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DIETARY ANTIOXIDANT SUPPLEMENTATION (ECONOMASE–BIOPLEX) TO ALLEVIATE ADVERSE IMPACTS OF OXIDIZED OIL ON BROILER MEAT QUALITY: A CHEMICAL, TEXTURAL, ENZYMATIC, AND PROTEOMIC STUDY

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By

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ABSTRACT OF DISSERTATION

DIETARY ANTIOXIDANT SUPPLEMENTATION (ECONOMASE–BIOPLEX) TO ALLEVIATE ADVERSE IMPACTS OF OXIDIZED OIL ON BROILER MEAT QUALITY: A CHEMICAL, TEXTURAL, ENZYMATIC, AND PROTEOMIC STUDY

This study investigated the influence of dietary antioxidants and quality of oil on the oxidative and enzymatic properties of chicken broiler meat stored in an oxygen-enriched package (HiOx: 80% O_2/20% CO_2) in comparison with air-permeable polyvinylchloride (PVC) or skin (SK) packaging systems during retail display 2–4 °C for up to 14, 7, and 21 d, respectively. Broilers were fed a diet either with a low-oxidized oil (peroxide vale POV 23 meq O_2/kg) or with a high-oxidized oil (POV 121 meq O_2/kg), supplemented with an antioxidant pack (200 ppm EconomasE and organic minerals Se, Zn, Cu, Mn, and Fe as in Bioplex) in substitution for vitamin E and inorganic minerals for 42 d.

In all packaging systems, lipid oxidation and protein oxidation were inhibited by up to 65% with an antioxidant-supplemented diet when compared to diets without antioxidant supplements. Antioxidant enzyme activities were significantly higher ($P < 0.05$) in the antioxidant-supplemented diets compared with control diets, regardless of oil quality.

Meat samples from the antioxidant-supplemented group, irrespective of oil quality, has less purge and cooking loss compared to control diets. In all packaging systems, meat shear force was higher ($P < 0.05$) for broilers fed high-oxidized diets than the low-oxidized groups. Comparison between muscle types (breast as white vs. thigh as red) showed a similar trend in muscle susceptibility to oxidized oil in the diet but greater protection of antioxidant supplements for thigh meat in both physiochemical and textural properties.

Dietary regimen influenced protein expression in broiler breast meat. Three protein spots from 2-dimensional gel electrophoresis, identified by mass spectrometry as glyceraldehyde 3-phosphate dehydrogenase, creatine kinase, and heat shock protein beta-1 were over-abundant in muscle from low-oxidized diets. The differential proteomes that

suggested down regulation of the genes encoding antioxidative proteins upon feeding oxidized oil may be implicated in the broiler meat quality deterioration during storage.

In summary, feeding diets with poor oil quality increased the vulnerability of lipids and proteins to oxidation in broiler breast and thigh meat during refrigerated and / or frozen storage in various packaging conditions, yet these effects were alleviated upon dietary supplementation with antioxidants.

KEYWORDS: Dietary Antioxidants, Oxidized Oil, Chicken Meat, Lipid and Protein Oxidation, Packaging Systems

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This dissertation is dedicated to my loving husband and my parents. The years of hard work, sacrifices, and frustration were only possible to withstand because of them.

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CHAPTER 1

INTRODUCTION

A key factor that determines consumers' acceptance or rejection of fresh meat and meat products is palatability, of which flavor and texture (juiciness and tenderness) are the primary quality traits. A central cause of quality deterioration during retail or home storage (refrigerated or frozen) is lipid and protein oxidation, which produces rancid off-flavors and decreases juiciness and tenderness. Meat quality is influenced by a variety of factors such as animal heredity, feeding system, nutritional status, pre-slaughter and slaughter condition, and meat process conditions (Anderson et al., 2005). Poultry meat, in particular, is much more susceptible to lipid oxidation due to the high proportion of polyunsaturated fatty acids (PUFAs). For this reason, the oxidative stability of poultry meat and meat products presents a great challenge to meat producers and processors.

Oxidation occurs ubiquitously in fresh and processed muscle foods due to the abundance of unsaturated phospholipids, heme pigments, metal catalysts, and various other oxidizing agents present in skeletal muscle. The losses in quality are manifested by adverse changes in flavor, color, texture and nutritive value, and the possible production of toxic compounds (Kanner et al., 1994). Modification of intracellular and membrane proteins in muscle can be readily modified by reactive oxygen species generated by lipid oxidation, metal- or enzyme-catalyzed oxidative reactions, and other chemical and biological processes (Lund et al., 2011). Lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipid fractions and generation of lipid radicals can consequently attack other muscle components, such as proteins.

Furthermore, the presence of transition metals, notably iron, is pivotal in facilitating the generation of radical species capable of abstracting a proton from an unsaturated fatty acid (Gray et al., 1996). Oxidative modification of proteins can cause fragmentation and conformational changes, ultimately modifying their physical and chemical properties such as water-binding in fresh meat (Rowe et al., 2004). Reduced water-binding activity in oxidatively stressed muscle tissue was identified using low-field NMR (Bertram et al. 2007). Similarly, Liu et al. (2009) viewed significant losses in hydration capacity of myofibrils when exposed to an oxidizing environment. The restriction in transverse swelling of myofibrils during salt irrigation was due to increased myosin cross-linking through disulfide bonds.

Previous studies have shown that overall quality and shelf life can be improved through dietary vitamin E supplementation. Dietary vitamin E has been found to decrease lipid oxidation and discoloration and reduce drip loss in pork (Guo et al., 2006), lamb (Macit et al., 2003) and poultry (Li et al., 2013) which may be attributed to the protection of myofibrillar proteins from oxidation. Furthermore, endogenous delivery of antioxidants may offer greater protection of unsaturated lipids and proteins compared with exogenous incorporation, since dietary antioxidants can be absorbed and distributed into muscle both inside the cell and at the membrane (Mitsumoto, 2000). Previous studies have also focused on the effect of vitamin C, selenium and synthetic antioxidants on growth characteristics or oxidative stability of lipids in meat (Engberg et al., 1996; Xiao et al., 2011). However, few studies have focused on the relationship between dietary feeding strategy and the overall quality of fresh meat during storage.

Meat quality is manifested through a complexity of biochemical processes in the muscle, which are influenced by various external factors in both the live animal and post-mortem. The implementation of proteomic tools such as two-dimensional gel electrophoresis coupled with mass spectrometry can aid in the achievement of sustainable animal production and improved and consistent product quality. Accordingly, the nutrigenomic approach has been introduced as a first step in the development of pro-active quality control and assurance systems to fulfill future demands from the industry and consumers. Nutrigenomics is the study of the effect of nutrition or dietary components on the transcriptome of cells and tissues. The broad scope of nutrigenomics involves studying the effect of nutrition or dietary components on the structure, integrity and function of the genome (Mutch et al., 2005). Currently, most of the nutrigenomic studies on meat animals, including poultry, are focused on the performance of animals in relation to specific dietary compounds, for example, their growth characteristics and carcass traits. Very little research has been done to establish the relationship between feeding bioactive compounds and the resulting quality of the end product – meat.

The aim of this research was to elucidate the *in situ* role of dietary antioxidant supplementation and quality of oil on the oxidative, enzymatic, and textural properties of fresh chicken meat stored in different atmospheres and to identify whether dietary regimen would influence protein expression. meat particle size reduction (grinding) would accentuate oxidation-induced alteration in water-binding properties of meat. The specific objectives of my dissertation research were:

1. To identify the likely benefits of dietary antioxidants for inhibiting oxidative changes and protecting eating quality of chicken meat packaged under modified atmospheres.

2. To determine how dietary antioxidant regimens would influence the storage stability and quality of chicken meat.

3. To assess how lipids, pigments, and proteins of different muscle fiber types (dark vs. light chicken meat) are affected by dietary oxidized oil and antioxidants.

4. To explore the molecular mechanism for the influence of dietary antioxidants on meat quality through proteomic and enzymatic tools.

CHAPTER 2

LITERATURE REVIEW

2.1. Broiler Production

Chickens are the most abundant birds in the world. Currently, poultry products (broiler meat in particular) have the highest per capita consumption in the United States. People eat various different poultry products including chicken wings, hot dogs, chicken nuggets, chicken-patties, fried, roasted, marinated chicken, etc… However, not all poultry products are consumed. For instance, eggs are used for the production of vaccines, antibodies, and pharmacological proteins. Part of the recent increase in poultry production can be attributed to the development of new, further-processed and value-added products.

In the United States, poultry houses are equipped with a nipple and cup waterer, which must remain fully functional, along with supplemental jug waterers. Feed is placed in the feeders and on paper placed on the floor of the pen to encourage young birds to eat. Generally, broilers are brooded in a portion of the house until a certain age before being given access to the entire barn. These facilities generally have litter floors and the amount of light the birds are exposed to is regulated.

2.1.1. Broiler Meat Quality Concerns and Issues. Over the past two decades, the consumption of poultry meat, particularly chicken, has increased dramatically (50%; from 60 lb/capita to 90 lb/capita) while the consumer demand for red meat (beef and pork) has remained stagnant over this period (USDA, 2009). Among the reasons are the relatively high feed conversion rate and being a “healthier” meat source (white meat) for

poultry as perceived by the consumer. Thus, the growing pressure on breeders, nutritionists and growers to increase the growth rate of birds, feed efficiency and size of breast muscle has placed additional stress on the developing birds potentially resulting in histological and biochemical modifications of the muscle tissue thereby impairing some meat quality traits (Petracci and Cavani, 2012). The most common concerns are associated with deep pectoral muscle disease, white striping, and pale, soft and exudate (PSE) – like conditions.

Today, poultry is marketed in about half the time and at twice the body weight compared to 50 years ago (Barbut et al., 2008), these improvements are primarily due to genetic selection of high heritable body weight. Yet there has been an increase in incidence of pectoral myopathies in concert with increased growth rate and muscle size. For instance, birds selected for breast muscle development have a significantly higher risk of deep pectoral muscle disease occurrence (Petracci and Cavani, 2012). Deep pectoral myopathy is an ischemic necrosis that develops in the *supracoracoideus* or *pectoralis minor* muscle due to the inability of the muscle mass to swell in response to increased circulation. The pectoral muscle in poultry is surrounded by an inelastic fascia and the sternum, thus when the muscles are exercised such as in wing flapping the muscle mass may increase in size to a point where the muscle itself becomes strangulated, resulting in the occlusion of the blood vessels and ultimately tissue necrosis (Bianchi et al., 2006). There is no consumer health issues associated with deep pectoral myopathy, except that the breast muscle is aesthetically undesirable. The loss of the most valuable part of the chicken carcass results in major losses to the poultry industry. To decrease the

incidence of pectoral myopathies reduced activity of wing flapping, triggered by feed and water depletion, loud noises, high heat, and high human traffic, should be addressed.

White striping is a recent defect in chicken breast meat that could negatively influence the purchasing decision of consumers. White striping refers to the occurrence of different degrees of white striations found parallel to the muscle fibers. Moderate to severe degrees of white striping can potentially reduce the visual acceptance and purchase intent of the consumer due to the fatty or marbled appearance of the product (Kuttappan et al., 2012). The condition is mainly associated with heavier birds. Bauermeister et al. (2009) reported a higher incidence in birds processed at 8 week than at 6 week of age, possibly due to their higher body weight at time of slaughter. Similarly, Kuttappan et al. (2012) reported higher degrees of white striping in thicker and heavier fillets, which is associated with increased growth rates. Furthermore, the histological studies conducted by Kuttappan et al. (2013) suggested that a higher degree of white striping is associated with muscle damage and myopathic changes, indicating that enhanced growth rate in birds could result in muscle damage which is manifested primarily as white striping. Lack of adequate development in the capillary or other supporting systems in fast growing birds may contribute to growth-induced myopathy (Mahon, 1999).

Another challenge in the broiler meat industry is the recent development of PSE or PSE-like meat. PSE is most commonly associated with pork products and is characterized by light color, flaccid texture, poor water-holding capacity and substantially reduced cooking yield. In swine, specific genetic mutations have been linked to the development of PSE: (1) the Ryanodine receptor and halothane gene mutation causes

Porcine Stress Syndrome (PSS) or Malignant Hyperthermia (MH), which results in a rapid and ultimate low pH at high temperatures; (2) Rendement Napole (RN) gene mutation results in a normal pH decline but to an ultimate low pH (Barbut et al., 2008). However, Strasburg and Chiang (2009) reported inconclusive data linking PSE-like conditions in poultry to a genetic mutation. Other hypotheses are considered as having the potential to cause PSE meat: (1) the nature and metabolism of the breast muscle; (2) the size of the muscle and muscle fibers (Dransfield and Sosnicki, 1999); (3) perimortem environmental conditions (Berri et al., 2005). Some studies have reported an elevated incidence of spontaneous or idiopathic myopathy and an increased susceptibility to stress-induced myopathy in rapidly growing strains of poultry (Sandercock et al., 2006). These pathologies are attributed to alterations in intracellular calcium homeostasis (Sandercock and Mitchell, 2003) and concurrent changes in the integrity of the sarcolemma, which may result from excessive muscle fiber hypertrophy and inadequate development of support tissues and vascular supply (McRae et al., 2007; McRae et al., 2006). Furthermore, faster growing and/or heavier birds are more susceptible to heat stress. Mujahid et al. (2005) reported an increase in superoxide free radical production in the skeletal muscle of birds exhibiting acute stress, which may induce muscle damage thereby altering cellular metabolism and tissue structure integrity, potentially resulting in PSE-like meat.

Today, with the advent of proteomics in muscle biology and meat science there are greater possibilities to further investigate and identify meat quality associated problems. Proteomic studies are becoming increasingly popular to study the relationship between genome and functional properties of meat. While genome contains information

on which genes and alleles are present in the genome, the proteome contains information on which genes are actually being expressed. The implementation of proteomic technologies will advance the meat science industry through improved quality of fresh and processed meat and meat products and advanced animal production methods.

2.1.2. Factors Affecting Meat Quality. To an average consumer, "meat quality" describes eating quality, which includes color, tenderness, juiciness, flavor, and the consistency of the meat in its raw and cooked states. Quality is a complex, multivariate property of meat that is influenced by animal heredity, feeding system, nutritional status, pre-slaughter and slaughter condition, and meat processing conditions (Anderson et al., 2005).

2.1.3. Pre-Slaughter Conditions. How an animal is handled prior to slaughter greatly impacts the quality of meat and meat products. Pork carcasses, especially, are known to possess either normal, Pale, Soft and Exudate (PSE) or Dark, Firm and Dry (DFD) conditions. PSE can occur in both pork and poultry and is characterized by an abnormally light color, poor fiber structure consistency, and low water0-holding capacity. This occurs due to an accelerated rate of glycolysis, post-mortem, resulting in a ultimate low pH. Genetic predispositions and high stress levels prior to slaughter are the main factors that contribute to PSE meat. Calmer animals will have lower body temperatures, which will help reduce protein denaturation. Eliminating wing flapping, vocalizations, and excessive movement as animals move from the farm into the holding pen can help reduce PSE. Also, reducing the length and roughness of transportation and human

handling can also help reduce the incidence of PSE. For poultry, automatic loaders and unloaders may be useful. Following transportation and unloading, animals should be given an extensive rest period before slaughter. DFD occurs more often in beef and is characterized by a dark, purplish red to black color, firm texture, and dry appearance. This usually occurs as a result if an animal's depleted glycogen reserves prior to slaughter, resulting in a high ultimate pH. In rested, calm cattle muscle glycogen levels will be 0.8% to 1.0% prior to slaughter. However, animals are exposed to long-term stress may have glycogen levels around 0.6%, thereby hindering post-mortem pH decline. Long-term stress in cattle depletes their glycogen reserves. In beef, the normal pH is between 5.4–5.7. However, DFD will have a higher pH range, 5.9–6.5. The depletion of muscle glycogen may be caused by a variety of severe pre-slaughter stresses including transport exhaustion, fear, climatic stress, aggressive behavior with young bulls, hunger, prolonged withholding of feed prior to slaughter, mixing of unfamilar animals and extreme adrenaline excitement (Miller, 2007). Replenishing muscle glycogen stored can reduce the incidence of DFD, but may take several days post stress.

2.1.4. Dietary Regimen. Like humans, diet plays an important role in the overall health and performance of an animal. Furthermore, nutrition has a regulatory effect on biological processes in muscle, which can influence the quality of meat and meat products (Anderson et al., 2005). Previous research has primarily focused on the performance of animals in relation to specific dietary compounds, for example, their growth characteristics and carcass traits. However, in the past few decades researchers

are focusing on the relationship between feeding bioactive compounds or specific waste products (i.e. dried distillers grain) and the resulting quality of the end product – meat.

Recently, consumers are pushing livestock producers to feed and raise farm animals without antibiotics, hormones, or synthetic feed additives. Therefore, bioactive compounds such as oligosaccharides, emulsifiers, carotenoids, vitamins, and minerals are used in animal feedstuff to either promote immunity, aid in digestion, and/or improve growth characteristics. When incorporated into feed and food components, the above bioactive compounds have a broad range of effects in animals. Historically, plants have been used for medicinal purposes by humans to treat aliments. Thus, there is global interest in harnessing bioactive properties of plants and their secondary compounds as alternatives to chemical, drugs and growth promoters (Durmic and Blache, 2012).

Antibiotic growth promoters in poultry feed increased weight gain, feed utilization, and overall well-being in birds (Gustafson and Bowen, 1997). However, the controversial subject of antibiotics in animal feed and the development of resistant bacteria led to a complete ban on antibiotics in poultry feed by the European Union, with the United States reducing and limiting the amount and type of antibiotics used (Sims et al., 2004). Today, global demands for "antibiotic-free" and "organic" poultry products are directing producers to search for alternative growth promoters.

Mannan oligosaccharides (MOS) is derived from the outer cell wall of yeast and has been reported to increase the body weight of turkeys, reduce large intestinal concentrations of pathogenic bacteria, such as *Clostridium perfingens* and *Escherichia coli*, and increase concentrations of mutualistic microflora, such as *Lactobacilli* (Sims et al., 2004). MOS and frutooligosaccharides act as a prebiotic in that they are nondigestible

feed ingredients that benefit the host through selective stimulation of the growth or metabolic activity of specific microflora (Gibson and Roberfroid, 1995; Ferket, 2004). Spring et al. (2000) and Ije et al (2001), reported that MOS can effectively suppress enteric pathogens, enhance immunity, and improve the integrity of the intestinal mucosa in broilers. Similarly, Baurhoo et al. (2007) described that purified lignin and MOS increased beneficial ceca microflora, villi height and number of goblet cells in the jejunum and lowered the population of *E.coli* in fecal material. Therefore, MOS, lignin, and possibly other products may serve as an alternative to the use of antibiotics as a growth promoter in poultry production.

Phytogenic additives are a new class of plant-derived products that are currently being used in animal feed to improve the performance of the livestock/ flock. These compounds are usually derived from fruits, vegetables, grains, spices, herbs, seeds, bark, etc… The use of feed additives is usually subject to restrictive regulations and is generally applied by the farmer to healthy animals for nutritional purposes throughout the entire feeding period (Windisch et al., 2008). Phytogenic compounds possess various phenolic compounds, of which some bear antioxidative properties. For instance, volatile oils from Labiaceae, such as rosemary, mint, thyme and oregano, have strong antioxidant activity due to the large amount of terpenes present. Specifically, rosemary contains rosmarinic acid and rosmarol (Cuppett and Hall, 1998). Fruits are rich in anthocyanins, and certain leaves, such as green tea are abundant in flavonoids, all of which have radical scavenging properties. The structure of the phenolic compound greatly influences its redox potential. Teissedre and Waterhouse (2000) reported that thyme has a high antioxidant activity, which was due to the presence of phenolic hydroxyl groups in

thymol, which act as hydrogen donors to peroxyl radicals thereby retarding lipid oxidation.

Phytogenic additives have also been reported to aid with digestion through the stimulation of gastric and intestinal secretions and enhanced enzyme activity (Platel and Srinivasan, 2004). Rao et al. (2003) reported that various spices and spice extracts enhanced pancreatic lipase and amylase in rats. Furthermore, rats fed anise oil showed accelerated glucose absorption (Kreydiyyeh et al., 2003). Essential oils added to dietary feed enhanced trypsin and amylase activities in broilers (Jang et al., 2004).

Finally, many of the aforementioned compounds also possess antimicrobial properties due to the potential of hydrophobic essential oils to penetrate the bacterial cell membrane, disintegrate membrane structures, and cause ion leakage (Windisch et al., 2008). Jamroz et al. (2006) reported that phytogenic feed additives stimulated intestinal secretion of mucus in broilers, which may impair the adhesion of pathogens and reduce gastrointestinal distress. However, the efficacy of the essential oils against pathogenic microbes is dose-dependent and may not be present at high enough levels in the dietary feed. Thus more research is needed in this area.

Dried distillers grain (**DDGS**) is a by-product from the fermentation and distillation of corn into bio-ethanol. DDGS has a high level of crude protein, lysine, methionine, and unsaturated fatty acids, which has been used in animal feed (Lemenager et al., 2006). Min et al. (2012) reported that feeding broilers up to 25% DDGS increased the ratio of polyunsaturated to saturated fatty acids in breast fillets, yet, samples from birds fed up to 10% DDGS had lower lipid oxidation, drip loss and cooking loss.

