Effects of Diet, Packaging and Irradiation on Protein Oxidation, Lipid Oxidation of Raw Broiler Thigh Meat

A.S. Leaflet R2761

Shan Xiao, Graduate Student; Wan Gang Zhang, Postdoctoral Research Associate; Eun Joo Lee, Assistant Scientist; Dong Ahn, Professor, Department of Animal Science

Summary and Implications

The effects of dietary treatment, packaging and irradiation singly or in combinations on the oxidative stability of broiler chicken thigh meat were studied. Lipid and protein oxidation of thigh meats from birds fed the diet supplemented with antioxidants (vitamin E + BHA) were significantly lower than those of the control while those from oxidized oil treatment were higher than the control. Vacuum-packaging slowed down while irradiation accelerated both lipid and protein oxidation of thigh meat during storage. Dietary antioxidants (vitamin E + BHA) and irradiation treatments showed stronger effect to lipid oxidation than protein oxidation. Significant correlation between lipid and protein oxidation in meat was found during storage. The results indicated that appropriate use of dietary antioxidants in combination with packaging could be effective in minimizing oxidative changes in irradiated raw chicken thigh meat.

Introduction

Oxidative deterioration is a major quality loss in muscle foods. The adverse effects of oxidation are not only involved in the economic loss but also are related to off-flavor development, discoloration, nutrient loss, and health risks. Muscle tissues have endogenous antioxidant mechanisms to control oxidative process *in vivo*. These antioxidants, which are classified as preventive antioxidants, continue to function but diminish their activities as postmortem time increases. Several intrinsic and extrinsic factors and post-slaughter processing and treatments including storage temperature, restructuring, nonmeat ingredient, packaging, and irradiation can influence the oxidative status of meat.

Chicken meat contains relatively high amounts of unsaturated fatty acids, which increase the concerns of oxidative deterioration. Many technologies had been developed to prevent or minimize oxidative changes in chicken meat: use of dietary strategy to improve the oxidative stability of chicken meat has been extensively studied. Among them, dietary supplementation of vitamin E is well accepted as an effective method to lower lipid oxidation and extend shelf-life of meat. Oxygen is the most common and essential component for the progress of lipid oxidation. Restructuring and grinding processes can increase the exposure of lipid to air. Many studies have shown that vacuum-packaging or oxygen-depleted modified atmosphere packaging could decrease lipid oxidation in meat during storage.

In order to meet the high energy demand for fast growing broliers, addition of oils or fats to broiler chicken diet 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 chicken meat because feeding diets containing high levels of oxidized fats increase oxidative stress in animal body.

With the approval of irradiation to improve the safety of poultry meat, the concern about negative quality changes (color, odor, tenderness, and lipid oxidation etc.) by irradiation has been raised. Some technologies have been used to minimize the negative effects of irradiation on poultry meat quality including adding antioxidant substances to poultry diet, modifying packaging method, and adding antioxidants to meat during processing.

During meat processing and storage, oxidative modification of amino acid residues and polypeptide backbone could result in physical and chemical changes including conformational stability, solubility, and the nutritional quality. Formation of carbonyls is one of the most prominent changes in oxidized muscle proteins. In fact, proteins in whole muscle are susceptible to oxidative changes during processing and storage because of the depletion of endogenous antioxidant. However, the effect of dietary treatments, package, and irradiation on protein oxidation in meat is limited. The purpose of this study is to evaluate the effects of dietary addition of antioxidants or oxidized oil, irradiation and different packaging singly or combination on the oxidative stability of broiler chicken thigh meat.

Materials and Methods

One hundred and twenty 29-day-old commercial broiler chicks were assigned into each of 12 floor pens. Four floor pens were randomly allotted to one of 3 experimental diets including control, oxidized diet, and antioxidants-fortified diet. Control diet was prepared with fresh animal-vegetable (AV) fat blend[1] with 25 IU vitamin E, oxidized diet was prepared after oxidizing the same animal-vegetable fat by exposing to room temperature for a long time until attaining peroxide value (PV) of 100, and the antioxidants-fortified diet was prepared with the fresh animal-vegetable fat supplemented with butylated hydroxyanisole (BHA, 200 ppm) and vitamin E (500 IU) per kg of feed. Each of the

diets was fed to the broilers for 2 weeks with free access to water and diet.

