-permeable packaging. This can be explained by the fact that irradiation can produce free radicals, and these free radicals initiate or accelerate protein oxidation in the presence of oxygen (Jo and Ahn, 2000; Rowe et al., 2004a). Significant interaction effects were also found for diet × packaging and diet × irradiation interactions on d 4. The combination of vacuum packaging and antioxidant was effective in preventing protein oxidation in chicken meat, and the results were consistent with those of Rowe et al. (2004a), who found that dietary supplementation of vitamin E prevented protein oxidation in irradiated beef.

Color

Many factors, such as the concentration of heme pigments (myoglobin in particular), oxidation status, and ligand formation of heme pigments, as well as physical characteristics (pH, temperature, storage time) of meat can contribute to variation in meat color (Qiao et al., 2001). Faustman et al. (1992) reported that lipid oxidation could promote myoglobin oxidation. Consequently, the factors affecting lipid oxidation in meat can also influence meat color.

Dietary supplementation of vitamin E increased the lightness (L*) of chicken thigh meat compared with the color of chicken thigh meat from the control group during 7 d of refrigerated storage (Table 5). Dietary addition of vitamin E was reported to improve the color stability of beef (Chan et al., 1996) and lamb (Guidera et al., 1997). However, other studies showed that dietary vitamin E supplementation did not improve the color stability of chicken (Coetzee and Hoffman, 2001; Zouari et al., 2010) and pork (Phillips et al., 2001). Adding oxidized oil to chicken diet did not affect the color of chicken meat during refrigerated storage (Table 5). Racanicci et al. (2008) also observed that meat color (L*, a*, and b*) during chilled storage was not affected by dietary treatments with oxidized poultry offal fat.

The effects of irradiation on meat color are well documented. Millar et al. (1995) and Nam and Ahn (2002)

Table 5. Effects of dietary treatment, packaging, and irradiation on color during refrigerated storage of chicken patties

Color	Storage time (d)	Control diet	Oxidized diet	Antioxidant diet	SEM	*P*-value
L*	1	53.08[c]	54.81[a]	54.31[ab]	0.42	<0.001
	4	53.36[b]	54.46[ab]	54.64[a]	0.42	<0.05
	7	52.21[b]	53.43[ab]	54.03[a]	0.44	<0.01
a*	1	13.14	13.02	12.79	0.20	NS[1]
	4	12.96	12.77	12.74	0.16	NS
	7	12.68	12.36	12.47	0.21	NS
b*	1	16.29	16.48	16.56	0.19	NS
	4	17.07	16.69	17.29	0.21	NS
	7	17.48	17.30	18.04	0.23	NS
		O_2-permeable packaging	Vacuum packaging			
L*	1	54.52	56.61		0.34	NS
	4	54.11	54.20		0.34	NS
	7	53.04	53.42		0.37	NS
a*	1	14.01[a]	11.96[b]		0.17	<0.001
	4	13.37[a]	12.28[b]		0.13	<0.001
	7	12.56	12.45		0.17	NS
b*	1	17.11[a]	15.77[b]		0.15	<0.001
	4	17.44[a]	16.60[b]		0.17	<0.05
	7	17.76	17.45		0.19	NS
		0 kGy of irradiation	3 kGy of irradiation			
L*	1	55.06[a]	53.07[b]		0.34	<0.001
	4	55.32[a]	52.99		0.43	<0.001
	7	53.57	52.88		0.37	NS
a*	1	13.39[a]	12.57[b]		0.19	<0.05
	4	12.51[b]	13.14[a]		0.13	<0.05
	7	12.08[b]	12.90[a]		0.17	0.001
b*	1	18.78[a]	14.11[b]		0.15	<0.001
	4	18.07[a]	15.97[b]		0.17	<0.001
	7	19.27[a]	15.94[b]		0.19	<0.001

[a–c]Means in the same row with different letters differ significantly.

[1]NS = no significant difference in means in the same row at the level of $P < 0.05$.

reported that irradiation increased the redness (a*) of turkey and chicken raw breast meat. The results of the present study indicated that irradiation decreased color lightness (L*) and increased the redness (a*) of thigh muscle during storage. Nam and Ahn (2002) postulated that the pigment responsible for pinking in irradiated turkey breast was CO-Mb.

In conclusion, dietary treatment with vitamin E and oxidized oil can directly influence the lipid and protein oxidation of chicken meat during refrigerated storage. Vacuum packaging minimized both lipid and protein oxidation. Irradiation showed negative effects on both lipid and protein oxidation, but its effects on protein oxidation were less pronounced than those on lipid oxidation. Dietary supplementation of antioxidants in combination with vacuum packaging was effective in minimizing the adverse oxidative effects of irradiation. In addition, dietary supplementation of vitamin E improved the color L* (lightness) value, but irradiation improved the a* (redness) value of chicken thigh meat.

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