2.2. Dietary Antioxidants and Meat Quality

With a relatively high proportion of polyunsaturated fatty acids (PUFA), poultry meat is much more susceptible to lipid oxidation than beef and pork. For this reason, the oxidative stability of poultry meat and meat products presents a great challenge to meat producers and processors. One of the novel approaches to overcoming oxidation and related problems with poultry meat and meat products is to feed the birds antioxidant diets. Enhancing diets with antioxidants and optimizing nutrient intake not only could reduce lipid oxidation but also may improve water-holding capacity and textural traits of meat. The use of dietary antioxidants has a distinct advantage over incorporation of antioxidants to meat through processing because dietary antioxidants absorbed by the bird can be effectively distributed in muscle (meat) both inside the cell and at the membrane, which is not possible if the antioxidants are incorporated into meat through blending and mixing. Several feeding studies have clearly demonstrated this difference and shown remarkable protection of some key quality parameters of meat by antioxidants formulated in animal feed. Vitamin E (α-tocopherol) is perhaps the best researched dietary antioxidant. It is an essential nutrient for the growth and health for animal species by functioning as an antioxidant in various biological systems. α-Tocopherol neutralizes free radicals slowing the propagation of lipid oxidation of the highly unsaturated fatty acids in the cellular and subcellular membranes (Burton and Traber, 1990). The molecular structure of α-tocopherol allows it to protect highly oxidizable PUFA from peroxidation by reactive oxygen species (ROS) produced by adjacent membrane-bound enzymes through radical delocalization. Aside from tocopherols, many other nutrients have been shown to enhance the quality of meat. Examples are magnesium, selenium,

vitamin C, vitamin A, creatine, and conjugated linoleic acids. Kietzmann and Jablonski (1985) reported that incorporation of magnesium into swine diets lowered plasma cortisol and catecholamine concentrations during stress. Swigert et al. (2004) found that magnesium supplemented diets improved pork quality. Pigs supplemented with magnesium produced lower purge loss, and a higher ultimate pH, potentially due to the lowering of stress induced hormones. Hamilton et al. (2003) also reported that supplementation with magnesium lowered Minolta *L** values, indicating darker muscle color, increased pH, and had lower drip loss compared to control treatments.

Antioxidants can potentially promote meat tenderness as well. Tenderness and associated juiciness (water-holding capacity) are important quality parameters of meat as well. Increased tenderness is attributed to postmortem proteolysis of key cytoskeletal proteins which is mediated by the calcium activated calpain enzyme system (calpain). Variations in muscle tenderness may be attributed to the activity rate of calpastatin, an enzyme that inhibits calpain. Studies have shown that calpain is remarkably susceptible to endogenous oxidants, such as hydrogen peroxide (Guttmann et al., 1997). When exposed to an oxidizing environment, m-calpain was readily inactivated, resulting in a reduced tenderness and decreased water-holding capacity of post-mortem beef muscle (Rowe et al., 2004). Although chicken dark meat (thigh; drumsticks) does not have a perceived toughness problem, the texture of chicken light meat (breast) is often considered less than desirable by many consumers. It is not clear whether dietary antioxidants could protect calpain, thereby ensuring normal proteolysis during post-mortem storage needed for desirable tenderness. Assuming dietary antioxidants can

promote the activity of muscle endogenous proteases, the meat flavor would improve as well because products of proteolysis (short peptides, amino acids) impart savory tastes.

2.2.1 Selenium. Selenium can be found within all cells and tissue of an animal and is an essential micronutrient for various metabolic functions, such as reproduction and thyroid hormone metabolism and cellular immune response. Selenium deficiency is the cause of various degenerative diseases in animals; most notable are skeletal and cardiac myopathies, and liver necrosis. Thus, mineral supplements are often incorporated into animal feedstuff to maintain optimal health of the animal. The extent to which selenium is absorbed from the gastrointestinal tract and its retention and distribution within the body varies with the species, chemical form, and amount ingested. Organic forms of selenium, such as selenocysteine and selenomethionine are more effectively absorbed than inorganic compounds like selenites or selenates (Kinal et al., 2012). It is a result of active transport of selenium amino acid complexes through epithelial cells of intestine compared with passive diffusion of selenates' or selenites' ions (Sunde, 1997). Also, dietary levels of selenium directly influence muscle selenium concentrations. Various studies have shown an increase in tissue Se levels upon increasing levels of supplementation (Qin et al., 2007). In animal feeds, naturally occurring selenium is primarily found as selenoamino acids, with selenomethionine (SeMet) compromising more than 50 % of total Se in many feed ingredients, (Schrauzer, 2003).

Selenocysteine is the form of Se present in selenoenzymes such as glutathione peroxidase (GsPx). SeMet can be incorporated into proteins in place of methionine, or be reformed to selenocysteine. Dietary methionine levels will affect the extent to which

SeMet is incorporated into general proteins (Butler *et al.,* 1989). GsPx can be found in different parts of the cell: cytosol, plasma or phospholipid, which is membrane bound (Daniels, 1996). As one of the primary antioxidant enzymes in mammals, GsPx protects cells and tissues against free radical damage and apoptosis. Four selenium atoms are covalently bound to cysteine residues in the enzyme glutathione peroxidase, which could potentially work synergistically with vitamin E. Glutathione peroxidase is an enzyme that catalyzes the reduction of hydrogen peroxide and lipid peroxides, thereby preventing oxidative damage. In humans, adequate intake of Se may decrease the risk of cancer, cardiovascular disease, and other immunodeficiencies (Hartikainen, 2005). Daun and Akesson (2004) noted an excellent correlation between glutathione peroxidase activity and Se content in tissues of cattle, pigs, and poultry. O'Grady et al. (2001) also reported an increase in glutathione peroxidase activity with increasing Se levels. Skrivanova et al. (2007) discovered an increase in oxidative stability in veal meat, along with increased glutathione peroxidase activity, when the animal was fed a Se-supplemented diet. Similarly, Benedetti et al. (2012) reported that rats fed high selenium bread had higher liver GsPx activity and lower hepatic MDA content after exposure to oxidative stress, attributed to the higher selenium intake.

2.2.2 Vitamin E. Vitamin E is the standard term used to describe at least eight naturally occurring compounds that exhibit the biological activity of α-tocopherols (Jensen and Lauridsen, 2007) This group comprises α β, γ, δ - tocopherol and tocotrienol. Vitamin E is a vital component of biological membranes with membrane-stabilizing properties and potent antioxidant activity. Furthermore, muscle health is dependent upon

an adequate supply of dietary vitamin E and although rare, vitamin E deficiency in humans is associated with muscle weakness, elevated creatine kinase (CK), and myopathy (Howard et al., 2011). In animals, early studies have reported profuse myocyte necrosis and lethal muscular dystrophy due to vitamin E deficiency.

There are two general mechanisms by which vitamin E could interact with cells or molecules to promote muscle health, (1) stabilizer or; (2) antioxidant. Vitamin E can act as a stabilizer in that the hydrophobic, phytal tail is anchored into the lipid bilayer and the chromonal ring lies at the membrane-water interface (Howard et al., 2011). Furthermore, the integration of vitamin E into the cell membrane may alter the bilayer physical properties potentially through fluidity (Neunert et al., 2010). The major biological evidence for a 'stabilizing' role is that erythrocyte lysis, induced by oxidative stress and other stressors is prevented by vitamin E supplementation and exacerbated by vitamin E depletion (Ahmad and Suhail, 2002). Moreover, the major symptom of vitamin E deficiency is hemolytic anemia. Often time premature newborns are commonly supplemented with vitamin E to stabilize their red blood cells and prevent hemolytic anemia. The second proposed mechanism of action for vitamin E is as a potent antioxidant. The chromanol head of vitamin E, located within the hydrophilic portion of the bilayer quenches free radicals and prevents potentially harmful phospholipid oxidation events. During strenuous exercise, skeletal muscle accumulates reactive oxygen species (ROS) and consequently increases lipid peroxidation, which can be alleviated through vitamin E supplementation (Sacheck et al., 2003). Interestingly, Howard et al. (2011) reported that vitamin E promotes plasma membrane repair of myoblasts. Thus,

vitamin E supplementation is not only required for homeostasis, but also may reduce oxidative deterioration of meat during processing and storage.

Vitamin E cannot be synthesized by animals and has to be supplied by the diet, thus its presence in body tissues is a reflection of dietary availability. Dietary vitamin E is commonly supplemented in the diet as α-tocopherol acetate, which is characterized by great stability during storage, feed processing, and passage through the forestomach of the animal. With dietary supplementation, the vitamin is properly physiologically incorporated within biomembranes, where its effect is maximal, making it a more effective source than when adding vitamin E as a postmortem supplement (Mitsumoto, 2000). Despite the possible influence of α-tocopherol on meat quality characteristics such as water retention and cholesterol oxidation, most studies have primarily concentrated on its effects on lipid oxidation and color stability (ref). Early studies have demonstrated delayed lipid oxidation and color deterioration of beef from cattle fed vitamin E supplementation diets (Morrissey et al., 2000). Ripoll et al. (2013) reported that dietary vitamin E supplemented lamb showed delayed metmyoglobin formation during storage compared to unsupplemented lamb and that the length of the finishing period feeding (supplemented with vitamin E) directly influenced muscle vitamin E content. Incorporation of α-tocopherol into pork patties through processing also showed some protective effect (Chen et al., 2008); however, the effect was not nearly as much as that through dietary method. Gao et al. (2010) also reported that feeding high levels of α-tocopherol lowered thiobarbituric acid-reactive substances production in the tissue and plasma of oxidatively stressed broilers. Recently, inclusion of recycled vegetable oils, highly oxidized, has provided animal meat producers an economically feasible means of

increasing the energy density of their livestock (Boler et al., 2012). However, adverse effects on overall meat quality can result from animals consuming diets composed of supplemented fat, typically high in polyunsaturated fatty acids (Gatlin et al., 2002). To alleviate the potential of greater susceptibility to lipid and protein oxidation, producers are adding antioxidants, particularly vitamin E, to feedstuff. Boler et al. (2012) reported that dietary vitamin E supplementation partially ameliorated the negative effects of feeding oxidized fat through the reduction of protein oxidation. Similarly, Xiao et al. (2011) and Li et al (2013) reported decreased lipid and protein oxidation, and improved tenderness, respectively, in chickens fed a vitamin E supplemented diet. Although, much research has been conducted evaluating the effects of dietary vitamin E and meat quality; the effects of other antioxidant-supplemented diets are much less known but should be investigated.

2.2.3 Algae. Fruits and vegetables contain various antioxidant and bioactive compounds, such as polyphenols, carotenoids, and isoprenoids that are beneficial to human health. In humans and animals, reactive oxygen species (ROS) are formed during metabolism and can cause oxidative stress. Under homeostatic circumstances, the antioxidant defense system efficiently neutralizes ROS, but in a challenged state the host's defense system will become compromised resulting in oxidative stress (Yeum et al., 2004). Consumption of products high in antioxidant and bioactive compounds has been reported to alleviate cellular stress (Cornish and Garbary, 2010; Halliwell and Gutteridge, 2007). Various studies have reported the health-promoting benefit of consuming fruits and vegetables (Escudero-Lopez, et al., 2013; Park et al., 2003).

Similarly, some marine organisms, such as seaweeds and algae, possess strong antioxidant activity, such as high potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH) radical scavenging activities and strong reducing ability (Cho et al. 2011). Simiarly, Sachrinda et al. (2007) reported high radical scavenging activity and singlet oxygen quenching activities of marine carotenoid and fucoxanthins. Takamatsu et al. (2003) stated that algae had similar chlorophyll, carotenoid, tocopherol derivatives and isopreoid compounds that were structurally similar to plant-derived antioxidants. The *in vivo* efficacy of algae-derived antioxidant activity was demonstrated in mice where topical and dietary treatment with polyphenols extracted from brown algae decreased UVB-induced skin tumor development (Hwang et al., 2006).

Several studies have reported that increased consumption of long chain fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can significantly improve cardiovascular health by helping maintain normal serum triacylglycerol levels and normal blood platelet reactivity (Breslow, 2006; Park and Harris, 2002). Highly unsaturated fatty acids such as DHA and EPA are intermittent in modern and specifically in western diets. Currently, the level of DHA found in animal products such as eggs, beef, pork, and chicken is minimal (Givens et al., 2006). Humans cannot efficiently synthesize DHA and EPA and thus must obtain these vital fatty acids from dietary sources. Although fish and algae is a primary dietary source of DHA and EPA, the consumption in the United States and in other parts of the world is low. A primary source of EPA and DHA are fish oil or flaxseed oil supplements, however, due to the threat of over-fishing and an enforcement of sustainable fishing practices alternative sources of DHA and EPA must be investigated. Therefore, there has been a recent push to

supplement algae in dietary animal feed to enrich eggs, fish, and possibly meat with DHA and EPA (Woods and Fearon, 2009). Fredriksson et al. (2006) reported that inclusion of up to 20% algae in the diet of laying hens significantly increased the amount of EPA and DHA and total carotenoid content in the egg yolk. Much of the fatty acid profile of the meat from monogastrics is greatly influenced by their diet. For instance, Min et al. (2012) reported that feeding broiler chickens up to 15% distillers dried grains with solubles (DDGS) increased the PUFA to saturated fatty acid ratio of meat as well as cooking loss and shear force. Thus, the incorporation of algae into an animal may increase the EPA and DHA content of the meat, but may also change the fatty acid and flavor profile. For example, meat from lamb fed a fish oil/algae diet had significantly higher levels of EPA and DHA, along with more volatile compounds, which was scored least favorable in a trained sensory panel compared to the control (Elmore et al., 2005).

2.3. Oxidative Processes

2.3.1. Mechanism of Lipid Oxidation. Lipid oxidation is a free radical chain reaction that occurs in three main steps: initiation, propagation, and termination. Initiation ensues when a radical or non-radical species abstracts a labile hydrogen atom from a methylene group of a lipid (LH) to form a lipid radical ($L^{\cdot}$). The abstraction of hydrogen atoms from fatty acid chains results in an unstable carbon radical, commonly known as an alkyl radical, which is stabilized through delocalization over the double bonds resulting in double bond shifting (Min and Ahn, 2005). Depending on the level of molecular oxygen present within a system the formed fatty acid radical can undergo various rearrangements. In the presence of oxygen, peroxyl radicals ($LOO^{\cdot}$) are primarily

generated, while under very low oxygen conditions, $L^{\cdot}$ can react with other molecules such as proteins or other lipids. During propagation $LOO^{\cdot}$ will abstract a hydrogen atom from neighboring lipids or fatty acids to form a hydroperoxide (LOOH) and a new lipid radical ($L^{\cdot}$). In food, hydroperoxides (ROOH) may be responsible for the development of off-flavors or for further reactions with other constituents such as proteins. Furthermore, formed hydroperoxides may undergo scission to form additional products including ketones, aldehydes, organic acids, and hydrocarbons. Some of these products retain a double bond, which, because of the preceding bond rearrangement, makes them highly reactive α, β unsaturated aldehydes (so called "-enals") (McIntyre and Hazan, 2010). These electrophilic species readily covalently modify nucleophilic groups on target proteins and they extensively derivatize reduced glutathione, thereby decreasing cellular antioxidant protection (McIntyre and Hazan, 2010). Lipid oxidation is terminated through the binding of two radical species to form a non-radical product. In the presence of oxygen, the predominant free radical is the peroxyl radical since oxygen will be added onto alkyl radicals at diffusion-limited rates (Fennema, 2008). Under atmospheric conditions termination of lipid oxidation may occur between peroxyl and alkoxyl radicals. In low oxygen environments, such as frying oils, termination reactions can occur between alkyl radicals to form fatty acid dimers. Furthermore, lipid oxidation products can yield polymers, which usually occur during high heating. The equation below summarizes the reactions of lipid oxidation.

$$\text{Initiation: } LH + O_2^{1} \rightarrow L^{\cdot}$$

$$\text{Propagation: } L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$$

$$\text{Termination: } LOO^{\cdot} + L^{\cdot} \rightarrow \text{Non-radical product}$$

2.3.2. Methods to Measure Lipid Oxidation. The acceptability of a food product depends on the extent to which deterioration has occurred. Researchers are also interested in determining the effects of certain processes or antioxidants on the stability of a product. Thus some criterion for assessing the extent of oxidation is required. Sensory analysis is one of the most sensitive techniques that provides data pertaining to practical applications, but is not useful for routine analyses and generally lacks reproducibility. Consequently, many chemical and physical methods have been developed to quantify oxidative deterioration with food products that correlate with off-flavor development. In food products abstraction reactions and rearrangements of alkoxyl and peroxyl result in the production of endoperoxides and epoxides as secondary products (Pike, 2003). Various methods have been developed to measure different compounds that form or degrade throughout lipid oxidative processes. Peroxide value, *p*-anisidine value, iodine value, volatile organic compounds, thiobarbituric acids, and conjugated dienes and trienes are common methods to assess lipid oxidation in food.

Peroxide value is one of the oldest and most commonly used methods to test for oxidative rancidity. It measures the concentration of peroxides and hydroperoxides formed during the initial stages of lipid oxidation and is defined as the milliequivalents (mEq) of peroxides per kilogram of sample. Peroxide value is measured through the addition of potassium iodide and lipids, where excess potassium iodide reacts with peroxides and the iodine is liberated. Titration with standardized sodium thiosulfate, will yield a colorless solution once all the excess iodine reacts with sodium molecules. The equation below summarizes the reaction of peroxide value.

$$ROOH + K^{+}I^{-} \rightarrow ROH + K^{+}OH^{-} + I_2$$

$$I_2 + \text{starch} + 2Na_2S_2O_3 \rightarrow 2NaI + \text{starch} + Na_2S_4O_6$$

blue colorless

Double bonds in lipids are changed from nonconjugated to conjugated bonds upon oxidation. Conjugated dienes give rise to an absorption peak at 230–235 nm, while conjugate trienes are measured at 270 nm. Ultraviolet detection of conjugated dienes is simple, fast, and useful for monitoring the early stages of oxidation. However, this method is less specific and sensitive compared to other methods, and the results may be affected by the presence of compounds absorbing in the same region, such as carotenoids (Shahidi and Zhong, 2005).

Primary oxidation products, such as hydroperoxides, are unstable and susceptible to decomposition. The decomposition of primary lipid oxidation products can give rise tp secondary products including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds (Shahidi and Zhong, 2005). Thiobarbituric acid reactive substances (TBARS) measures malondialdehyde, a compound formed during the degradation of polyunsaturated fatty acids. Malondialdehyde reacts with thiobarbituric acid to form a colored complex that can be measured spectrophotometrically.

The *p*-anisidine value (*p*-AnV) method estimates the amount of α- and β-unsaturated aldehydes (mainly 2-alkenes and 2,4-dienals), generated during the decomposition of hydroperoxides (Pike, 2003). The reaction with *p*-anisidine reagent with aldehydes, under acidic conditions forms yellowish products that absorb at 350 nm.

Finally, carbonyl compounds, including aldehydes and ketones, are major contributors to off-flavors and odors associated with rancidity. Volatile compounds can be measured using gas chromatography, while total carbonyls can be measured using a colorimetric assay with 2,4-dinitrophenylhydrazine (DNPH). DNPH reacts with carbonyl compounds to form a yellow hydrazine. Because food is a dynamic system, two or more methods should be used to draw a more complete picture.

2.3.3 Impact of Lipid Oxidation on Meat Quality. Muscle foods are prone to lipid oxidation since they contain both unsaturated lipids and pro-oxidant components. In meat, lipids are present as either intermuscular or intramuscular fat. Intermuscular fat is generally stored in specialized connective tissues as a large deposit, while intramuscular fat is integrated into the tissue and widely dispersed (Love and Pearson, 1971). Of the muscle lipid fractions, the polar phospholipids contain the highest proportion of unsaturated fatty acids, which is primarily responsible for lipid oxidation in muscle foods. Lipid oxidation is a major cause of quality deterioration in meat and meat products. Undesirable changes in color, flavor, and nutritional value occurs as lipids, present in meat, oxidize and interact with other constituents, such as pigments, proteins, carbohydrates and vitamins. Pigment and lipid oxidation are interrelated, and ferric hemes are believed to promote lipid oxidation (Faustman et al., 2010). Iron and ascorbic acid may also function as prooxidants in meat. Sodium chloride accelerates oxidation of the triglycerides, although the mechanism of salt catalysis is not completely known. Cooked meat undergoes rapid deterioration due to tissue lipid oxidation. Refrigerated and frozen fresh meats are also susceptible to lipid and protein oxidation, which causes

quality losses due to 'freezer-burn.' Protein denaturation and cross-linking may result from lipid oxidation in stored freeze-dried meat. With increased consumption of prepackaged raw meat and precooked convenience meat items, control of oxidation has become increasingly important. Antioxidants, such as vitamin E, and chelating agents, such as phosphates, are the most effective inhibitors of lipid oxidation (Mitsumoto, 2000).

2.3.4. Mechanism of Protein Oxidation. In the ground state, molecular oxygen contains two, highly reactive unpaired electrons. Formation of free radical intermediates occurs in oxidation reactions involving oxygen in the ground state (Fridovich, 1972). Of primary concern are the effects of reactive oxygen species (ROS) and reactive substances (RS), which are capable of oxidizing cellular proteins, lipids, and nucleic acids, resulting in cellular membrane instability. ROS and RS may be generated by a wide variety of physiological and non-physiological processes, such as the formation of hydrogen peroxide by endogenous oxidases, conversion of H_2O_2 to $OH^{\bullet}$ by metal catalyzed oxidation systems, formation of alkyl radicals, peroxides, and aldehydes during lipid peroxidation, among others (Berlett and Stadtman, 1997). In live tissue, aerobic cells are able to defend themselves against ROS damage through various enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase (Fridovich, 1972). However, over time the accumulation of oxidatively modified proteins may lead to a number of physiological disorders and diseases. The sites of free radical attack occur at the amino acid side chains and peptide backbone on proteins, which lead to protein polymerization and fragmentation.