The birds were slaughtered at the end of experiment, deboned, and thigh meats from each treatment were ground twice through a 3-mm plate, and were prepared. Half of the patties from each treatment were packaged in vacuum bags (nylon/polyethylene, 9.3 ml O_2/m^2/24 h at 0 °C) and the other half packaged in oxygen-permeable bags (polyethylene, 2,300 mL/m^2/24 h, 4 x 6, 2 MIL), and irradiated at 3.0 kGy using a Linear Accelerator Facility. Color, lipid and protein oxidation were determined at 1, 4 and 7 days after irradiation. Lipid oxidation was determined by a TBARS method Protein oxidation was determined using the DNPH method. Data were analyzed by the procedure of generalized linear model using SAS 9.1 software.

Results and Discussion

Two weeks of dietary vitamin E supplementation (500 IU/kg) lowered lipid oxidation in thigh muscle up to 7 d refrigerated storage ($p < 0.001$, Table 1). Consumption of oxidized oil can increase oxidative stress in live birds, which drives the chickens to utilize antioxidants such as vitamin E to protect themselves from the oxidative stress. In addition, the oxidized oils that the birds have consumed can directly destruct or denature vitamins and increase the susceptibility of gastrointestinal tract or other tissues to lipid oxidation. Therefore, consumption of oxidized oil can result in decreased accumulation of antioxidants like vitamin E in muscle of broiler chicken. The decreased levels of vitamin E and other antioxidants eventually could decrease the antioxidant ability of meat samples during postmortem storage.

Adding fat/oil to animal diets not only supplies energy but also provides other benefits such as increasing the absorption of fat-soluble vitamins, increasing peristaltic movements of intestine, and improving the palatability of the diets. However, vegetable oils are highly susceptible to lipid oxidation due to their high content of PUFA and will affect the oxidative stress of livestock if they are oxidized.

When broiler chickens were fed the rations containing 5% oxidized animal-vegetable oil blend, which exposed for a long period at room temperature, for 2 weeks did not affect the performances of broilers. However, the increase of TBARS in meat from chickens fed a diet added with oxidized oil was significantly greater than that of control diet after 7 days of storage at 4 °C (Table 2). Dietary consumption of oxidized lipids can increase the absorption of lipid hydroperoxides and/or secondary oxidation products (dienes and trienes) in the intestinal lumen. Therefore, lipids hydroperoxides and secondary oxidation products would be present in plasma, lymph and muscle, which would lead to increased α-tocopherol turnover in animal tissues. Therefore, the increased level of lipid oxidation in meat from chickens fed oxidized diets could be related to the decreased α-tocopherol content in chicken muscle.

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. Compared to oxygen-permeable packaging, thigh meats packaged in vacuum bags significantly reduced lipid oxidation at 1, 4 and 7 d refrigerated storage (Table 2). Especially at Days 4 and 7, the TBARS values of vacuum-packaged chicken meat were very low compared with those with oxygen-permeable packaged ones (Table 2). As the storage time increased and intrinsic antioxidants diminished, vacuum-packaging played more pronounced role in reducing lipid oxidation than oxygen-permeable packaging.

The result showed that 3 kGy irradiation significantly increased lipid oxidation in chicken thigh meat during refrigerated storage for 7 days (Table 2). Chicken muscle contains approximately 75% water and irradiation can produce free radicals such as $^{\bullet}OH$, $^{\bullet}H$, H_3O^+ by the radiolysis of water. These radicals can modify unsaturated fatty acids or triglycerides and then initiate lipid oxidation. Moreover, formation of peroxyl compounds in irradiated meat may negatively affect some sensitive components such as vitamins. Therefore, the increased lipid oxidation in irradiated meat, in some degree, is attributed to decreased level of α-tocopherol. Vacuum-packaging effectively prevented lipid oxidation by irradiation (Table 2). 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.