All amino acid residues of proteins are theoretically subject to attack by free radicals and nonradical ROS. However, some amino acids such as cysteine and methionine are particularly sensitive to oxidation by almost all forms of ROS. Under mild conditions, cysteine residues are converted to disulfides and methionine residues are converted to methionine sulfoxide (MeSOX) residues (Berlett and Stadtman, 1997). Aromatic amino acid residues are also among the preferred targets for ROS attack. Oxidative cleavage of proteins by the α-amidation pathway or by oxidation of glutamyl side chains leads to the formation of protein carbonyl derivatives (Stadtman and Berlett, 1997).

Protein carbonyls are highly reactive groups, whether they were derived from proteins or originate from a non-protein source. Carbonyl groups from oxidized proteins will react with an electron dense protein molecule (i.e. the free amino group of a lysine residue) forming a covalent cross-linkage (Feeney et al., 1975). In oxidized muscle, the carbonyl-amino cross-linking (Schiff base) between protein molecules causes polymerization and aggregation that can be non-dissociable. Mild or complete unfolding of protein molecules due to oxidation can increase the exposure of nonpolar residues, leading to hydrophobic association of proteins. Peptide bond cleavage may also occur through ROS attack on the side chains of glutamic acid and aspartic acid residues (Garrison, 1987). The abstraction of a hydrogen atom from side chain carbon atoms of both amino acid residues leads to the formation of peptide fragments.

2.3.5. Methods to Measure Protein Oxidation. Oxidative reactions results in various modifications of the protein, such as carbonyl formation and amino acid

alterations. The colorimetric DNPH assay is a universal technique applied when measuring protein oxidation in muscle food systems. In this method, DNPH reacts with protein carbonyls to generate hydrazones and the absorbance is read at 370 nm (Levine et al., 1990).

All amino acids are potential targets of oxidative modifications; however, sulfur-containing and aromatic amino acid residues are particularly sensitive to oxidation, which can be measured through a variety of methods. For instance, cysteine residues (i.e. protein thiols) can cross-link, forming disulfide bonds. These changes can be assessed using gel electrophoresis with and without the addition of a reducing agent, such as β-mercaptoethanol, and Ellman's reagent (5,5'-dithiolbis-(2-nitrobenzoic acid): **DTNB**). Adding a reducing agent to the sample and seeing a recovery in band width and intensity is indicative of disulfide bond formation. DTNB is used o quantify the number or concentration of thiol groups present in a sample. Thiols react with DTNB and at neutral or alkaline pH will ionize to form the yellow compound, NTB^{2-}. Losses in tryptophan residues are measured fluorescently, where decreases of tryptophan are determined by the emission spectra recorded from 300 to 400 nm at the excitation wavelength of 283 nm (Lund et al., 2011). Tyrosine residues are susceptible to dimerization linked by 1,3-dityrosine, a highly fluorescent molecule that is resistant to acid hydrolysis and protease activity (Saeed et al., 2006). Dityrosine is measured by fluorescent measurements at 325 nm excitation and 400 nm emission or reversed phase high pressure liquid chromatography (**RP-HPLC**). HPLC attached to a fluorescence detector is the general method used for the analysis of amino acids and formation of dityrosine. For instance, tyrosine peaks will elute at 25 min, while dityrosine elutes at 31 min (Saeed et al., 2006).

Changes to protein molecular structure due to oxidation can be investigated by multidimensional nuclear magnetic resonance (**NMR**) spectroscopy, electron spin resonance (**ESR**), and fourier transform infrared spectroscopy (**FTIR**). ESR can analyze materials with unpaired electrons, such as radicals, while NMR and FTIR can be utilized to study conformational changes in oxidized proteins. NMR is used to assess chemical modification of proteins at an atomic level, while FTIR can pinpoint specific chemical modifications and help assess their impact on the global secondary structure of a protein.

2.3.6. Impact of Protein Oxidation on Meat Quality. Oxidation is the leading cause of quality deterioration in muscle foods due to the formation of rancid odor, off flavors, and off color. Fresh and processed muscle foods are susceptible to oxidative processes due to the high concentrations of heme pigments, unsaturated lipids, metal catalysts, and various oxidizing agents. These agents may function as a precursor or catalyst for the production of reactive oxygen species and reactive substances.

Myosin molecules are made up of approximately 4500 amino acid residues and 40 residues are cysteine (Strehler et al., 1986). When exposed to oxidative conditions, as much as one-third of free sulfhydryls are lost (Srinivasan and Xiong 1996; Wang et al., 1997). Ooizumi and Xiong (2004) investigated the impact of hydroxyl radicals on the conformational and biochemical characteristics of chicken myofibrillar proteins. Exposure to hydroxyl radicals led to progressive aggregation inside and between myosin molecules. Extreme cross-linking may be the cause of reduced tenderness in meat products. Kim et al. (2010) reported a decrease in tenderness and juiciness in beef steaks

packaged in high-oxygen MAP, which resulted from protein oxidation and polymerization of intermolecular cross-links between myosin.

Amino acids with reactive side chains are susceptible to lipid radicals and their by-products. Lipid oxidation deteriorates food quality and alters protein functionality because it affects different components such as lipids, proteins, vitamins, and other compounds. Proteins may react with oxidized lipids through two different mechanisms: (1) free radicals produced by cleavage of hydroperoxides react with proteins forming protein free radicals and subsequent by-products; (2) secondary products of lipid oxidation (i.e. aldehydes, ketones) react with ε-amino groups forming protein aggregates (Tironi et al., 2002). Malondialdehyde (MDA) is a major secondary product formed from lipid oxidation. MDA is able to react with NH_2 groups of proteins, nucleic acids, and phospholipids. The formation of covalent bonds cause these molecules to cross-link, thus inactivating and modifying their physiochemical properties (Aubourg 1993). However, oxidative attacks of amino acids in muscle tissue may occur independently of lipids. Heme iron (Fe^{2+}) is proposed to complex with a metal binding site on the protein which reacts with H_2O_2 to generate oxygen species that attack side chains of amino acid residues (Stadtman and Oliver, 1991). Uchida et al. (1992) reported the polymerization of collagen under copper- and iron- H_2O_2 oxidizing systems. Furthermore, metal-catalyzed oxidation results in the loss of enzyme activity and increase in carbonyl groups (Levine et al., 1990).

Formation of protein aggregates through covalent and noncovalent forces are one of the major consequences of protein oxidation by ROS and other oxidizing agents. The conversion of amino acid residues, such as histidine, into carbonyl derivatives, and the

formation of disulfide linkages reduce the functionality of proteins (Xiong and Decker, 1995). Liu et al. (2000) reported an increase in carbonyls, amines, disulfide bonds, myosin polymerization and a decrease in thermal stability and gel forming ability in myofibrils oxidized with $FeCl_3/H_2O_2$/ascorbate. Whether from red meat, poultry, or fish species, previous studies have shown that under oxidative conditions, all muscle proteins are susceptible to oxidative damage caused by lipid oxidation byproducts, metal ions, and other prooxidants indigenous to muscle or generated during meat processing (Decker et al., 1993; Wan et al., 1993).

CHAPTER 3

DIETARY ANTIOXIDANT SUPPLEMENTATION ENHANCES LIPID AND PROTEIN OXIDATIVE STABILITY OF CHICKEN MEAT THROUGH PROMOTION OF ANTIOXIDANT ENZYME ACTIVITY

3.1. Summary

Recent nutrigenomic studies have shown that animal nutrition can have a major influence on tissue gene expression. Dietary antioxidant supplements can enhance the quality of meat through modification of tissue metabolic processes. This study investigated the influence of dietary antioxidants and quality of oil on the oxidative and enzymatic properties of chicken broiler breast meat stored in an oxygen-enriched package (HiOx: 80% O_2/20% CO_2) in comparison with air-permeable polyvinylchloride (PVC) or skin (SK) packaging systems during retail display at 2–4 °C for up to 21 d. Broilers were fed a diet either with a low-oxidized oil (peroxide value POV 23 meq O_2/kg) or with a high-oxidized oil (POV 121 meq O_2/kg), supplemented with an antioxidant pack (200 ppm EconomasE and organic minerals Se, Zn, Cu, Mn, and Fe as in Bioplex) in substitution for vitamin E and inorganic minerals for 42 d. Lipid and protein oxidation and tissue enzymatic activity were analyzed. In all packaging systems, lipid oxidation (TBARS) was inhibited by up to 32.5% ($P < 0.05$) with an antioxidant-supplemented diet when compared to diets without antioxidants, particularly in the HiOx and PVC systems. Protein sulfhydryls were significantly protected by antioxidant diets, e.g., by 14.6% and 17.8% for low-and high-oxidized dietary groups, respectively, in PVC d 7 samples. Glutathione peroxidase, catalase, and superoxide dismutase activities were significantly higher ($P < 0.05$) in antioxidant-supplemented diets compared with the basal diet,

regardless of oil quality. Also, serum carbonyls were lower ($P < 0.05$) in broilers fed a low-oxidized antioxidant-supplemented treatment. The results demonstrate that dietary antioxidants can minimize the oxidative instability of proteins and lipids, and the protection may be linked to improved cellular antioxidant enzymatic activity.

3.2. Introduction

Oxidation is a result of natural metabolic processes but excessive formation of reactive species, such as free radicals, can damage to important biomolecules (i.e., lipids, proteins, and nucleic acids) in the body of humans and animals alike. The rate of oxidation increases in result to the following: (1) high intake of oxidized lipids and pro-oxidants; (2) deterioration of sensitive polyunsaturated fatty acids (**PUFAs**); and (3) low intake of antioxidative nutrients (Morrissey et al., 1998; Smet et al., 2008). In muscle foods, oxidative reactions continue post-mortem and are a leading cause of quality deterioration during processing and storage. With a relatively high proportion of PUFAs, poultry meat is more susceptible to oxidative processes, specifically lipid oxidation, than beef or pork. Therefore, incorporation of dietary antioxidants, such as vitamin E and selenium (**Se**) in poultry feed, has been implemented to achieve optimal growth performance, reproduction, and meat quality.

A major challenge in broiler production is the expense of in raising birds, where 60–75% of the total incurred cost is in feed alone (Tahir and Pesti, 2012). In recent years, the rising cost of raw materials and energy has driven up the prices of vitamin E and animal feedstuff, forcing producers to find less expensive alternative dietary sources. Often for economical poultry rearing, highly oxidized, recycled vegetable oils (e.g., reused frying oils) are often used as an added fat source in poultry feed to increase the energy density (Tavárez et al., 2011). Xiao et al. (2011) reported that EconomasE (**EcoE**), an algae-based antioxidant containing Se yeast, reduced the amount of vitamin E required in broiler feed without compromising growth performance, overall health, or meat quality. Unfortunately, vegetable oils rich in PUFAs are highly susceptible to

oxidative deterioration. The products of lipid oxidation can decrease the nutrient content of the feed by reacting with proteins, lipids, and fat-soluble vitamins, which may even form toxic products that can adversely affect broiler performance and health (Engberg et al., 1996). Crespo and Garcia (2002) reported that broilers deposit PUFAs into their tissues similar to the rates present in their diet, and Eder et al. (2003) showed that dietary thermoxidized oils suppressed gene expression of lipogenic enzymes in rats. Hence, utilizing oxidized oil in broiler feed may result in decreased shelf-life and quality consistency of meat due to the potential suppression of gene expression for antioxidant enzymes.

Recently, nutrigenomic studies coupled with proteomic investigations have indicated a potential link between dietary nutrients and the expression of specific enzymes and metabolites in muscle (Hesketh, 2008). Li et al. (2009) reported that dietary supplementation with α-tocopherol improved meat tenderness and reduced lipid oxidation in broiler breast and thigh meat. However, the influence of dietary antioxidants on the genetic and regulatory mechanisms which define metabolic and physiological changes in muscle tissue is complex and poorly understood. The present study was designed to assess the influence of dietary EcoE/Se/ organic mineral-based antioxidants and quality of oil on the oxidative and enzymatic properties of chicken broiler breast meat. To relate the study to *in situ* situations, harvested meat was packaged and stored in an oxygen-enriched (**HiOx**: 80% O_2/20% CO_2), air-permeable polyvinylchloride (**PVC**), or skin (**SK**) packaging systems during retail display at 2–4 °C for up to 21 d.

3.3. Material and Methods

3.3.1. Materials

A commercial algae-based antioxidant pack containing Se yeast as in EcoE and organic minerals as in Bioplex, was supplied by Alltech Inc. (Nicholasville, KY). Soybean oil was acquired from a local retailer, and the initial peroxide value (POV), determined according to AOCS (2007), was 23 meq O_2/kg. To create oxidized oil, aluminum pans (41×13×4 cm) each containing 5 kg of the above oil were heated in a convection oven at 95 °C ± 5 °C for up to 7 d. The POV of the oxidized oil was monitored intermittently. When the POV reached the target level (120 meq O_2/kg), heating was discontinued and the oxidized oil was cooled to room temperature. The actual POV of the final cooled oil was 121 meq O_2/kg and it was used immediately for diet preparation. All chemicals (reagent grade) were purchased from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

3.3.2. Broiler Production

All procedures used in the study herein were approved by the University of Kentucky Animal Care and Use Committee. Three independent feeding trials (n = 3) over a two-year period were performed. In each, 960 male broilers were raised from 1 to 42 d of age and randomly placed in 48 floor pens with 20 birds per pen. Each pen was randomly designated one of four dietary treatments consisting of feeding: (1) basal diet–low oxidized oil (**LO**); (2) basal diet–low oxidized oil, supplemented with antioxidants (**ALO**); (3) basal diet–high oxidized oil (**HO**); (4) basal diet–high oxidized oil, supplemented with antioxidants (**AHO**). Broilers were randomly distributed into the 4

dietary groups with 12 replicate pens for each diet. Each pen was equipped with a feeder, a nipple drinker line and a litter of soft wood shavings. Birds consumed feed in mash form and water on an *ad libitum* basis. A starter diet containing 22% crude protein (CP) and 3,120 kcal/kg was fed from 0–21 d of age and a grower diet containing 20% CP and 3,150 kcal/kg was fed from 21–42 d of age (Table 3.1 and Table 3.2). Photoperiod consisted of 23 h of light and 2 h of dark throughout the experiment.

3.3.3. Meat Preparation, Packaging, and Storage

After 42 d of feeding, one broiler from each of the 48 pens (4 diets × 12 pens) was randomly selected, humanely harvested, de-feathered, then chilled in ice slurries for 1.5 h. Both sides of the breast (*Pectoralis major*) were then removed and skinned. Per diet, one randomly selected broiler breast was placed in a Cryovac black processor tray, CS977 (22×17×4 cm; Sealed Air Corporation, Elmwood Park, NJ) and sealed with Cryovac Lidstock 1050 MAP ethylene vinyl alcohol film (1.0 mil, < 20 cc/m^2/24 h oxygen transmission rate at 4.4 °C using an InPack Junior A10 packaging machine (Ross Industries Inc., Midland, VA). A gas mixture of 80% O_2/20% CO_2 (Scott-Gross Company Inc., Lexington, KY) was used for the HiOx packaging. For PVC, one breast per diet was placed on #2 supermarket white polystyrene trays (20.8×14.5×2.3 cm in dimension; Pactive LLC; Lake Forest, IL) and overwrapped with an air-permeable polyvinylchloride film (15,500–16,275 cm^3/m^2/24 h oxygen transmission rate at 23 °C; E-Z Wrap Crystal Clear PVC Wrap, Koch Supplies, North Kansas City, MO). For SK, broiler breasts were packaged using Cryovac black processor trays and sealed with a

Table 3.1. Composition of the experimental diets

Nutrient	LO		ALO		HO		AHO	
	Starter	Grower	Starter	Grower	Starter	Grower	Starter	Grower
ME, kcal/kg	3120	3150	3120	3150	3120	3150	3120	3150
CP, %	22	20	22	20	22	20	22	20
Lysine, %	1.24	1.11	1.24	1.11	1.24	1.11	1.24	1.11
TSAA, %	0.90	0.72	0.90	0.72	0.90	0.72	0.90	0.72
AvP, %	0.45	0.41	0.45	0.41	0.45	0.41	0.45	0.41
Ca, %	1.00	0.90	1.00	0.90	1.00	0.90	1.00	0.90
Vitamin E, IU/kg	50	50	10	10	50	50	10	10
[1]Se, ppm	0.30	0.30	–	–	0.30	0.30	–	–
[2]Zn, ppm	100	100	25	25	100	100	25	25
[2]Cu, ppm	125	125	31	31	125	125	31	31
[2]Mn, ppm	90	90	23	23	90	90	23	23
[2]Fe, ppm	80	80	20	20	80	80	20	20
[3]EconomaseE, ppm	–	–	200	200	–	–	200	200
Soybean oil, %, LO	4.4	3.4	4.4	3.4	–	–	–	–
Soybean oil, %, HO	–	–	–	–	4.4	3.4	4.4	3.4

[1]As in Selenite.
[2]As inorganic minerals for Diets LO and HO; as in Bioplex for Diets ALO and AHO.
[3]A tocopherol-Se based antioxidant blend.

Table 3.2. Ingredient and nutrient composition (as-fed basis) of the basal diet

Item	Starter diet (1–21d)	Grower diet (22–42d)
Ingredient	% of diet	% of diet
Corn	53.41	61.26
Soybean meal (48%CP)	38.00	31.40
Soybean oil (low or high oxidized)	4.40	3.40
Salt	0.45	0.45
Limestone	1.33	1.30
Dicalcium phosphate	1.76	1.54
DL-Methionine	0.15	0.15
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
Nutrient (calculated values)		
AME_n, kcal/kg	3.12	3.15
Protein, %	22.00	20.00
Ca, %	1.00	0.90
Nonphytate P, %	0.45	0.41
TSAA, %	0.90	0.72
Lysine, %	1.24	1.11

[1]Supplied per kg diet for all diets: 11,025 IU of vitamin A (retinyl acetate), 0.0882 mg of vitamin D_3 (cholecalciferol), 0.91 mg of vitamin K_3 (2-methyl-1, 4-naphthoquinone), 2 mg of thiamin, 8 mg of riboflavin, 55 mg of niacin, 18 mg of Ca pantothenate, 5 mg of vitamin B_6 (pyridoxines), 0.221 mg of biotin, 1 mg of folic acid, 478 mg of choline, 28 µg of vitamin B_{12} (cyanocobalamin).Vitamin E (DL-α-tocopheryl acetate): 50 IU/kg for LO & HO diets, 10 IU/kg for ALO & AHO diets

[2]Supplied per kg diet for LO & HO diets: 80 mg Fe as $FeSO_4 \cdot H_2O$, 90 mg Mn as $MnSO_4 \cdot H_2O$, 125 mg Cu as $CuSO_4 \cdot 5H_2O$, 100 mg Zn as ZnO, 0.35 mg I as KIO_3, and 0.30 mg Se as sodium selenite.
Supplied per kg diet for ALO & AHO diets: 20 mg Fe as Bioplex Fe, 23 Mn as Bioplex Mn, 31 mg Cu as Bioplex Cu, 25 mg Zn as Bioplex Zn, 0.35 mg of iodine as KIO_3, and 0.30 mg Se as Selplex.

Cryovac V834HB polyolefin film (4.0 mil, 1 cc/m^2/24 h oxygen transmission rate at 23 °C). All packaged samples were stored in a retail display cooler (2–4 °C) for up to 21days and received approximately 1076 lux of warm white fluorescent light to stimulate retail storage conditions. Storage time was defined in the present study started from the day of breast meat collection (day 0) and ended after 7 day (PVC), 14 day (HiOx), and 21 day (SK).

3.3.4. Antioxidative Minerals and Vitamin in Muscle Tissue

Eight broilers per dietary treatment (total of 32 per trial) were humanely harvested, and the *Pectoralis major* muscle tissue was removed and stored at –20 °C until use. Selenium content was measured according to Olson et al. (1975) as detailed by Cantor and Tarino (1982). Zinc (**Zn**) level was determined as described by Montaser and Golightly (1992). Vitamin E was determined using the procedure of Liu et al. (1996).

3.3.5. Lipid Oxidation

Lipid oxidation in stored muscle samples was measured as thiobarbituric acid-reactive substances (TBARS) according to Sinnhuber and Yu (1977). The TBARS concentration, using a molar extinction coefficient of 152,000 M/cm for the chromophore, was expressed as mg MDA per kg muscle.

3.3.6. Protein Oxidation

Because myofibrillar proteins are responsible for most of the meat quality attributes important to broilers, i.e., water-holding, tenderness, and texture (Xiong, 2000), this

muscle protein group was selected for protein oxidation analysis. Myofibrils were isolated from meat on the appropriate storage days using a rigor buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM K_2HPO_4 (pH 7.0) as previously described (Xiong et al., 2000). Protein concentration was determined by the Biuret method. Myofibril pellets were kept on ice and all the measurements were completed within 24 h of isolation.

Protein carbonyls were measured according to the 2,4-dinitrophenylhydrazine (DNPH) colorimetric method as described by Levine et al. (1990). The carbonyl content expressed as nmol per mg protein was calculated using a molar absorption coefficient of 22,000 M/cm for the formed protein hydrazones. Sulfhydryls were determined using 5,5' dithio-bis(2-nitrobenzoic acid) (DTNB) (Ellman, 1959). Total sulfhydryl content was calculated using the molar extinction coefficient of 13,600 M/cm and expressed as nmol per mg protein.

3.3.7. Antioxidant Enzymes

Six broilers per dietary treatment (total of 24 per trial) were humanely harvested. Immediately following exsanguination, aliquots of *Pectoralis major* muscle samples (approximately 5 g each) were removed from each broiler, cryogenically frozen in liquid N_2 (–196 °C), and stored in a –80 °C freezer until use. Upon enzyme analysis, partially thawed muscle samples were mixed into 20 mL of chilled buffer (0.05 M Tris-HCl, 1 mM EDTA, pH 7.0) and homogenized for 30 s at 75,000 rpm with a Model PT 10/35 Polytron homogenizer fitted with a PTA-20TS generator (Kinematica Ag, Switzerland). The homogenate was centrifuged for 10 min at 10,000 × g at 4 °C and the supernatant

was filtered through 4 layers of grade 10 mesh cheese cloth. Protein content was measured using the Biuret method. Cu–Zn superoxide dismutase (**SOD**) activity was determined according to Marklund and Marklund (1974) and Gatellier et al. (2004) using the inhibition of pyrogallol in a basic medium. Catalase (**CAT**) activity was measured by the rate of H_2O_2 disappearance according to Aebi (1974). Glutathione peroxidase (**GsPx**) activity was determined according to Beutler (1957).

3.3.8. Serum Protein Carbonyls

The chemical composition of blood serum, which is influenced by diet, is often used as an indicator of the nutritional status of an animal (Liotta et al., 2003). Hence, blood samples were taken from broilers by cardiac puncture immediately postmortem. Samples were allowed to clot at room temperature before centrifugation at 1,2000 × *g* for 15 min to separate serum. The serum was transferred to nalgene tubes, cryogenically frozen in liquid nitrogen, and then stored in an –80 °C freezer. Serum was thawed at 4 °C in darkness then protein carbonyl content was determined according to Levine et al. (1990) as stated above.

3.3.9. Statistical Analysis

Three independent animal feeding trials (n = 3) over a two-year period each with duplicate or triplicate muscle sample analyses were conducted. Data were subjected to analysis of variance (**ANOVA**) using the Statistix software 9.0 (Analytical Software, Tallahassee, FL) with general linear model's procedure to determine the significance of main treatment factors (diet, packaging systems, and storage time). Least Square

Differences (**LSD**) all-pairwise multiple comparisons were performed to separate the means when a treatment effect was found significant ($P < 0.05$). In addition, interactions between diet, packaging, and storage time were analyzed.

3.4. Results and Discussion

3.4.1. Tissue Antioxidative Minerals and Vitamin

Minerals and vitamins are generally added to animal feedstuff for growth, maintenance, and sustenance of life; yet the bioavailability, absorption, and distribution of these micronutrients in various tissues may vary with feed quality. Breast meat was chosen for the analysis since it is the most valuable cut on the bird and a greater distribution of antioxidative minerals and vitamins to this area may help improve oxidative stability. The effects of dietary antioxidant supplementation and oil quality on Se, Zn, and vitamin E content in broiler breast tissue can be seen in Table 3.3. Diets with antioxidant supplementation (ALO, AHO) significantly ($P < 0.05$) increased tissue Se content compared to the basal diet (LO, HO). There was no significant ($P < 0.05$) difference in tissue Zn and vitamin E levels among dietary treatments. However, birds fed a high-oxidized diet had slightly lower zinc and vitamin E levels compared to their respective counterparts. Boler et al. (2012) reported lower vitamin E levels in the back fat of barrows fed a high-oxidized corn oil diet supplemented with synthetic antioxidants compared to the fresh corn oil diet supplemented with synthetic antioxidants.

Table 3.3. Effects of dietary antioxidants and oil quality on tissue vitamin and mineral content of broilers.

Diet	Se (ppb)	Zn (ppm)	VE (µg/g)
LO	170.78[b]	6.85	134.18
ALO	300.68[a]	6.67	128.98
HO	172.10[b]	6.58	127.36
AHO	328.94[a]	6.37	121.13
P - value	< 0.0001	0.3449	0.4891

[ab] Means (n = 3) between dietary treatments without a common lowercase superscript differ significantly ($P < 0.05$).

3.4.2. Lipid Oxidation

For all dietary treatments, lipid oxidation increased throughout the first 7 d of storage under each packaging condition (Table 3.4). HiOx and PVC meat samples exhibited significant signs of microbial spoilage after 14 and 7 d, respectively, and therefore not analyzed beyond these storage times. Compared with the HO dietary treatments, regardless of antioxidant supplementation, the low-oxidized samples (LO, ALO) had lower TBARS values in all packaging conditions, in agreement with Tavárez et al. (2011) who reported a reduced TBARS production in retail display breast meat from broilers fed a commercial blend of ethoxyquin and propyl gallate. On d 14, the TBARS value of the HO dietary group was significantly higher ($P < 0.05$) compared with the LO samples packaged under HiOx. Furthermore, HiOx and PVC produced higher amounts of TBARS ($P < 0.05$) than SK throughout storage. Delles et al. (2011) reported similar oxidative susceptibility and subsequent higher TBARS values of pork muscle packaged in HiOx and PVC compared with vacuum packaging (similar to SK). Samples from birds fed an antioxidant supplemented diet, regardless of oil quality, showed lower TBARS formation compared with basal dietary regimes. Other studies focusing on natural antioxidants have also shown that feeding broilers high levels of α-tocopherol (De Winne and Dirinck, 1996) and selenium (Ryu et al., 2005) delay the onset of oxidative off-flavor formation in chicken meat during storage.

Lipid oxidation in muscle foods occurs primarily in the highly unsaturated phospholipids of the subcellular membranes (Frankel, 1980). The molecular structure of α-tocopherol allows it to protect highly oxidizable PUFAs through neutralization of free radicals in the cellular and subcellular membranes (Liebler, 1993). The lower levels of

Table 3.4. Effects of diets on lipid oxidation (TBARS, mg/kg MDA) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet	HiOx				PVC		SK			
	0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	0.046^{c}	0.172^{b}	0.315^{aAB}	0.305^{aB}	0.212^{b}	0.278^{aB}	0.116^{b}	0.262^{a}	0.242^{a}	0.247^{aAB}
ALO	0.064^{c}	0.136^{b}	0.279^{aB}	0.269^{aB}	0.177^{b}	0.232^{aB}	0.116^{b}	0.229^{a}	0.214^{a}	0.204^{aB}
HO	0.05^{c}	0.208^{b}	0.379^{aA}	0.399^{aA}	0.254^{b}	0.352^{aA}	0.158^{b}	0.298^{a}	0.277^{a}	0.279^{aA}
AHO	0.053^{c}	0.165^{b}	0.348^{aAB}	0.342^{aAB}	0.23^{b}	0.288^{aAB}	0.133^{b}	0.267^{a}	0.269^{a}	0.256^{aAB}
P - value	0.3284	0.2651	0.0508	0.0129	0.0826	0.015	0.0773	0.7571	0.1877	0.0298
Packaging		*	*	*	*	*	*	*	*	

[a–c] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[AB] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

TBARS formation in the antioxidant supplemented diets may be attributed to a similar membranal protective effect as that of α-tocopherol. Furthermore, tissue vitamin E or Zn levels did not significantly differ between diets (Table 3.3), indicating that an algae/Se/organic mineral-based antioxidant can be an effective vitamin E and Zn replacement.

3.4.3. Protein Oxidation

Dietary intake of oxidized oil has been reported to increase the oxidative stress *in vivo* and potentially cause an imbalance between the production of reactive oxygen species (**ROS**) and the defense mechanism of an animal's body (Hayam et al., 1995; Boler et al., 2012). Furthermore, oxidized oil may damage dietary vitamins and increase the susceptibility of the gastrointestinal tract and other tissues to lipid and protein oxidation (Sheehy et al., 1994; Zhang et al., 2011b). Proteins are a major target of reactive oxygen species; the accumulation of oxidized products in the muscle tissue leads to meat quality deterioration. Hence, oxidative chemical modifications, including reduced tryptophan fluorescence, loss of sulfhydryl groups, intra- and inter-molecular crosslinks, and formation of carbonyl derivatives, have a detrimental effect on meat quality (Xiong, 2000).

In the present study, muscle tissue protein carbonyl content increased during storage for all dietary treatments and all packaging conditions (Table 3.5). The carbonyl level in HO samples, irrespective of packaging condition, was higher than those in LO samples. However, muscle samples from antioxidant-supplemented diets had lower carbonyl content compared with the basal group. For example, SK samples after 21 d had

Table 3.5. Effects of diets on protein carbonyl formation (nmol/ mg protein) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet			HiOx			PVC			SK	
	0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	0.189^{dB}	0.565^{cAB}	1.004^{b}	1.145^{a}	0.508^{bAB}	0.925^{aB}	0.387^{cB}	0.751^{bBC}	0.92^{a}	1.025^{aB}
ALO	0.167^{cB}	0.472^{bB}	0.994^{a}	1.009^{a}	0.417^{bB}	0.844^{aB}	0.314^{cC}	0.674^{bC}	0.934^{a}	0.947^{aC}
HO	0.235^{cA}	0.707^{bA}	1.05^{a}	1.116^{a}	0.625^{bA}	1.065^{aA}	0.46^{cA}	0.842^{bA}	0.945^{b}	1.092^{aA}
AHO	0.203^{dAB}	0.608^{cAB}	0.927^{b}	1.127^{a}	0.538^{bAB}	0.949^{aAB}	0.41^{cAB}	0.792^{bAB}	0.968^{a}	1.046^{aAB}
P - value	0.0257	0.0397	0.6058	0.2299	0.0386	0.0124	0.0007	0.0035	0.8269	0.0002
Packaging		*	*	*	*	*	*	*	*	

[a–c] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[AB] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

lower amounts of carbonyls ($P < 0.05$) in the ALO group compared with LO. The impact of packaging systems and storage time on protein carbonyl formation was overall similar to that of TBARS, suggesting a possible relationship between lipid oxidation and protein carbonyl formation. Malondialdehyde (MDA), a secondary dicarbonyl product of lipid oxidation, can interact with amine groups in proteins, generating protein-bound carbonyls (Buttkus, 1967; Hidalgo et al., 1998). The loss of TBARS formation after 7 d and, conversely, the increase in protein carbonyl content, suggests that some of the MDA bound to amino acid side chains forming extra carbonyl compounds.

Sulfhydryls from cysteine residues are highly susceptible to oxidation by most forms of ROS and provide an additional assessment of protein oxidation (Lund et al., 2011). As shown in Table 3.6, samples from broilers fed an antioxidant treatment (ALO, AHO), regardless of oil quality or packaging condition, showed greater protein sulfhydryl retention compared with their respective controls (LO, HO). Furthermore, samples from broilers fed a HO diet, initially (d 0) already had a lower ($P < 0.05$) sulfhydryl content compared with LO samples; this oxidation-associated effect was extended to stored meat packaged under all three conditions, i.e., 14 d for HiOx, 4 d for PVC, and 4, 7, and 21 d for SK. Significant ($P < 0.05$) losses of sulfhydryls occurred from d 0 to d 4 in HiOx and PVC for all dietary treatments, whereas SK samples remained relatively constant. By d 7, all packaging systems showed significant ($P < 0.05$) losses in protein sulfhydryls. The reduced carbonyl formation and sulfhydryl disappearance in antioxidant-supplemented samples may be due to reduced reactive species formation *in vivo*. EcoE functions as a natural antioxidant by altering the expression of various gene transcripts, which may increase the total antioxidant capacity in the serum of broilers (Xiao et al., 2011).

Table 3.6. Effects of diets on free sulfhydryl (nmol/ mg protein) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet	HiOx				PVC		SK			
	0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	87.28^{aB}	70.23^{bBC}	52.95^{c}	45.72^{dA}	66.12^{bB}	51.54^{cB}	83.75^{aA}	70.72^{bA}	52.28^{c}	48.99^{cA}
ALO	93.48^{aA}	77.49^{bA}	52.24^{c}	40.43^{dAB}	71.54^{bA}	59.09^{cA}	85.36^{bA}	70.23^{cA}	58.08^{d}	53.44^{dA}
HO	81.16^{aC}	65.00^{bC}	50.46^{c}	38.08^{dB}	61.81^{bB}	44.36^{cC}	76.34^{aC}	58.44^{bB}	56.79^{b}	39.87^{cB}
AHO	82.41^{aC}	72.93^{bAB}	55.92^{c}	37.81^{dB}	63.76^{bB}	52.27^{cB}	79.37^{aB}	64.81^{bAB}	57.98^{c}	41.66^{dB}
P - value	0.0000	0.0017	0.3061	0.0225	0.0039	0.0000	0.0000	0.0071	0.3389	0.0001
Packaging		*	*	*	*	*	*	*	*	

[a–d] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A–C] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

Selenium, an antioxidant mineral, has strong antioxidant properties and serves as a cofactor for glutathione peroxidase, an enzyme that catalyzes the reduction of hydrogen peroxide and lipid peroxides, thereby preventing oxidative damage. Finally, various studies have reported beneficial effects of organic minerals on broiler performance, health and meat quality that may be attributed to its greater bioavailability and absorption compared to inorganic minerals (Castellini et al., 2002; Bao et al., 2007; Aksu et al., 2011).

3.4.4. Serum Protein Carbonyls

Dietary PUFAs greatly influence serum lipid concentrations, lipid profiles, and lipid metabolism by regulating gene expression through alteration of transcription factors involved in the absorption, extracellular transport, cellular uptake, metabolism, and elimination of lipids in the animal (Jump, 2002; Ringseis and Eder, 2005). Dietary supplementation with oxidized oil resulted in higher levels of protein carbonyl formation in blood serum (Figure 3.1), in agreement with Zhang et al. (2011b), who reported that 5% inclusion of oxidized animal-vegetable fat in broiler diets increased plasma carbonyl content. Low-oxidized samples supplemented with antioxidants (ALO) showed significantly lower ($P < 0.05$) serum carbonyl content compared with all other dietary groups. The greater serum carbonyl content in broilers fed a HO diet may be attributed to the propagation of dietary lipid peroxides and subsequent attack of blood serum proteins, such as albumin, upon the transportation of fatty acids by chylomicrons to various tissues in the bird. Hence, feeding broilers a high-oxidized diet increases oxidative stress, *in vivo*.

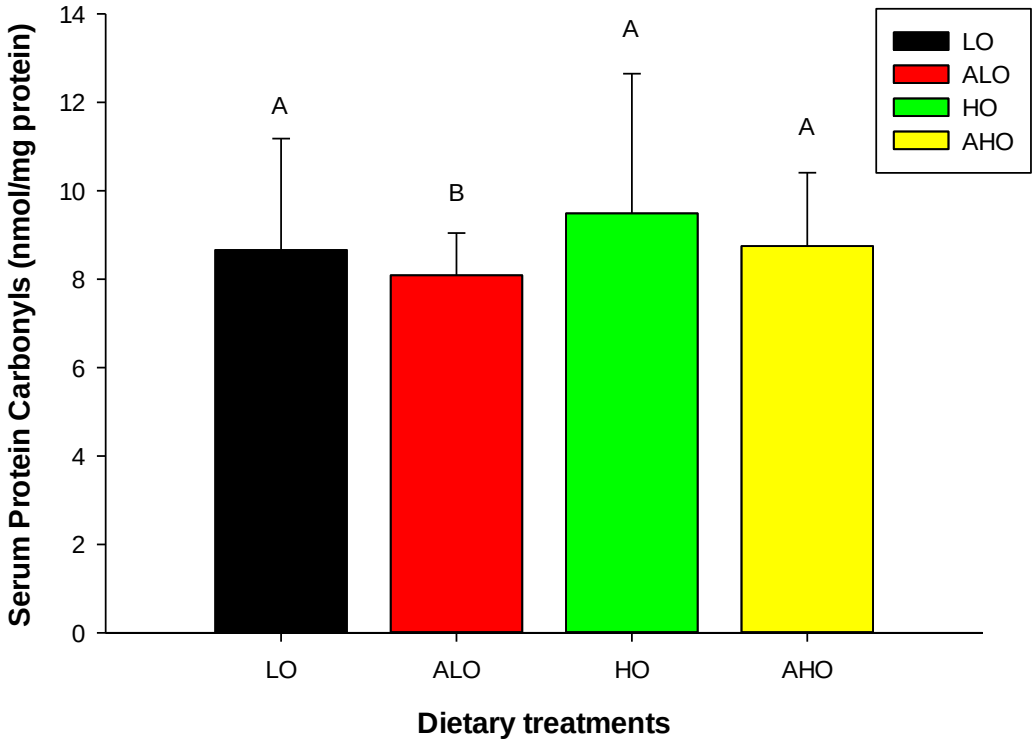

Figure 3.1. Effects of dietary treatments on carbonyl content isolated from blood. Different uppercase letters (A,B) indicate significant difference ($P < 0.05$) of means ($n = 3$) between dietary treatments.

3.4.5. Antioxidant Enzyme Activities

Oxidative stress is the imbalance between the production and degradation of ROS, such as superoxide anion, hydrogen peroxide, and lipid peroxides. Enzymatic inactivation of ROS in muscle tissue is mainly achieved by SOD, CAT, and GsPx with each having a unique mechanism. SOD and CAT are antioxidant enzymes that directly react with radical species, whereas GsPx regenerates oxidized antioxidants. Although the metabolic pathways for oxidized lipids after ingestion has not been fully elucidated, some studies have suggested that absorption of lipid peroxides is dependent upon antioxidant defense enzymes that metabolize lipid derivatives in the mucosa of the digestive tract (Aw and Williams, 1992) and in the liver (Takahashi et al., 2002; Zalejska-Fiolka et al., 2010).

Ingestion of the high-oxidized diets (HO, AHO) resulted in reduction in the activity of SOD, CAT, and GsPx compared with their respective low-oxidized counterparts (Figure 3.2). Specifically, SOD activity was significantly ($P < 0.05$) higher in samples from antioxidant supplemented diets than the basal diet. Gatellier et al. (2004) reported an increase in tissue SOD activity from bovine fed a high vitamin E pasture diet. Interestingly, the presence of antioxidants in the high-oxidized diet alleviated some of the negative effects of oxidized oil on SOD, resulting in similar SOD enzymatic rates between LO and AHO samples, which may be attributed to the gene upper regulation in response to antioxidant supplementation. There was no significant correlation between SOD and CAT activity (Figure 3.2) and zinc and vitamin E levels (Table 2). Tissue samples from broilers fed a high-oxidized treatment without antioxidant supplementation (HO) showed the lowest ($P < 0.05$) SOD activity. CAT showed a similar trend to that of SOD. In agreement, Srivastava et al. (2010) noticed a significant decline in tissue SOD

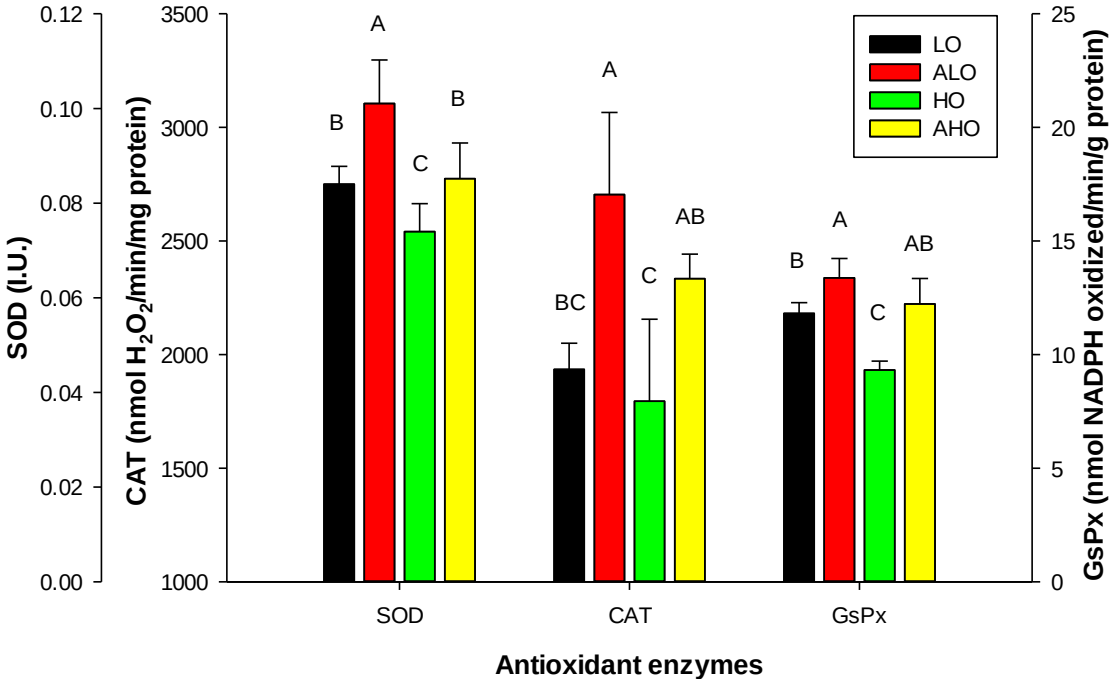

Figure 3.2. Effects of dietary treatments on tissue antioxidant enzyme activities (SOD, CAT, GsPx) in broiler meat. Different uppercase letters (A–C) indicate significant difference ($P < 0.05$) of means ($n = 3$) between dietary treatments.

and CAT activity in rats fed repeatedly boiled sunflower oil. The antioxidant treatment groups ALO and AHO had higher CAT reactivity compared with LO and HO, respectively. Prolonged dietary stress may cause oxidative tissue damage as evidenced by loss in the activity of the coupled antioxidant enzymes, SOD and CAT. DaCosta and Huang (2007) reported a general decline in SOD and CAT antioxidant enzyme activities and an increase in lipid oxidation of grass plant species upon drought stress, indicating that the production of free radicals may exceed the scavenging capacity of the antioxidant defense system under extreme stress conditions.