Dietary vitamin E or oxidized oil, different package methods, and irradiation significantly influenced protein oxidation of thigh meat during the 7d refrigerated storage (Table 1). 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. Protein oxidation is also free radical chain reactions, which 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. The protein damages in postmortem muscle can lead to the functional changes of proteins including gel-forming ability, meat-binding ability, emulsification capacity, solubility, viscosity, and water-holding capacity, which can significantly impact the quality of meat.

Previous studies reported strong interactions between protein and lipid oxidation in muscle foods, and oxidative reactions could be easily transferred from lipids to proteins. The primary (hydroperoxides) and secondary lipid oxidation products (aldehydes and ketones) can react with proteins and further induce protein oxidation. Therefore, the factors that affect lipid oxidation could contribute to protein oxidation as well. The chicken meat with higher lipid oxidation also showed greater protein oxidation (Tables 1

and 2). The correlation between lipid and protein oxidation was statistically significant ($p < 0.001$). Feeding high levels of vitamin E (500 IU/kg feed) to broilers significantly ($p < 0.001$) lowered protein oxidation (detected by carbonyl) in thigh patties at Days 1 and 4 of refrigerated storage (Table 2). This may be due to the fact that the addition of vitamin E in diet led to the accumulation of α-tocopherol in chicken muscle, which could prevent oxidative damages in muscle proteins. Thigh meat patties contained relatively higher concentration of PUFA and were subjected to grinding during preparation, which increased oxidative reactions due to incorporation of oxygen and contact of oxidative catalysts with lipids. However, during 1-, 4- and 7-day of storage periods, meats from vitamin E treatment had lower lipid and protein oxidation (Table 1) than control at the respective storage days (Table 2). This suggested that as the storage time progressed, Vitamin E played more pronounced role on lipid oxidation than protein oxidation.

Broilers fed oxidized diets (5% oxidized oil) produced higher amounts of carbonyls than vitamin E and control treatments at 1 and 4d storage (Table 2). In some degree, protein oxidation is initiated as lipid oxidation increased. Vacuum-packaging protected chicken meats from protein oxidation, in some extent, because of decreased lipid oxidation during storage.

Irradiation at 3 kGy significantly increased protein oxidation in chicken thigh meat during the 7d refrigerated storage (Table 2). Irradiation can produce hydroxyl radicals by splitting water molecules in chicken muscle. These radicals react with 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. Irradiation can also break the organized structure of proteins by splitting hydrogen and –S-S- bridges. Moreover, the secondary and tertiary structure of proteins can be destroyed due to the reduction of –S-S- bond or the oxidation of –SH group. These chemical changes not only depend upon the structure and state of proteins but also irradiation conditions such as dose, dose rate, temperature and presence of oxygen. At 1, 4, and 7 days of refrigerated storage, irradiation increased carbonyl values of chicken patties over non-irradiated meat (Table 2). However, irradiation increased TBARS values several-folds at the respective storage time over non-irradiated meat (Table 1). It was shown that irradiation significantly increased both lipid and protein oxidation in chicken meat during refrigerated storage, but its effects on lipid oxidation were much greater than that on protein oxidation. Carbonyl contents were found to be higher in meat samples from irradiation and oxygen permeable package. 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.

Table 1. Effects of dietary treatment, packaging and irradiation on lipid oxidation of chicken thigh patties during refrigerated storage[1]

Storage Time (d)	Treatment	TBARS (mg MDA/kg meat)			SEM	*P*
	Diet	Control	Oxidized	Antioxidant		
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
	Packaging	O_2-permeable	Vacuum			
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
	Irradiation	0 kGy	3 kGy			
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

[1]On the same row, means with different letters differ significantly. n=4.

Table 2. Effects of dietary treatment, packaging and irradiation on protein oxidation of chicken thigh patties during refrigerated storage[1]

Storage Time (d)	Treatment	Carbonyl (nmol/mg protein)			SEM	*P*
	Diet	Control	Oxidized	Antioxidant		
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
	Packaging	O_2-permeable	Vacuum			
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
	Irradiation	0 kGy	3 kGy			
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

[1]On the same row, means with different letters differ significantly. n=4.