Glutathione peroxidase is a key antioxidant enzyme within most cells that reduces hydrogen peroxide to water and lipid peroxides to their respective alcohols (Sies, 1999). Compared with basal dietary regimes, regardless of oil quality, the antioxidant treatment groups (ALO, AHO) had significantly ($P < 0.05$) higher GsPx activity compared with the basal dietary group (LO, HO). HO had the lowest GsPx activity. Bansal et al. (2005) reported a decrease in glutathione reductase activity as well as total glutathione content in the liver of rats fed nitrosamine compounds, a hepatic carcinogen. However, rats pre-treated with oral doses of vitamin E showed improved glutathione reductase activity and increased levels of total glutathione content, indicating an elevation in antioxidant activity and a counteraction against nitrosamine-induced oxidative stress. As shown in Table 3.2, birds fed diets supplemented with the algae/Se/organic mineral-based antioxidants had higher levels of tissue Se, coinciding with a higher reactivity of GsPx (Figure 3.2). Zhang et al. (2011a) also found that oral doses of selenium increased blood and serum GsPx activity.

The reduction of the tissue antioxidant enzyme efficacy as a consequence of feeding oxidized oil may be attributed to chemical toxicity of oxidized PUFAs. It appears that the attenuated enzyme activity may be ultimately related to the alteration or reduction in gene expression and transcription. Ringseis and Eder (2005) evidenced reduced transcription of proteosomal and lysosomal enzymes in rats fed oxidized cholesterol. The proteosomal pathway is responsible for the protein repair mechanism required to rescue oxidized proteins and prevent cellular cytoxicity. Hence, a loss in proteasome function may contribute to the accumulation of oxidized proteins and loss in the activity of the antioxidant defense mechanism (Grune et al., 2003) and ultimately reduce meat quality. It appears that the observed differences between dietary treatments (LO, ALO, HO, AHO) in muscle tissue TBARS, protein carbonyls, and sulfhydryls were associated with CAT, SOD and GsPx antioxidant enzyme activity. Figure 3.3 depicts a schematic diagram of the potential interaction of oxidized oils with respect to broiler meat oxidative stability and antioxidant enzyme potential. Nevertheless, more work is needed to fully clarify the mechanism of dietary antioxidants and oxidized oil on the metabolic and physiological changes in muscle tissue.

3.5. Conclusion

The results indicate that feeding diets with high-oxidized oil increased the vulnerability of lipids and proteins to oxidation and reduced the activities of tissue antioxidant defense enzymes. However, the dietary supplementation with an algae/Se/organic mineral-based antioxidant blend, negated these effects. Furthermore, dietary antioxidant

supplementation imparted a protective barrier against oxidation of broiler breast meat under HiOx, PVC, and SK packaging conditions throughout retail display. The improved oxidative stability appears to be associated with enhanced cellular antioxidant enzymatic activity and reduced ROS propagation *in vivo*. Further research is warranted to establish the precise *in situ* relationship between dietary antioxidants, tissue enzyme activity and meat quality.

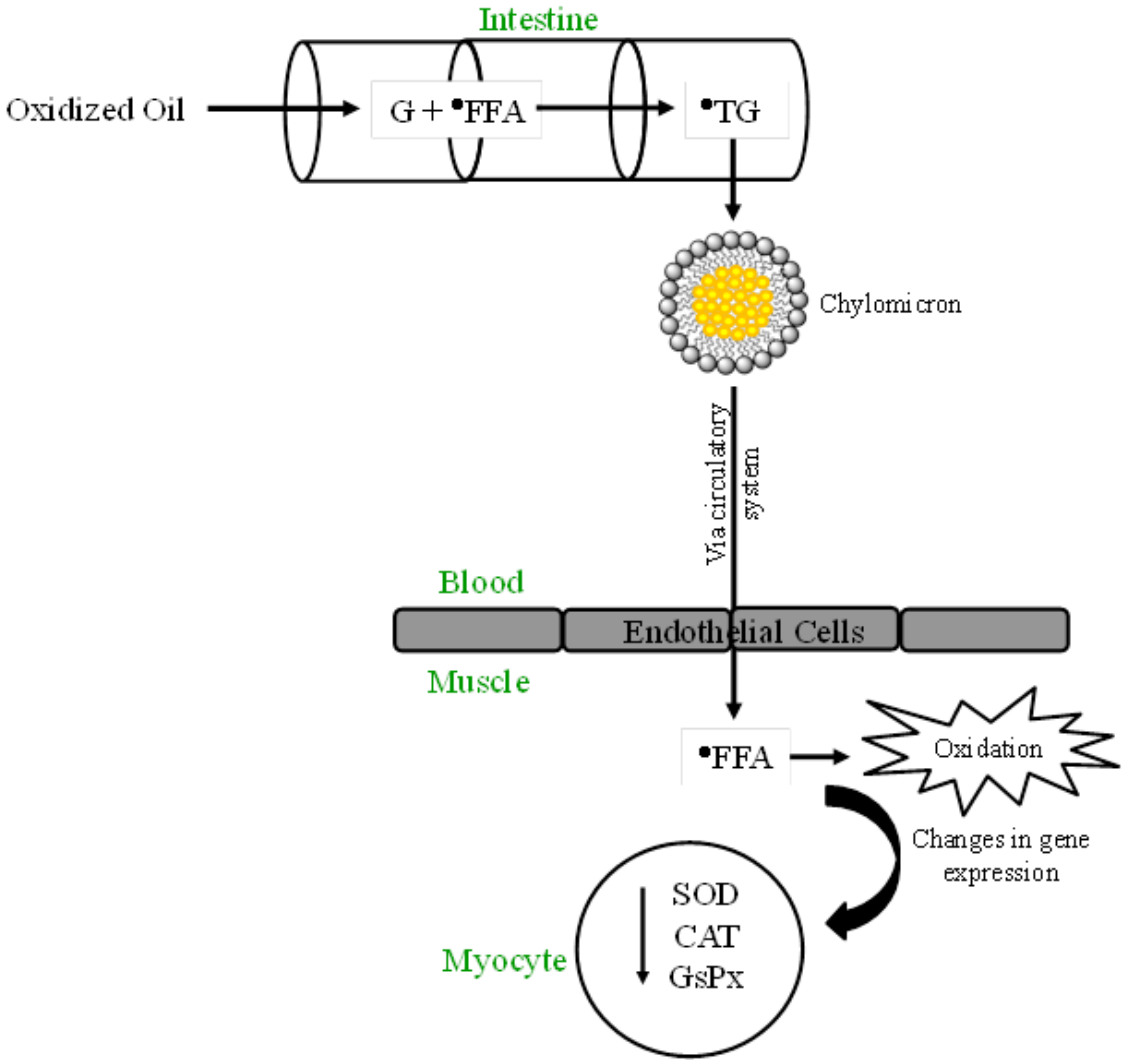

Figure 3.3. Schematic diagram of the mechanism of dietary oxidized oil on muscle tissue oxidation

CHAPTER 4

AUGMENTATION OF WATER-HOLDING AND TEXTURAL PROPERTIES OF BREAST MEAT FROM OXIDATIVELY STRESSED BROILERS THROUGH DIETARY ANTIOXIDANT REGIMENS

4.1. Summary

Previous studies have shown that the inclusion of antioxidants in broiler diets reduced the adverse muscle physicochemical changes associated with feeding oxidized oil. In the present study, the impact of dietary antioxidants and level of oil oxidation on textural attributes of chicken meat stored in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during retail display at 2–4 °C for up to 14, 7, and 21 days, respectively, was assessed. Broilers were fed a diet with either a low-oxidized oil (peroxide value POV 23 meq O_2/kg) or high-oxidized oil (POV 121 meq O_2/kg), supplemented with an antioxidant pack (200 ppm EconomasE and organic minerals Se, Zn, Cu, Mn, and Fe as in Bioplex) for 42 d. Fatty acids and radical scavenging activities of the dietary feed were analyzed. Meat color, pH, myofibrillar protein profile, purge loss, cooking loss, and shear force were measured. Diets with high-oxidized oil had reduced ($P < 0.05$) stearic, linoleic, and linolenic acid content compared diets with low-oxidized oil, regardless of antioxidant supplementation. Yet, as expected, the presence of antioxidants imparted greater radical scavenging capacity. Meat color and pH were variable between dietary treatments throughout storage. Although electrophoresis revealed significant losses of myosin due to feeding high-oxidized diets, the losses were alleviated by the antioxidant supplementation ($P < 0.05$). Meat samples

from chicks fed antioxidant containing diets, irrespective of oil quality, had less (P < ?) purge and cooking loss compared to those fed control diets (no antioxidants). In all packaging systems, meat shear force was higher for broilers fed high-oxidized diets than those fed low-oxidized diets. The results clearly demonstrate the beneficial effects of dietary antioxidant supplementation on the quality of broiler meat negatively impacted by dietary oxidized oil under different package systems.

4.2. Introduction

Consumer demand for healthier, lower fat meat and meat products has led to increased consumption of poultry over the past few decades. Recent studies have shown poultry meat can be a potential functional food for human health because it contains many bioactive substances, such as conjugated linoleic acid (**CLA**), vitamins and antioxidants, and a beneficial *n*-6 to *n*-3 polyunsaturated fatty acids (**PUFAs**) ratio (Grashorn, 2007). The burgeoning demand for poultry meat has resulted in increased pressure on breeders to produce larger birds in less time. Currently, poultry meat is market ready in approximately half the time with double the weight compared to 50 years ago (Barbut et al., 2008). Selection for rapid muscle growth has placed added stress on the growing birds that may have resulted in histological and biochemical modifications of broiler muscle tissue leading to poor, inconsistent meat quality (Barbut et al., 2008). Birds grown at a faster rate have been reported to possess inconsistent fiber quality, and overly tender/ soft chicken meat (Macrae et al., 2006).

In order to reach fast growing potential of modern broiler breed, high energy and protein diets are required. In the past few decades, broiler producers have routinely added fat to commercial diets to increase the overall energy density (Engberg et al., 1996). Yet these fats, particularly vegetable oils which are rich in PUFAs, are highly susceptible to oxidative deterioration in the storage and processing conditions of feedmills. Previous studies have shown that oxidized dietary fat negatively influences broiler performance, health, and meat quality (Engberg et al., 1996; Tavárez et al., 2011; Xiao et al., 2011). The susceptibility of lipids and proteins to oxidative processes plays a crucial role on the quality of fresh meat throughout storage and cooking. A key factor that determines

consumers' acceptance or rejection of fresh poultry meat and meat products is palatability, of which flavor and texture (juiciness and tenderness) are the primary quality traits. Central causes of quality deterioration during retail or home storage (refrigerated or frozen) are lipid and protein oxidation, which produce rancid flavors and decrease juiciness and tenderness. Quality losses in fresh meat products are generally characterized by discoloration, flavor deterioration, reduced juiciness and tenderness and loss of nutrients.

Poultry meat is extremely susceptible to lipid oxidation due to the relatively high proportion of PUFAs. One approach to overcoming oxidation and its related problems is to enhance the diet with synthetic antioxidants (Smet et al, 2008; Tavárez et al., 2011; Xiao et al., 2011). However, increasing health concerns over the use of chemical additives in food has shifted consumer preferences toward more natural and organic products. Thus, dietary supplementation with synthetic antioxidants may be deemed undesirable by consumers. Yet, the use of dietary antioxidants has a distinct advantage over direct incorporation of antioxidants into meat during processing because nutritional antioxidants absorbed by the bird can be effectively distributed in muscle (meat) both inside the cell and at the membrane (Mitsumoto, 2000).

Enhancing diets with natural antioxidants and optimizing nutrient regimen could not only reduce oxidative damage that occurs in the live animal but also may improve the water-holding capacity and textural traits of meat and meat products. Our previous study, Chapter 3, showed enhanced lipid and protein oxidative stability of breast meat of broilers fed a natural algae and organic mineral -based antioxidant blend. However, few studies have investigated the impact of dietary antioxidants and oil quality on meat

sensory attributes. Therefore, this study investigated the influence of dietary antioxidants and oil quality on the physical-chemical properties of chicken breast meat stored in either an oxygen-enriched (**HiOx**: 80% O_2/20% CO_2), air-permeable polyvinylchloride (**PVC**), or skin (**SK**) packaging system during retail display at 2–4 °C for up to 21 days. Broilers were fed either a diet with a low-oxidized oil (23 meq O_2/kg) or high-oxidized oil (121 meq O_2/kg) supplemented with or without an antioxidant blend for 42 days.

4.3. Materials and Methods

4.3.1. Materials

A commercial algae-based antioxidant containing Se yeast as in EcoE and organic minerals as in Bioplex, was supplied by Alltech Inc. (Nicholasville, KY). Soybean oil was acquired from a local retailer, and the initial peroxide value (POV), 23 meq O_2/kg, was determined according to AOCS (2007). Thermally oxidized oil was prepared as detailed in Chapter 3.3.1. The final POV of the pooled oxidized oil was 121 meq O_2/kg which was used immediately for diet preparation. All chemicals (reagent grade) were purchased from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

4.3.2. Animals and Experimental Design

Animals were raised, fed, harvested, and packaged according to Chapter 3.3. Briefly, nine hundred and sixty male broilers raised from 1 to 42 d of age were randomly placed in 48 floor pens with 20 birds per pen. Each pen was randomly designated one of four dietary treatments: (1) basal diet–low oxidized oil (**LO**); (2) basal diet–low oxidized,

supplemented with antioxidants (**ALO**); (3) basal diet–high oxidized oil (**HO**); (4) basal diet–high oxidized oil, supplemented with antioxidants (**AHO**); for a total of 4 treatments with 12 replicate pens. Each pen was equipped with a feeder, a nipple drinker line, and a litter of soft wood shavings. Birds consumed feed in mash form and water on an ad libitum basis. A starter diet containing 22% crude protein (CP) and 3,120 kcal/kg was fed from 1–21 d of age and a grower diet containing 20% CP and 3,150 kcal/kg was fed from 21–42 d of age (Table 3.1). Photoperiod consisted of 22 h of light and 2 h of dark during the entire experiment.

After 42 d of feeding, one broiler from each pen (48 total pens: 4 diets × 12 pens) was randomly selected, humanely slaughtered, de-feathered, then chilled in ice slurries for 1.5 h. Both sides of the breast (*Pectoralis major*) were removed and skinned. One randomly selected broiler breast, per diet, was packaged in either HiOx: 80% O_2 and 20% CO_2, PVC, or SK packaging, as detailed by Chapter 3.3.3.

4.3.3. Dietary Feed Analysis

The antioxidant potential of the water and lipid soluble fractions of the dietary feed were assessed using the radical cation ABTS (2,2'-Azino-bis[3-ethylbenzthiazoline-6-sulfonic acid]) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activities. The ABTS radical scavenging activity of the water-soluble fraction of the dietary feed was determined by a decolorization assay (Pellegrini et al., 1999). The lipid soluble fraction was extracted with methanol: chloroform (2:1) extraction according to Bligh and Dyer (1959). The chloroform layer was isolated and used to assess the $DPPH^{\bullet}$ radical scavenging activity according to Brand-Williams, et al. (1995). The degree of radical

scavenging activity of the dietary feed samples was calculated based on a Trolox standard curve and the results were expressed as Trolox equivalent antioxidant capacity (TEAC, μM).

The fatty acid content was marked as fatty acids methyl esters and determined by Hewlett Packard series 6890, Model G1530A gas chromatogram (Agilent Technologies, Santa Clara, CA).

4.3.4. Instrumental Color

Colorimetric values (*L**, *a**, *b**) of the surface of chicken breast meat were determined using a Chroma Meter CR-300 equipped with a 1-cm aperture, Illuminant C (Minolta, Osaka, Japan). Colorimetric measurements were taken in triplicate random locations.

4.3.5. pH

From the center of each chicken breast, a portion was excised and minced in a Model 51BL31 micro blender (Waring Commercial, Torrington, CT) at low speed setting for 15 sec. Aliquots of 5 g of the minced meat samples were mixed with 15 mL deionized water in a 50 mL conical tube, then homogenized for 30 s at 75000 rpm with a Polytron PT 10/35 fitted with PTA-20TS generator (Kinematica Ag, Switzerland). The pH of the slurry was measured with an accuFlow flushable junction Ag/AgCl reference electrode (Fisher Scientific, Pittsburgh, PA.). All samples were measured in triplicate.

4.3.6. Preparation of Myofibrils

Myofibrils were isolated from chicken breast meat on the appropriate storage day and packaging treatment using a rigor buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM K_2HPO_4 (pH 7.0) as described by Xiong et al. (2000). Protein concentration was determined by the Biuret method. Myofibril pellets were stored on ice under refrigeration and used within 72 h of isolation.

4.3.7. Gel Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli (1970) on myofibril samples with a 10% acrylamide resolving gel and a 3% stacking gel. To each gel lane, 25 μg of protein (1 mg/ mL final protein concentration) was loaded. Selected protein bands, notably myosin heavy chain (MHC), were quantitatively analyzed using the UN–SCAN–IT Gel™ digitizing software (Ver. 6.1, Silk Scientific Corp., Orem, Utah, U.S.A.).

4.3.8. Purge Loss

During storage, a certain amount of exudate was expelled from fresh, whole chicken breast in all packages. The percent loss of liquid, expressed as purge loss (%), was calculated from the weight difference of fresh meat samples before and after respective storage days.

4.3.9. Cooking Loss and Muscle Shear Force

Chicken breast meat was baked in a 176 °C oven until the internal temperature reached 77 °C, approximately 25 min. Cooking loss was defined as the percentage of

meat juice lost during cooking; the values were obtained from the weight of breasts before and after cooking. Cooked breasts were stored at 2–4°C for textural analysis, the following day core samples measuring 1.27 cm in diameter were removed from the breast meat parallel to the direction of the muscle fiber. To evaluate tenderness, cores were placed in an EZ-Test Model Shimadzu Instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a Warner-Bratzler shearing device and a 500 N load cell and crosshead speed of 100 mm/min. The results were expressed in N.

4.3.10. Statistical Analysis

Over a two year period three independent feeding trials (n = 3), each with duplicate or triplicate sample analyses were performed. Data were subjected to analysis of variance (**ANOVA**) using the Statistix software 9.0 (Analytical Software, Tallahassee, FL) with general linear model's procedure to determine the significance of main treatment factors (diet, packaging systems, and storage time). Least Square Differences (**LSD**) all-pairwise multiple comparisons were performed when a treatment effect was found significant ($P < 0.05$).

4.4. Results and Discussion

4.4.1. Dietary Feed

The fatty acid profile was measured to assess the influence of oxidized oil on feed quality. The fatty acid composition of dietary feed samples differed between low-oxidized oil and high-oxidized oil, yet the addition of antioxidants did not show appreciable differences (Table 4.1). The stearic acid (C18:0) content was significantly (*P*

Table 4.1. Fatty acid profile of treatment diets.

Fatty Acid (mg/g)	Dietary Treatment			
	LO	ALO	HO	AHO
C18:0	4.2 ± 0.39[a]	4.01 ± 0.31[ab]	3.64 ± 0.37[c]	3.74 ± 0.31[bc]
C18:1	14.37 ± 1.07[a]	14.35 ± 1.06[a]	13.64 ± 0.91[a]	13.82 ± 0.95[a]
C18:2	52.99 ± 8.32[a]	49.35 ± 9.92[a]	43.28 ± 7.91[b]	41.07 ± 10.98[b]
C18:3	14.25 ± 0.76[a]	13.16 ± 0.65[ab]	12.7 ± 0.52[b]	10.65 ± 1.21[c]

[a–c] Means (n = 2) between dietary treatments without a common lowercase superscript differ significantly ($P < 0.05$).

< 0.05) lower in HO samples compared with LO, while there was no significant differences in oleic acid (C18:1) content between dietary treatments. Comparison between feed samples revealed a significantly ($P < 0.05$) lower amount of linoleic acid (C18:2) and α-linolenic acid (C18:3) content in HO and AHO compared to LO and ALO samples, respectively. Similarly, Kowalski (2007) reported a greater loss in linoleic acid content compared to oleic acid in heated sunflower and olive oil. Fatty acids with a higher degree of unsaturation are more susceptible to oxidation compared to saturated counterparts. Thus, feed with high-oxidized oil had lower levels of linoleic and α-linolenic acid content due to losses imparted by thermal oxidation.

The $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging assays have been widely used for the assessment of antioxidant activity of various foods and food systems. In this study, both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays were used to assess the water and lipid soluble fractions of the dietary feed, respectively. The $ABTS^{\bullet+}$ radical scavenging activity was significantly ($P < 0.05$) greater in ALO samples compared to all other dietary treatments (Figure 4.1). AHO showed slightly greater scavenging activity compared to HO samples. The greater radical quenching ability of the antioxidant supplemented diets, regardless of oil quality, may be attributed to either Se yeast or algae in EcoE. Se incorporation in a variety of foods, such as yeast, green tea, rice, and algae have been shown to enhance antioxidant activity (Xu et al., 2003; Xu and Hu, 2004; Chen and Wong, 2008). Chen and Wong (2008) reported greater $ABTS^{\bullet+}$ radical scavenging activity of extracts isolated from Se-enriched *Spirulina platensis*, a blue-green microalgae. Moreover, algae contain natural antioxidants, such as polyphenols that act as potent radical quenchers (Wang et al., 2009), which may contribute to the greater $ABTS^{\bullet+}$ scavenging ability of the water soluble

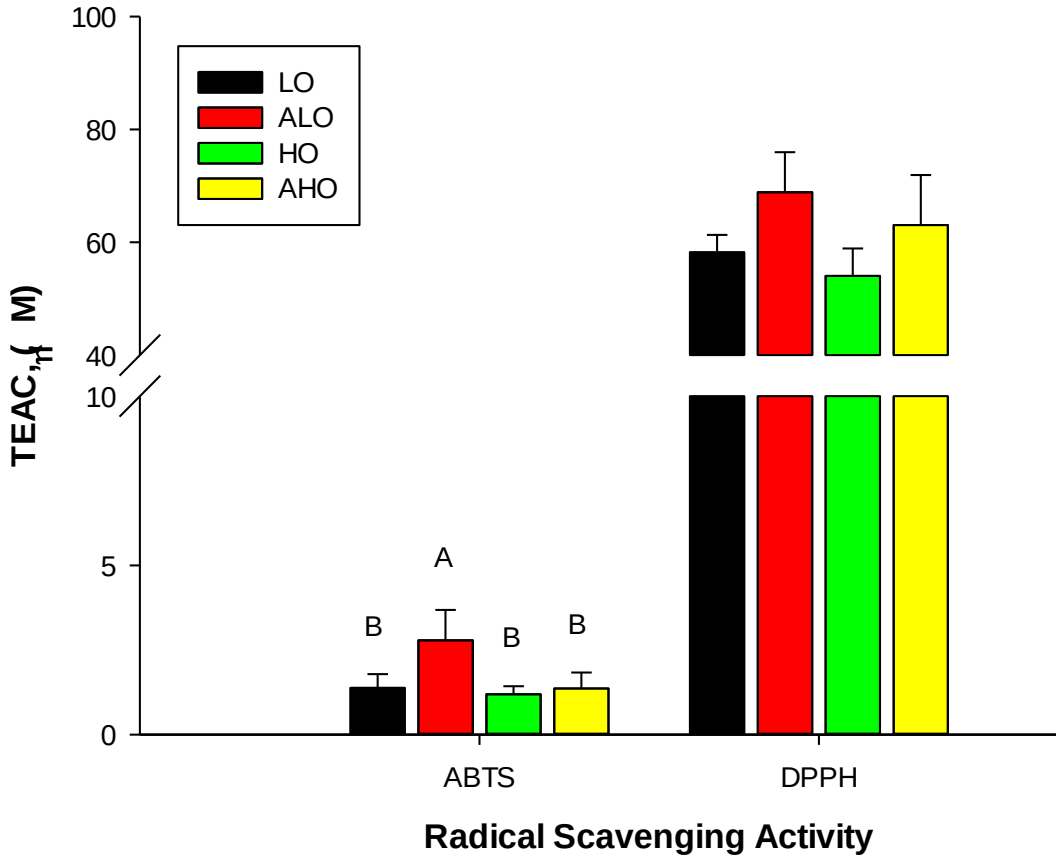

Figure 4.1. Effects of oxidized oil on radical scavenging activities (ABTS, DPPH) of experimental diets. Different uppercase letters (A–B) indicate significant difference ($P < 0.05$) between dietary treatments ($n = 2$).

fraction of EcoE supplemented diets.

DPPH$^{\bullet}$ radical-quenching capacity was measured in the lipid soluble fraction and although the results were not significantly different, the greatest antioxidant activity was noted in ALO samples while HO samples had the lowest scavenging capacity. The greater radical scavenging activity of the antioxidant supplemented treatments, irrespective of oil quality, may be attributed to the algae component of the antioxidant. Sulphated polysaccharides, such as astaxanthin and fucoxanthin, can be found in algae and have been reported to possess excellent antioxidant potential (Zhao et al., 2008).

4.4.2. Instrumental Color and pH

Meat discoloration is a complex process that is influenced by a variety of internal (myoglobin concentration, heme redox stability, oxidation status) and external (pH, temperature, packaging atmosphere) factors (Faustman et al., 2010). In the present study, the composition of packaging atmospheres significantly ($P < 0.05$) impacted the surface color of fresh chicken meat during storage (Table 4.2). While the colorimetric L^* (lightness) value of all fresh meat samples did not show appreciable changes ($P > 0.05$) during storage, there were notable differences in a^* (redness) and b^* (yellowness) values. The colorimetric a^* values for LO samples showed a significant ($P < 0.05$) reduction after the first 4 days in HiOx and PVC samples, but the a^* value remained unchanged for ALO samples, potentially indicating a more stable myoglobin structure due to reduced lipid oxidation. Our previous study, Chapter 3, reported reduced lipid oxidation in the antioxidant supplemented groups, regardless of oil quality. The colorimetric b^*

Table 4.2. Effects of diets on surface color (*L**, *a**, *b**) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

	Diet	HiOx				PVC			SK			
		0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
L*	LO	61.16	63.8	63.77	64.86	61.2	63.03	62.56	61.68	63.98	60.31^{B}
	ALO	64.04	62.63	63.84	65.02	61.47	62.02	63.83	61.79	62.44	61.95^{B}
	HO	62.19	63.26	64.54	64.91	61.4	62.95	64.25	62.22	63.7	64.18^{A}
	AHO	61.48	64.85	64.24	65.54	61.85	61.6	61.29	63.73	63.04	65.13^{A}
	P - value	0.1556	0.2067	0.9500	0.9122	0.9431	0.6671	0.2437	0.2766	0.4397	0.0004
	Packaging		*	*	*	*	*	*	*	*	
	Diet	HiOx				PVC			SK			
		0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
a*	LO	12.48^{a}	8.67^{b}	8.22^{b}	9.36^{b}	9.80^{b}	8.89^{b}	11.4	11.97	12.16	13.50^{A}
	ALO	10.62	10.04	10.45	10.01	9.54	9.08	11.2	11.47	11.31	10.92^{B}
	HO	10.98^{a}	8.84	9.19	9.39	9.73^{ab}	8.46^{b}	10.7	11.57	11.1	11.06^{B}
	AHO	11.26^{a}	8.27^{b}	8.46^{b}	9.29^{b}	9.43	9.82	11.2	10.92	11.93	10.71^{B}
	P - value	0.114	0.0878	0.3509	0.8748	0.9493	0.7306	0.7525	0.7978	0.5364	0.0002
	Packaging		*	*	*	*	*	*	*	*	
	Diet	HiOx				PVC			SK			
		0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
b*	LO	6.66^{bB}	13.01^{a}	12.27^{aB}	13.15^{a}	12.96^{a}	13.65^{a}	9.33^{aA}	8.42^{a}	9.96^{a}	9.76^{a}
	ALO	9.53^{bA}	13.63^{a}	11.79^{aB}	12.55^{a}	12.23^{a}	13.36^{a}	8.30^{bAB}	8.14^{b}	9.29^{b}	11.89^{a}
	HO	7.94^{bAB}	12.47^{a}	12.29^{aB}	11.72^{a}	12.60^{a}	13.01^{a}	9.54^{abA}	8.31^{b}	10.29^{a}	9.58
	AHO	8.20^{bAB}	12.28^{a}	13.32^{aA}	13.06^{a}	12.79^{a}	13.69^{a}	7.56^{cB}	9.87^{ab}	9.51^{ab}	11.91^{a}
	P - value	0.0274	0.2105	0.0315	0.1244	0.6756	0.8077	0.0162	0.3298	0.4942	0.0724
	Packaging		*	*	*	*	*	*	*	*	

[a–c] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A,B] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

(yellowness) values of meat samples in all packaging systems showed an increase over time with SK samples resulting in the least pronounced changes. The appreciable increase in b^* values in all samples throughout storage indicates that neither antioxidant supplementation nor oxidized oil significantly influenced the yellowness of chicken breast meat during retail display. Similarly, Zouari et al. (2010) reported no improvement in the color stability of chicken meat supplemented with vitamin E.

Exposure to fluorescent lighting during retail display can increase lipid and myoglobin oxidation, due to photooxidation and microbial growth (Gatellier et al., 2001). Liu et al (1995) and Chen et al. (2010) reported that dietary vitamin E supplementation improved the color stability of beef and pork, respectively. However, the influence of antioxidant supplementation or oxidized oil did not greatly influence the color of chicken breast meat. More appreciable changes may be seen in chicken thigh meat, which has a higher concentration of heme pigment.

Dietary treatment had no ($P > 0.05$) apparent influence on the pH of muscle samples (Table 4.3) during the first 7 d of storage. On d 14, the low-oxidized samples packaged in HiOx, regardless of antioxidant supplementation (LO, ALO), had higher pH values compared to high-oxidized samples. However, on d 21, antioxidant supplemented samples, regardless of oil quality (ALO, AHO), had higher pH values compared to the basal treatment group. Although there were decreases in the basal dietary treatment during storage the magnitude of the changes appeared to be unsubstantial. The consistent pH values between different dietary treatments and packaging systems, which averaged 5.90 ± 0.19, reflected a high homogeneity between the samples. Furthermore, the results are relatively consistent with the color of the muscle samples (Table 4.2), which showed

Table 4.3. Effects of diets on pH in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet	HiOx				PVC		SK			
	0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	6.07^{aA}	5.87^{b}	5.80^{b}	6.00^{aA}	5.83^{bB}	5.88^{aB}	5.92	5.80^{C}	5.73	5.73^{C}
ALO	5.94^{aB}	5.91	5.84	5.91^{AB}	5.96^{aA}	5.86^{bB}	6.04	5.91^{A}	5.79	5.91^{AB}
HO	6.04^{AB}	5.92	5.77	5.81^{B}	5.88^{B}	5.86^{B}	5.87	5.82^{BC}	5.8	5.83^{BC}
AHO	5.94^{B}	5.89	5.8	5.88^{B}	5.89^{B}	5.93^{A}	5.94	5.85^{B}	5.77	5.98^{A}
P - value	0.0425	0.9431	0.1071	0.0097	0.0045	0.0023	0.1202	0.0005	0.2287	0.0037
Packaging			*	*		*		*	*	

$^{a–c}$ Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

$^{A–C}$ Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

insignificant changes in L^* values in all packaging systems throughout storage. Swatland (2008) reported that pH influenced the color of breast muscle; low pH scatters back more light, resulting in a pale color, while a high pH allows more light to be transmitted into the tissue, leading to a darker color.

4.4.3. Protein Aggregation and Losses

To identify how antioxidant supplementation and dietary oxidized oil would affect protein- protein interactions during storage under different oxygen atmospheres, isolated myofibrils were subjected to SDS–PAGE. The results revealed significant, time-dependent losses of myosin heavy chain (MHC) and concomitant production of high molecular weight (MW) polymers for all dietary treatments and packaging systems throughout storage (Figure 4.2, top panel). The electrophoretic patterns of the samples of all dietary treatments from HiOx and PVC for the first 7 d appeared similar. Specifically, the loss of the MHC of the HO group was more extensive than AHO. However, for the first 7 d of storage all samples stored under SK showed less appreciable MHC losses compared to HiOx and PVC. When the samples were treated with +βME (Figure 4.2, bottom panel), the MHC was nearly fully recovered, thus polymerization of myosin through disulfide bond cross linkages was largely responsible for the disappearance of MHC.

The above results were in agreement with the sulfhydryl analysis of our previous study (Table 3.5), supporting that oxidation of sulfhydryls contributed to myosin aggregation. Furthermore, skeletal muscle undergoing protein thiol oxidation may cause changes in protein function, contributing to dystrophic pathology and altered muscle

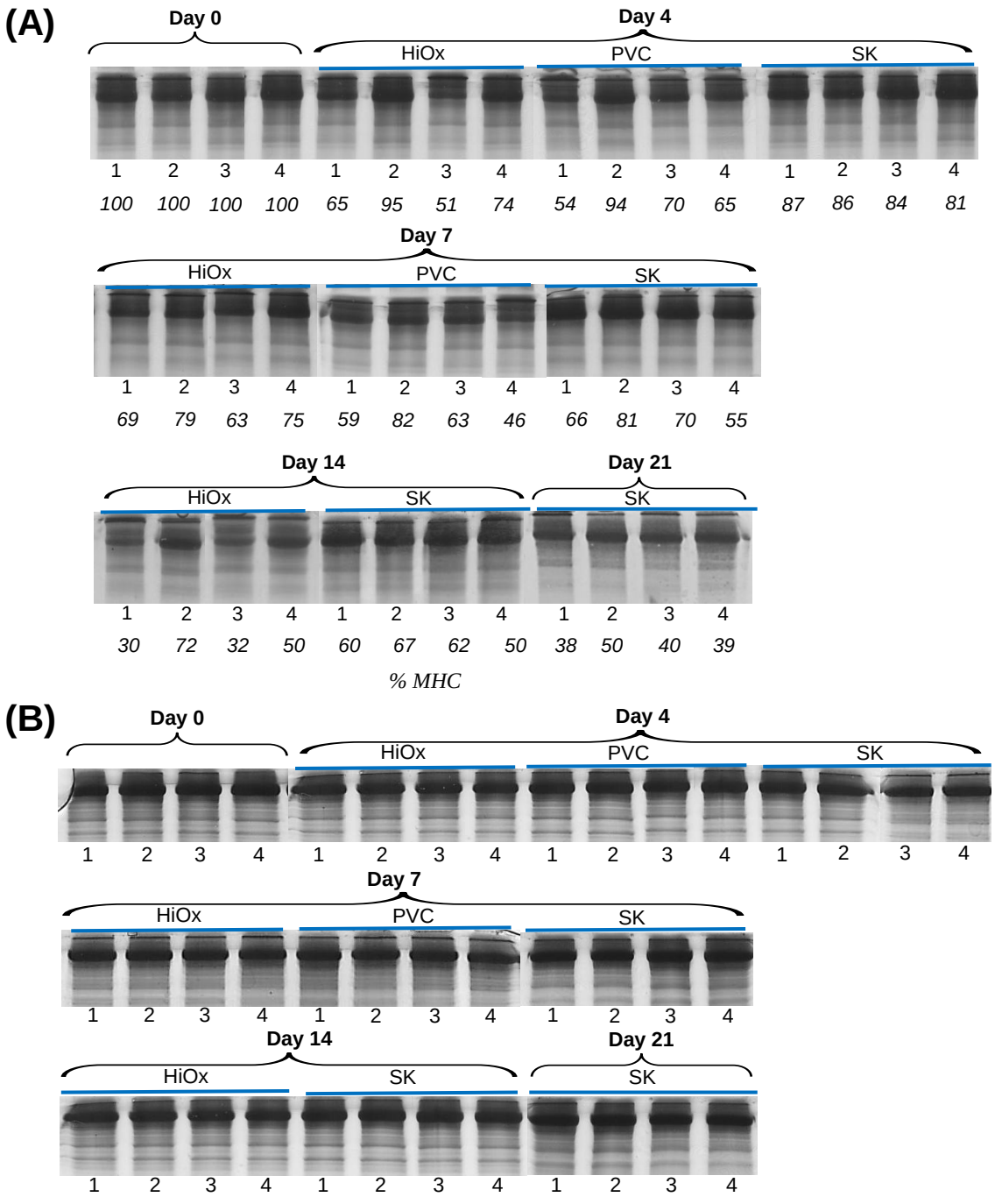

Figure 4.2. Effect of dietary treatments on the SDS-PAGE patterns of myosin heavy chain in myofibrils isolated from fresh chicken breast meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C. Electrophoresis was run under non-reducing (–βME: A) and reducing (+βME: B) conditions. (1) LO; (2) ALO; (3) HO; (4) AHO.

performance (Prakash et al., 2009). Terrill et al. (2013) identified elevated protein thiol oxidation in mice with greater susceptibility to muscular myopathies. In the present study, the results indicated a protective effect, albeit small, of dietary antioxidants against myosin oxidation during storage and an antagonistic effect of dietary oxidized oil on protein oxidation. The electrophoretic data indicated that the SK packaging system, which allowed for a minimal amount of oxygen transmission, was not capable of preventing myosin loss due to disulfide bond formation. MHC appeared to be prone to the oxidative conversion from monomers to disulfide cross-linked polymers, in agreement with various studies (Xiong et al., 2009; Estevez, 2011). Similarly, Dhanarajan et al. (2011) reported that oxidative stress activates protein degradation and the losses in actin and myosin were associated with selective muscle dystrophy. Protein aggregates that can be formed through disulfide cross-linkages, Schiff base adducts, dityrosine formation, and carbon-carbon covalent bonds (Berlett & Stadtman, 1997) have been reported to reduce water-holding capacity and tenderness of fresh pork and beef, respectively, during storage (Lund et al., 2007; Liu et al., 2010).

4.4.4. Water-Holding Capacity

The amount of exudate (purge loss) was lower in antioxidant supplemented samples (ALO, AHO), regardless of oil quality or packaging system, compared to the basal group (LO, HO), respectively (Table 4.4). Within the first 7 d of storage, the amount of purge loss differed significantly ($P < 0.05$) only between diets packaged in HiOx. On the other hand, PVC and SK samples did not show an appreciable amount of

Table 4.4. Effects of diets on purge loss (%) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet	HiOx			PVC		SK			
	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	1.125^{c}	2.003^{bAB}	2.777^{aA}	1.313^{b}	2.18^{aAB}	1.141^{b}	1.363^{b}	2.519^{aA}	2.096^{aBC}
ALO	0.986	1.298^{B}	1.814^{B}	1.100^{b}	1.744^{aB}	1.033^{b}	1.226^{b}	1.48^{abB}	1.799^{aC}
HO	1.419^{b}	2.586^{aA}	3.179^{aA}	1.523^{b}	2.647^{aA}	1.502^{b}	1.581^{b}	2.391^{aA}	2.935^{aA}
AHO	1.123	1.938^{AB}	2.065^{B}	1.258^{b}	2.383^{aA}	0.957^{c}	1.436^{b}	1.824^{bAB}	2.525^{aAB}
P - value	0.3246	0.0056	0.0008	0.2305	0.0431	0.1122	0.4472	0.0158	0.0029

$^{a–c}$ Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

$^{A–C}$ Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

There was no significant difference ($P < 0.05$) of packaging or diet × packaging interaction.

purge loss for the first 7 days of storage; a significant difference ($P < 0.05$) between dietary treatments was not observed until d 14 in SK, consistent with a lower degree of protein oxidation. Similarly, Zhang et al. (2011) reported a higher percentage of drip loss of breast meat from broilers fed a diet with 5% oxidized oil compared to the control and antioxidant-supplemented samples. Several studies have reported a loss in ability of fresh muscle to retain endogenous and exogenous (brine marinated) water upon oxidation of myofibrillar proteins (Xiong et al., 2010; Delles et al., 2011). Myofibrils isolated from broilers fed a control diet exhibited a greater tendency to produce protein carbonyls when compared to broilers fed an antioxidant supplemented treatment (Table 3.4). Thus, the aggregation of myosin (Figure 4.2) and the increase in protein carbonyl formation appeared to contribute to the reduced water-holding capacity (WHC), potentially through a reduction of the myofibrillar lattice (Liu et al., 2011). Microscopic examination with fluorescent immunohisochemical staining revealed an increase in protein carbonyls between the periphery and inside the cells and an increase in extracellular space due to the disintegration of collagen fibers during refrigerated storage of beef (Astruc et al., 2007). Similarly, in humans, excessive oxidative stress may cause irreversible oxidative damage to proteins and lipids, characterized by muscle weakness and progressive skeletal muscle wasting and degeneration (Terrill et al., 2013). Therefore, cellular damage to proteins due to oxidative stress, *in vivo*, may contribute to a weakening of skeletal muscle and ultimately reduce textural properties in meat.

The amount of moisture loss during cooking directly influences the palatability (i.e. juiciness and tenderness) and overall consumer acceptance of a product. Although the results from cooking loss (Table 4.5) did not show a clear trend in moisture loss upon

Table 4.5. Effects of diets on cooking loss (%) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet			HiOx			PVC			SK	
	0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	22.88^{A}	20.23	19.94	23.00	16.96^{B}	20.8^{A}	22.52	20.44	22.48	20.31^{AB}
ALO	17.58^{B}	19.00	18.88	20.66	13.87^{C}	17.24^{AB}	18.87	17.08	19.23	16.52^{B}
HO	21.92^{A}	20.85	20.77	22.59	21.84^{A}	19.64^{A}	20.78	19.74	21.19	24.32^{A}
AHO	20.84^{AB}	18.98	20.67	21.44	18.39^{B}	15.28^{B}	20.24	18.76	20.37	16.79^{B}
P - value	0.0392	0.5273	0.4215	0.4389	0.0011	0.0509	0.2012	0.1975	0.2266	0.0138
Packaging		*	*	*	*	*	*	*	*	

[a] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A–C] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

cooking, it was noted that ALO muscle stored for 4 days under PVC had the lowest percent cooking loss compared to all other dietary treatments. This may be attributed to the protective effect of the antioxidant supplementation against tissue oxidative damage, resulting in reduced protein carbonyls (Table 3.3), and the retention of free sulfhydryls (Table 3.4) and myosin heavy chain (MHC) (Figure 4.2). The denaturation of myofibrillar proteins can result in reduced water-holding capacity due to loss of integrity of the myofibrillar structure (Liu et al., 2010). Thus, the higher cooking loss in broiler breast meat from the high-oxidized group is indicative of higher levels of protein oxidation, which could change the structure and biochemical function of proteins through fragmentation, aggregation, and polymerization (Estévez, 2011). Furthermore, Sülzle et al. (2004) reported that heating fats at a lower temperature for a long period generated mainly primary lipid peroxidation products, which have a severe effect on lipid metabolism. Absorption of primary and secondary lipid oxidation products can react with proteins, potentially leading to oxidative processes that generate protein carbonyls, peptide scission, and protein polymers. Therefore, consumption of oxidized oils can result in protein damage in postmortem muscle leading to functional changes of proteins, such as reduced water-holding capacity.

4.4.5. Warner-Bratzler Shear Force

The shear force values increased during storage regardless of dietary treatment or packaging conditions (Table 4.6). In HiOx, specifically, shear force values of muscle samples from antioxidant supplemented diets, regardless of oil quality, were lower than the basal dietary group and differed significantly ($P < 0.05$) on d 4 and 14 of storage.

Table 4.6. Effects of diets on textural properties (shear force, N) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2 °C.

Diet	HiOx				PVC		SK			
	0 d	4 d	7 d	14 d	4 d	7 d	4 d	7 d	14 d	21 d
LO	11.12^{cB}	17.17^{bAB}	19.39^{bA}	23.58^{aB}	13.16^{bBC}	18.98^{cAB}	11.63^{d}	11.63^{cBC}	20.06^{bB}	22.63^{aAB}
ALO	10.67^{dB}	14.02^{cC}	17.06^{bB}	20.85^{aC}	12.98^{bC}	17.71^{aB}	13.04^{c}	13.04^{cC}	17.95^{bC}	20.18^{aB}
HO	13.29^{cA}	19.09^{bA}	21.52^{bA}	26.41^{aA}	16.95^{bA}	20.06^{aA}	13.88^{c}	13.88^{bA}	23.67^{aA}	24.81^{aA}
AHO	11.8^{cAB}	15.37^{bBC}	20.29^{aA}	22.45^{aBC}	15.46^{bAB}	19.83^{aA}	12.14^{c}	12.14^{bAB}	20.16^{aB}	21.69^{aB}
P - value	<0.0001	0.0004	0.003	0.0005	0.008	0.0431	0.3554	0.0035	0.0001	0.0076
Packaging		*	*	*	*	*	*	*	*	

[a–d] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A–C] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction

Numerous studies have found significant correlations between protein carbonyl content and increased instrumental texture (decreased tenderness) in beef (Rowe et al., 2004; Zakrys et al., 2009). Protein oxidation has been reported to reduce meat tenderness through decreased proteolytic degradation through endogenous enzyme inactivation and/or increased protein cross-linkages through disulfide bond formation (Lund et al., 2011). The results were in agreement with the electrophoresis data (Figure 4.2) that showed a loss of the MHC through disulfide bond cross-linking correlated with a decrease in tenderness throughout storage. Furthermore, the loss of the MHC was less pronounced in ALO samples compared to LO, in concert with the shear force values that were lower in ALO than LO samples. Indicating that protein oxidation, specifically through disulfide bond formation in the MHC, has a negative impact on tenderness of cooked chicken breast meat.

4.5. Conclusion

In summary, addition of high-oxidized oil in dietary feed altered the fatty acid profile and lowered the radical-scavenging capacity, which was improved with the addition of antioxidants. Furthermore, dietary antioxidant supplementation alleviates the negative impact of high-oxidized oil on meat quality, specifically through increased water-holding capacity and tenderness of broiler meat during refrigerated storage under various packaging conditions. Additional research is needed to provide a more complete picture of meat quality during the farm to food conversion. This could lead to the development of more consistent meat products, increased consumer confidence, and cost-effective feed formulations.

CHAPTER 5

INFLUENCE OF DIETARY ANTIOXIDANT SUPPLEMENTATION ON THE OXIDATIVE STABILITY OF DIFFERENT BROILER MUSCLE TYPES

5.1. Summary

Studies have shown that animal nutrition can have a major impact on tissue gene expression, consequently affecting the quality of meat and meat products. This study investigated the influence of dietary antioxidants and quality of oil on the oxidative and physiochemical properties of chicken broiler breast and thigh meat stored in either an oxygen-enriched (HiOx: 80% O_2/20% CO_2) or an air-permeable polyvinylchloride (PVC) packaging system during retail display at 2–4 °C for up to 14 and 7 days, respectively. Broilers were fed 42 days a diet with either a low-oxidized (peroxide value 23 meq O_2/kg) or high-oxidized (121 meq O_2/kg) oil, supplemented with or without an algae/selenium-based antioxidant (EconomasE) with organic minerals (Se, Zn, Cu, Mn, and Fe as in Bioplex). Lipid and protein oxidation, myofibrillar protein profile, and purge loss were analyzed. In both packaging systems, lipid oxidation (TBARS) was inhibited by up to 65 and 57% in chicken breast and thigh, respectively, with an antioxidant-supplemented diet compared to those without antioxidant. In both breast and thigh samples, protein sulfhydryls and water-holding capacity (purge loss) were better protected by the antioxidant dietary treatment, regardless of oil quality. Thigh muscles had up to 7-fold greater TBARS formation and more extensive losses in myosin heavy chain compared to breast samples. Antioxidant supplementation showed a greater protective effect against lipid oxidation and water-holding capacity in the high-oxidized

group. The results suggest that dietary antioxidants can minimize the negative impact of oxidized oil on broiler meat quality, and this protection was more pronounced for thigh than for breast muscle, indicating inherent variations between muscle fiber types.

5.2. Introduction

To the average consumer, “meat quality” describes eating quality, which includes color, tenderness, juiciness, flavor, and consistency of meat in its raw and cooked states. Quality is a complex, multivariate property of meat that is influenced by animal heredity, feeding system, nutritional status, pre-slaughter and slaughter conditions and meat processing conditions (Anderson et al., 2005). To consumers, the most important meat sensory attributes are flavor, tenderness and juiciness all of which are directly influenced by the oxidative stability of lipids and proteins during processing and storage. Poultry meat has a higher proportion of polyunsaturated fatty acids (**PUFAs**) compared to beef or pork and is more susceptible to lipid oxidation. However, the continuous demand for high-quality, shelf-stable meat and meat products in the United States calls for the development of new strategies beyond traditional animal production practices. Previously, synthetic antioxidants such as butylated hydroxytoluene (**BHT**), butylated hydroxyanisole (**BHA**), and ethoxyquin (**EQ**) have been incorporated as an additive in animal feed to hinder rancidity but has also be found in the resulting meat and meat products (Błaszczyk, et al., 2013). A growing number of consumers are concerned about the use of the aforementioned antioxidants due to their possible carcinogenicity. Recently, the incorporation of natural antioxidants in dietary feed to optimize nutrient regimens has been investigated (Avila-Ramos et al., 2013; Luna et al., 2010).

Myofibrillar proteins play an important role in the quality of meat, specifically tenderness and juiciness. The functional properties of myofibrillar proteins (i.e. water-binding) are influenced by fiber type, and several studies have revealed large variations in the quality between red and white meat of chicken (Xiong, 2005; Lesiów and Xiong,

2003). Compared to white meat, red meat contains a larger amount of lipids and higher concentration of heme protein, inorganic iron and mitochondria, which act as catalysts for lipid and protein oxidation. Therefore, the aim of this study was to investigate the effects of dietary antioxidants on oxidative stability of both white (breast) and red (thigh) chicken meat packaged and stored in an oxygen-enriched package (**HiOx**: 80% O_2/20% CO_2) in comparison with air-permeable polyvinylchloride (**PVC**) during retail display at 2–4 °C for up to 14 d.

5.3 Materials and Methods

5.3.1. Materials

A commercial algae-based antioxidant containing Se yeast (**EcoE**) and organic minerals, as in Bioplex, was supplied by Alltech Inc. (Nicholasville, KY). The initial peroxide value (PV) of soybean oil, acquired from a local retailer, was 23 meq O_2/kg as determined according to AOCS (2007). Oxidized oil was prepared as detailed in Chapter 3.3.1. Briefly, the above oil (5 kg) was heated in a convection oven at 95 °C ± 5 °C for up to 7 d. The peroxide value of the oxidized oil was monitored intermittently, until the PV reached the target level of 120 meq O_2/kg; the final PV upon addition to dietary feedstuffs was 121 meq O_2/kg. Chemicals (all reagent grade) were purchased from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

5.3.2. Broiler Production

All procedures used in the study herein were approved by the University of Kentucky Animal Care and Use Committee. Over a two-year period three independent feeding trials (n = 3) were performed in which birds were raised from 1 to 42 d of age. In each trial, nine hundred and sixty female broilers were randomly placed in 48 floor pens with 20 birds per pen, each randomly designated one of four dietary treatments consisted of feeding: (1) basal diet–low oxidized oil (**LO**); (2) basal diet–low oxidized oil, supplemented with antioxidants (**ALO**); (3) basal diet–high oxidized oil (**HO**); (4) basal diet–high oxidized oil, supplemented with antioxidants (**AHO**). Broilers were randomly distributed into the 4 dietary groups with 12 replicate pens for each diet. Each pen was equipped with a feeder, a nipple drinker line, and a litter of soft wood shavings. Birds consumed feed in mash form and water on an *ad libitum* basis. A starter diet containing 22% crude protein (CP) and 3,120 kcal/kg was fed from 0–21 d of age and a grower diet containing 20% CP and 3,150 kcal/kg was fed from 21–42 d of age (Table 3.1). Photoperiod consisted of 22 h of light and 2 h of dark throughout the experiment.

5.3.3. Meat Preparation, Packaging, and Storage

After 42 d of feeding, one broiler from each of the 48 pens (4 diets × 12 pens) was randomly selected, humanely harvested, de-feathered, then chilled in an ice slurry for 1.5 h. Both sides of the breast (*Pectoralis major*) and thighs (*Biceps femoris*) were then removed, skinned, vacuum packaged (99% vacuum) and stored in a –30 °C freezer until use. Per diet, one randomly selected broiler breast or thigh (deboned) was placed in a Cryovac black processor tray, CS977 (22×17×4 cm; Sealed Air Corporation, Elmwood

Park, NJ) and sealed with Cryovac Lidstock 1050 MAP ethylene vinyl alcohol film (1.0 mil, < 20 cc/m^2/24 h oxygen transmission rate at 4.4 °C) using an InPack Junior A10 packaging machine (Ross Industries Inc., Midland, VA). A gas mixture of 80% O_2/20% CO_2 (Scott-Gross Company Inc., Lexington, KY) was used for the HiOx packaging. For PVC, one breast per diet was placed on #2 supermarket white polystyrene trays (20.8×14.5×2.3 cm in dimension; Pactive LLC; Lake Forest, IL) and overwrapped with an air-permeable polyvinylchloride film (15,500–16,275 cm^3/m^2/24 h oxygen transmission rate at 23 °C; E-Z Wrap Crystal Clear PVC Wrap, Koch Supplies, North Kansas City, MO).

5.3.4. Lipid Oxidation

Lipid oxidation in stored muscle samples was measured as thiobarbituric acid-reactive substances (**TBARS**) according to Sinnhuber and Yu (1977). The TBARS concentration, using a molar extinction coefficient of 152,000 M^{-1} cm^{-1} for the chromophore, was expressed as mg MDA per kg muscle.

5.3.5. Protein Oxidation

Because myofibrillar proteins are responsible for most of the meat quality attributes important to broilers, i.e., water-holding, tenderness, and texture (Xiong, 2000), this muscle group was selected for protein oxidation analysis. Myofibrils were isolated from meat on the appropriate storage days using a rigor buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM K_2HPO_4 (pH 7.0) as previously described (Xiong

et al., 2000). Protein concentration was determined by the Biuret method. Myofibrillar pellets were kept on ice and all analyses were completed within 24 h of isolation.

Protein carbonyls were measured according to the 2,4-dinitrophenylhydrazine (**DNPH**) colorimetric method as described by Levine et al. (1990). The carbonyl content expressed as nmol per mg protein was calculated using a molar absorption coefficient of 22,000 M^{-1} cm^{-1} for the formed protein hydrazones. Sulfhydryls were determined using 5,5' dithio-bis(2-nitrobenzoic acid) (**DTNB**). Total sulfhydryl content was calculated using the molar extinction coefficient of 13,600 M^{-1} cm^{-1} and expressed as nmol per mg protein.

5.3.6. Instrumental Color

Colorimetric values (*L**, *a**, *b**) of the meat samples were determined using a Chroma Meter CR-300 equipped with a 1-cm aperture, Illuminant C (Minolta, Osaka, Japan). Colorimetric measurements were taken in triplicate, each at a random location of the surface of the breast meat.

5.3.7. pH

From the center of each chicken breast or thigh, a portion was excised and minced in a Model 51BL31 micro blender (Waring Commercial, Torrington, Conn.) at low speed setting for 15 sec. Aliquots of 5 g of the minced meat samples were each mixed with 15 mL deionized water in a 50 mL conical tube, then homogenized for 30 s at 75000 rpm with a Polytron PT 10/35 fitted with PTA-20TS generator (Kinematica Ag, Switzerland).

The pH of the slurries was measured with an accuflow flushable junction Ag/AgCl reference electrode (Fisher Scientific, Pittsburgh, PA.).

5.3.8. Preparation of Myofibrils

Myofibrils were isolated from chicken breast or thigh meat on their appropriate storage day and packaging treatment using a rigor buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM K_2HPO_4 (pH 7.0) as described by Delles et al. (2011). Protein concentration was determined by the Biuret method. Myofibrillar pellets were stored on ice under refrigeration and used within 24 h of isolation.

5.3.9. Gel Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (**SDS–PAGE**) was performed according to Laemmli (1970) on myofibril samples with a 10% acrylamide resolving gel and 3% acrylamide stacking gel. To each gel lane, 25 μg of protein (1 mg/mL final protein concentration) was loaded.

5.3.10. Purge Loss

During storage, a certain amount of exudate was expelled from fresh, whole chicken breast and thigh in all packages. The percent loss of liquid, expressed as purge loss (%), was calculated from the weight difference of fresh meat samples before and after respective storage days.

5.3.11. Statistical Analysis

Over a two-year period, three independent feeding trials (n = 3), each with duplicate or triplicate muscle sample analyses were performed. Data were subjected to analysis of variance (ANOVA) using the Statistix software 9.0 (Analytical Software, Tallahassee, FL) with general linear model's procedure to determine the significance of dietary treatment, packaging systems and storage time. Least Square Differences (**LSD**) all-pairwise multiple comparisons were performed when a treatment effect was found significant ($P < 0.05$). In addition, interactions between diet, packaging, and storage time were analyzed.

5.4 Results and Discussion

5.4.1. Lipid Oxidation

Fat or oil is commonly added to poultry diets to increase energy density, yet other benefits have been reported such as improved absorption of fat-soluble vitamins, and peristalsis of the intestine, and increased feed intake (Baião and Lara, 2005). However, vegetable oils are highly susceptible to oxidation due to their high content of PUFAs. Previous studies have shown that dietary supplementation of oxidized oil adversely affected the growth and development of chickens (Engber et al., 1996) and reduced chicken meat quality (Racanicci et al., 2008). The decrease in animal viability and overall quality of chicken meat may be attributed to increased susceptibility to lipid oxidation. Consumption of oxidized oils can destroy important dietary vitamins and nutrients and increase oxidative stress *in vivo*, which may potentially reduce the efficacy of the antioxidative system in muscle tissue. Table 3.2 and Figure 3.2 reported reduced tissue

selenium content and glutathione peroxidase activity in the breast tissue of broilers fed a high oxidized oil diet. Thus, consumption of oxidized lipids may increase the absorption of lipid-derived radicals into the bloodstream, propagating oxidative reactions throughout various tissues of the bird, including skeletal muscle, thereby influencing meat quality during storage.

In the present study, lipid oxidation increased throughout the first 4 d of storage for all dietary treatments, packaging conditions, and muscle types (Table 5.1). Compared to breast, thigh samples had significantly higher TBARS content ($P < 0.05$) due to the greater amount of lipids and high concentrations of heme proteins and inorganic iron, both of which are catalysts for lipid and protein oxidation. Similarly, sardines underwent rapid lipid oxidation due to the high content of polyunsaturated fatty acids (Chaijan et al., 2006). Supplementation with antioxidants exhibited a greater protective effect in thigh samples packaged in HiOx compared with breast samples, which may be attributed to the higher concentration of myoglobin present in red (thigh) fibers compared with white (breast). Oxidation of myoglobin produces superoxide anions and hydrogen peroxides which can further react with iron to produce hydroxyl radicals, thus contributing to greater oxidative susceptibility of red fibers compared with white fibers (Chaijan et al., 2008). Dietary antioxidant treatment groups (ALO, AHO) had lower TBARS values under all packaging conditions when compared to basal dietary regimes, regardless of oil quality (LO, HO). Previous studies have shown that feeding broilers high levels of selenium (Ryu et al., 2005) or tocopherols (Smet et al., 2008) delayed the onset of off-flavor formation and reduced lipid oxidation. TBARS values of HO thigh meat stored in HiOx were significantly higher ($P < 0.05$) than all other dietary treatments throughout

Table 5.1. Effects of diets on lipid oxidation (TBARS, mg/kg MDA) in broiler breast (white) and thigh (red) meat packaged in oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C.

Muscle Type	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
White	LO	0.050^{cB}	0.232^{cAB}	0.660^{b}	0.979^{aA}	0.050^{cB}	0.174^{b}	0.340^{aB}
	ALO	0.054^{cB}	0.191^{bB}	0.463^{a}	0.342^{bB}	0.054^{cB}	0.138^{b}	0.330^{aB}
	HO	0.095^{dA}	0.274^{cA}	0.547^{b}	0.787^{aAB}	0.095^{cA}	0.264^{b}	0.535^{Aa}
	AHO	0.065^{bB}	0.186^{bB}	0.512^{a}	0.505^{aB}	0.065^{cB}	0.197^{b}	0.476^{aA}
	P - value	0.013	0.024	0.188	0.032			
	Packaging		NS	*			NS	*
	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
Red	LO	0.31^{cAB}	0.85^{bcB}	1.14^{bAB}	2.37^{a}	0.31^{bAB}	0.53^{ab}	0.73^{aAB}
	ALO	0.34^{bAB}	0.77^{bB}	0.78^{bB}	2.47^{a}	0.34^{AB}	0.41	0.49^{B}
	HO	0.41^{dA}	1.08^{cA}	1.69^{bA}	2.80^{a}	0.41^{A}	0.58	0.94^{A}
	AHO	0.41^{cB}	0.75^{bB}	0.74^{bB}	2.09^{a}	0.41^{bB}	0.69^{a}	0.68^{a}
	P - value	0.049	0.011	0.007	0.098	0.049	0.452	0.013
	Packaging		NS	NS			NS	NS

$^{a–c}$ Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

^{AB} Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

storage, most likely due to dietary oxidized oil increasing the susceptibility of PUFAs to oxidation (Engberg et al., 1996). Furthermore, samples packaged under HiOx showed a higher degree of lipid oxidation compared to PVC.

5.4.2. Protein Oxidation

Due to the presence of oxidizable lipids, heme pigments, transition metal ions, and oxidative enzymes, muscle foods are highly susceptible to oxidation, which can result in the generation of protein carbonyls. The DNPH method has been widely employed for the quantification of carbonyl compounds (Lund et al., 2011). As shown in Table 5.2, protein carbonyl content was significantly ($P < 0.05$) higher in thigh samples compared to breast, in agreement with the TBARS data (Table 5.1). The greater oxidative stability in breast samples may be attributed to not only a lower amount of lipids, myoglobin, and iron, but also to a higher concentration of indigenous antioxidants, such as carnosine, anserine, and antioxidative enzymes (Chan et al., 1994). Carnosine and anserine are rich in white (breast) fibers, but relatively deficient in red (thigh) fibers (Davey, 1960). HO thigh samples, packaged in HiOx and PVC exhibited greater sensitivity to protein oxidation compared to breast samples. For example, on 7 d, HO thigh samples packaged in HiOx had an 85.6% higher carbonyl content compared to HO breast samples. However, the protective effect of antioxidant supplements was similar between both breast and thigh samples most likely due to a masking effect from freezing of samples prior to analysis. Benjakul and Bauer (2001) suggested that the freeze-thaw process damages cellular membranes and heme-proteins, resulting in the release of pro-oxidants, which would be intensified in predominately red fiber meats. Similarly, Xiao et al. (2011)

Table 5.2. Effects of diets on protein carbonyl formation (nmol/ mg protein) in broiler breast (white) and thigh (red) meat packaged in oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C.

Muscle Type	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
White	LO	0.37	0.53	0.52	0.83	0.37	0.74	0.60
	ALO	0.33	0.38	0.36	0.90	0.33	0.55	0.34
	HO	0.55	0.76	0.67	1.39	0.55	0.57	0.63
	AHO	0.25	0.61	0.52	0.92	0.25	0.75	0.75
	P - value	0.008	0.008	0.393	0.001	0.008	0.0661	0.013
	Packaging		NS	NS			NS	NS
	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
Red	LO	1.78	2.42	3.40	5.53	1.78	1.90	3.10
	ALO	1.23	2.23	2.96	4.72	1.23	2.49	2.88
	HO	2.09	2.88	4.26	5.00	2.09	2.88	3.70
	AHO	1.80	2.54	3.73	4.76	1.80	2.28	3.83
	P - value	0.004	0.304	0.014	0.007	0.004	0.067	0.002
	Packaging		NS	NS			NS	NS

[a–d] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A–C] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

reported that broilers fed a 5% oxidized animal-vegetable fat produced higher amounts of carbonyls compared to the control. Furthermore, a promoting effect of HiOx on the formation of protein carbonyls in both breast and thigh samples was observed, although there was no significant difference ($P > 0.05$) between packaging treatments. Throughout storage the inhibitory effect of dietary antioxidant supplementation was much weaker or even negligible in the high-oxidized (HO, AHO) breast samples packaged in PVC. Carotenoids are fat-soluble antioxidants and may have a greater protective effect against lipid oxidation than protein oxidation. Salminen et al. (2006) postulated that antioxidants primarily protect lipids against oxidative reactions. Furthermore, the greater efficacy of the antioxidant supplementation against protein oxidation in the HiOx packaging treatment may be due to the augmented oxidative environment. Throughout storage there was an overall increase in protein carbonyl content of breast and thigh samples in both HiOx and PVC packaging. Microscopic examination of beef during storage revealed an increase in protein carbonyls along the periphery of cells throughout ten days of refrigerated storage (Astruc et al., 2007).

Sulfhydryls from cysteine residues are highly susceptible to oxidation and provide an additional assessment of protein oxidation. As shown in Table 5.3, there were significant losses in sulfhydryl content within the first 7 d of storage for all samples. Thigh meat showed greater losses in sulfhydryl content compared to breast meat, which is in agreement with the TBARS (Table 5.1) and carbonyl (Table 5.2) data. Furthermore, antioxidant dietary supplementation, regardless of oil quality, packaging type, or muscle sample (ALO, AHO), showed greater protein sulfhydryl maintenance compared with the basal dietary treatment. Samples from broilers fed high dietary oxidized oil (HO, AHO)

Table 5.3. Effects of diets on free sulfhydryl (nmol/ mg protein) in broiler breast (white) and thigh (red) meat packaged oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C.

MuscleType	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
White	LO	58.97	52.77	43.06	24.09	58.97	52.84	27.94
	ALO	62.68	57.18	44.02	26.86	62.68	54	35.25
	HO	52.74	46.08	31.23	18.8	52.74	46.64	34.51
	AHO	57.21	49.61	39.29	19.24	57.21	49.19	37.48
	P - value	0.020	< 0.0001	< 0.0001	< 0.0001	0.020	< 0.0001	< 0.0001
	Packaging		NS	*			NS	*
	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
Red	LO	57.87	42.97	36.25	14.04	57.87	34.88	31.03
	ALO	60.96	45.69	36.03	17.94	60.96	44.83	32.79
	HO	40.59	41.99	23.31	21.25	40.59	34.02	30.59
	AHO	44.19	38.6	27.65	17.08	44.19	39.39	32.13
	P - value	< 0.0001	0.011	0.001	0.128	< 0.0001	0.000	0.522
	Packaging		NS	NS			NS	NS

[a–c] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[AB] Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

showed greater sulfhydryl destruction compared to the low oxidized oil regimen. The loss in sulfhydryls may be attributed to the formation of disulfide bonds due to oxidatively induced cross-linking. Antioxidants, such as carotenoids and selenium, may minimize the negative effects of dietary and environmental stresses placed on the birds throughout rearing. Specifically, the aforementioned antioxidants can accumulate in various tissues and either delocalize radicals, reduce peroxides, or serve as cofactors for antioxidative enzymes, thereby slowing the propagation of lipid oxidation of the highly unsaturated fatty acids in the cellular and subcellular membranes and concertedly reducing protein oxidation.

Other chemical modifications as a result of protein oxidation include loss of tryptophan and sulfhydryl groups and formation of intra- and inter-molecular crosslinks, which detrimentally affects meat quality (Xiong, 2000). The gel electrophoresis (SDS-PAGE) results revealed significant, time-dependent losses of myosin heavy chain (MHC) and concurrent production of high molecular-weight (MW) polymers for all dietary treatments, packaging systems, and muscle type throughout storage (Figure 5.1, top panel). Thigh samples showed significantly notable losses of the MHC, initially and throughout storage, compared to breast. Frozen storage appeared to exacerbate myofibrillar denaturation in thigh samples more greatly than breast. Breast and thigh samples from broilers fed a high-oxidized diet (HO, AHO) packaged in HiOx had more extensive losses of MHC compared to low-oxidized samples, which was apparent in the first 4 d of storage. Electrophoretic patterns from chickens fed an antioxidant supplemented diet showed less extensive losses of the MHC compared to their respective controls. When the samples were treated with +βME (Figure 5.1, bottom panel), the

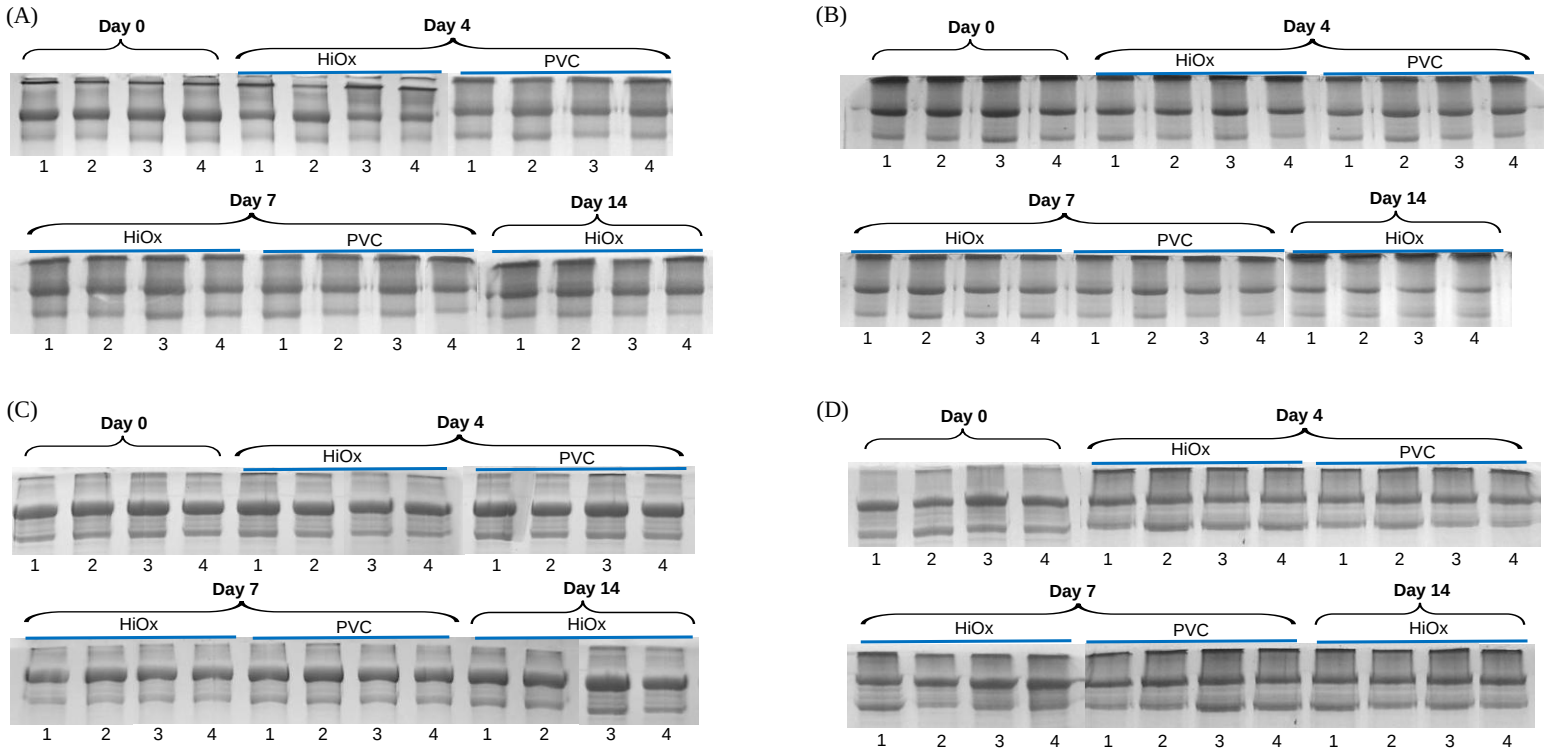

Figure 5.1. Effect of dietary treatments on the SDS–PAGE patterns of myosin heavy chain in myofibrils isolated from pre-frozen chicken breast (A, B) or thigh (C, D) meat in oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C. Electrophoresis was run under non-reducing (–βME: A, C) and reducing (+βME: B, D) conditions.

MHC was nearly fully recovered, thus polymerization of myosin through disulfide bond cross linkages was largely responsible for the disappearance of MHC. The above results were in agreement with the sulfhydryl analysis (Table 5.3), supporting that oxidation of sulfhydryls contributed to myosin aggregation. The results indicated a protective effect of dietary antioxidants against protein oxidation during storage and a negative influence of dietary oxidized oil on protein oxidation. The greater oxidative stability in the algae/Se/organic mineral-based antioxidant supplemented dietary group may be attributed to either: (1) the neutralization of free radicals by algae based carotenoids, or (2) increase in glutathione peroxidase activity, thereby slowing the propagation of lipid and protein oxidation.

5.4.3. Meat Quality

To investigate the impact of dietary antioxidant supplementation and oil quality on water retention properties during storage, the amount of exudate (purge loss) from breast and thigh meat were measured (Table 5.4). In PVC, only, breast samples from antioxidant-supplemented diets, regardless of oil quality (ALO, AHO), had lower amounts of purge compared to LO and HO, respectively. A similar effect was noted in chicken thigh samples packaged under HiOx. The larger amount of percent purge loss from the basal dietary group (LO, HO) may be attributed to a higher degree of protein oxidation. Oxidatively induced formation of disulfide bonds within myosin and between myosin molecules has been shown to decrease water-holding capacity of myofibrils (Kim et al., 2010; Liu et al., 2010). Specifically, disulfide cross-linkages between myosin tails

Table 5.4. Effects of diets on purge loss (%) in broiler breast (white) and thigh (red) meat packaged in oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C.

Muscle Type	Diet		HiOx		PVC	
		Day 4	Day 7	Day 14	Day 4	Day 7
White	LO	0.23	0.66	0.98	0.17	0.34
	ALO	0.19	0.46	0.34	0.14	0.33
	HO	0.27	0.55	0.79	0.26	0.54
	AHO	0.19	0.51	0.51	0.20	0.48
	P - value	0.826	0.488	0.051	0.840	0.937
	Packaging	*	*		*	*
	Diet		HiOx		PVC	
		Day 4	Day 7	Day 14	Day 4	Day 7
Red	LO	4.23	6.41	9.62	5.23	7.73
	ALO	3.14	4.88	7.04	4.05	6.39
	HO	3.99	7.19	11.71	6.63	6.02
	AHO	3.30	5.71	11.36	4.79	7.97
	P - value	0.840	0.158	0.634	0.585	0.840
	Packaging	NS	NS		NS	NS

[ab] Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).
There was no significant difference ($P < 0.05$) of diet × packaging interaction.

were shown to be a major constraint in myofibrillar swelling during salt marination, which negatively influenced water-holding capacity due to an enlargement of intercellular gaps (Liu et al., 2010). In the present study, the amount of purge loss increased, overall, throughout storage for all samples and packaging conditions, in parallel with the protein carbonyl content (Table 5.2). However, variations in purge loss during storage may be due to the inconsistent size of the chicken breast (238.7 ± 34.5 g) and thigh (81.4 ± 11.1 g) samples. Furthermore, chicken breast samples had a greater amount of purge loss compared to thigh samples. The difference was most likely due to the greater amount of water present in the breast sample size (167.09 ± 24.2 g) compared to thigh meat sample size (57.0 ± 7.8 g).

The influence of pH on the water-holding capacity of chicken breast or thigh samples was negligible, as shown in Table 5.5. The consistent pH values between different dietary treatments, muscle types, and packaging systems, which averaged 5.96 ± 0.18 in breast samples and 6.00 ± 0.16 in thigh samples, reflected a high homogeneity between broiler birds. Furthermore, dietary treatment had a small effect on pH during storage. A significant difference ($P < 0.05$) was noted only on d 7 HiOx breast sample and d 4 PVC and d 14 HiOx thigh samples.

The surface meat color of the breast and thigh samples, expressed by L^*, a^*, and b^*, varied between dietary regimen, muscle type, and packaging treatment (Table 5.6). The composition of packaging atmospheres significantly ($P < 0.05$) impacted the a^* (redness) in both breast and thigh samples, but only influenced L^* (lightness) in thighs and yellowness (b^*) in breast meat. While the colorimetric L^* value of breast samples

Table 5.5. Effects of diets on pH in broiler breast (white) and thigh (red) meat packaged in oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C.

Muscle Type	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
White	LO	5.94^{bc}	5.89^{c}	6.16^{aA}	6.08^{ab}	5.94	5.71	5.96
	ALO	5.97^{c}	6.06^{a}	6.08^{abB}	5.98^{bc}	5.97	6.03	6.04
	HO	5.97	5.82	5.98^{C}	5.96	5.97	5.85	6.00
	AHO	5.89^{bc}	5.70^{c}	6.11^{aB}	6.04^{ab}	5.89^{a}	5.79^{b}	6.00^{a}
	P - value	0.888	0.153	<0.0001	0.096	0.888	0.235	0.235
	Packaging		NS	*			NS	*
	Diet	HiOx				PVC		
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
Red	LO	6.02	6.02	5.92	6.06^{A}	6.02^{a}	6.04^{aB}	5.76^{b}
	ALO	6.07^{ab}	6.13^{a}	5.88^{b}	5.94^{bB}	6.07^{ab}	6.16^{aAB}	5.87^{b}
	HO	6.11^{a}	6.14^{a}	5.88^{b}	5.95^{bB}	6.11^{b}	6.22^{aA}	5.82^{c}
	AHO	6.09^{a}	6.11^{a}	5.89^{b}	5.97^{bB}	6.09^{a}	6.16^{aAB}	5.85^{b}
	P - value	0.266	0.108	0.397	0.002	0.266	0.002	0.081
	Packaging		*	*			*	*

$^{a–c}$ Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

^{AB} Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

Table 5.6. Effects of diets on surface color (*L**, *a**, *b**) in broiler breast (white) and thigh (red) meat packaged in oxygen-enriched (HiOx) or air-permeable polyvinylchloride (PVC) packaging systems during refrigerated storage at 2 °C.

	Fiber Type	Diet	HiOx				PVC		
			Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
		LO	61.94^{c}	63.91^{bA}	63.06^{b}	68.71^{aA}	61.94	60.56	62.65
		ALO	61.00^{ab}	59.7^{bcB}	63.87^{a}	58.27^{cB}	61.00	61.26	60.94
	White	HO	59.41^{b}	60.33^{bB}	63.19^{a}	62.3^{aA}	59.41^{b}	61.86^{ab}	63.11^{a}
		AHO	60.43	60.92^{B}	62.57	55.8^{B}	60.43^{b}	62.24^{a}	63.41^{a}
		Package		NS	NS			NS	NS
L* Value		Diet	HiOx				PVC		
			Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
		LO	50.53^{c}	55.92^{bc}	57.9^{ab}	63.78^{a}	50.53^{b}	51.89^{ab}	59.52^{a}
		ALO	56.01^{b}	58.63^{b}	57.90^{b}	66.20^{a}	56.01	56.86	61.50
	Red	HO	56.88^{b}	58.24^{b}	60.39^{b}	66.10^{a}	56.88^{b}	55.63^{b}	62.53^{a}
		AHO	50.54^{c}	57.95^{bc}	59.44^{b}	66.01^{a}	50.54^{b}	59.94^{b}	60.30^{a}
		Package		*	NS			*	NS
		Diet	HiOx				PVC		
			Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
		LO	9.77^{a}	11.79^{aB}	12.03^{a}	4.65^{bB}	9.77	11.83	9.29
		ALO	12.45	13.86^{A}	11.42	12.54^{A}	12.45	12.16	11.34
	White	HO	11.46^{ab}	12.96^{aA}	11.97^{ab}	10.31^{bAB}	11.46	12.39	11.34
		AHO	12.37	13.03^{A}	13.09	12.78^{A}	12.37^{a}	11.07^{ab}	9.61^{b}
		Pkg		*	*			*	*
a* Value		Diet	HiOx				PVC		
			Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
		LO	17.53^{ab}	17.62^{a}	14.43^{b}	10.98^{c}	17.53	17.44	14.69
		ALO	15.92^{a}	16.67^{a}	14.55^{b}	10.81^{c}	15.92^{a}	16.90^{a}	13.74^{b}
	Red	HO	14.86^{b}	17.28^{a}	14.9^{b}	9.42^{c}	14.86^{ab}	16.50^{a}	13.3^{b}
		AHO	15.04	17.44	15.17	9.54	15.04	15.75	14.22
		Pkg		*	*			*	*
		Diet	HiOx				PVC		
			Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
		LO	8.80	12.31	11.66	11.9	8.80^{c}	12.61^{b}	15.93^{a}
		ALO	10.88^{c}	12.93^{b}	14.95^{a}	12.07^{b}	10.88	12.65	14.02
	White	HO	9.62^{b}	14.04^{a}	14.60^{a}	11.77^{b}	9.62^{c}	12.39^{b}	15.56^{a}
		AHO	10.55^{b}	14.23^{a}	12.95^{a}	12.63^{a}	10.55^{c}	11.91^{b}	14.86^{a}
		Pkg		*	NS			*	NS
b* Value		Diet	HiOx				PVC		
			Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7
		LO	11.73	14.10	11.27	9.83	11.73	11.7	10.56
		ALO	10.01	14.18	11.8	10.3	10.01	9.87	10.3
	Red	HO	8.21	12.83	11.44	8.95	8.21^{b}	12.02^{a}	8.95^{ab}
		AHO	8.80^{b}	11.75^{b}	12.17^{a}	9.42^{b}	8.80	10.33	9.67
		Package		NS	NS			NS	NS

$^{a–c}$ Means (n = 3) between days within the same diet (same row) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

^{A,B} Means (n = 3) between diets on the same day (same column) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

* Means between packaging systems on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction

varied throughout storage, there was a notable increase in thigh surface color. However, significant differences ($P < 0.05$) between dietary groups were apparent only in breast meat. For breast meat packaged in HiOx, the *a** value of the antioxidant supplemented group remained unchanged throughout storage, potentially indicating a more stable myoglobin structure and reduced oxidation. Although, the lower concentration of myoglobin present in the breast samples may have impacted the results. Various endogenous and exogenous factors influence meat color, such as pH, muscle source, presence of antioxidants, lipid oxidation, temperature, and packaging conditions (Suman and Joseph, 2013). However, thigh samples packaged in HiOx showed a greater reduction in redness in the high-oxidized dietary group (HO, AHO), compared with the low-oxidized treatments (LO, ALO). The colorimetric *b** (yellowness) values of breast meat samples in all packaging systems showed an overall increase over time, yet thigh samples showed an initial increase then decrease throughout storage. The appreciable increase in *b** values in all samples throughout storage indicates that neither antioxidant supplementation nor oxidized oil significantly influenced the yellowness of chicken breast meat during retail display. Similarly, Ryu et al. (2005) reported no improvement in the color stability of chicken meat supplemented with vitamin E or selenium.

5.5. Conclusion

The results indicate that dietary antioxidant supplementation imparts a protective barrier against oxidation of broiler breast and thigh meat under both HiOx and PVC packaging conditions throughout retail storage, thereby minimizing the negative impact of oxidized oil on broiler meat quality. The improved water-holding capacity of meat, the

most notable benefit, can be attributed to the reduced protein oxidation and retention of the myosin heavy chain. Chicken thigh meat showed more extensive oxidative damage due to the higher amount of lipids, heme proteins, and inorganic iron, compared to chicken breast meat.

CHAPTER 6

DIFFERENTIATION OF CHICKEN SARCOPLASMIC PROTEOME AS INDUCED BY DIETARY ANTIOXIDANT SUPPLEMENTATION

6.1. Summary

Animal nutrition can have a major influence on tissue gene expression, and dietary antioxidant supplements can enhance the quality of meat through modification of tissue metabolic processes. This study investigated differences in the sarcoplasmic proteome of breast muscle from broiler chickens fed diets of different Redox potentials. Broilers were fed either a diet with a low-oxidized (peroxide value 23 meq O_2/kg) or high-oxidized (121 meq O_2/kg) oil, supplemented with an antioxidant pack (200 ppm EconomasE and organic minerals Se, Zn, Cu, Mn, and Fe as in Bioplex) for 42 d. Protein expression was analyzed using 2-dimensional gel electrophoresis and mass spectrometry. Statistical analysis indicated that 4 protein spots were differentially abundant ($P < 0.05$) between dietary treatments with spot intensity difference of 1.5-fold or more. Glyceraldehyde 3-phosphate dehydrogenase and creatine kinase were overabundant in birds fed low-oxidized oil supplemented with antioxidants. Triose phosphate isomerase and heat shock protein beta-1 were more abundant in birds fed a diet without antioxidants. Dietary regimen with antioxidants influenced protein expression in broiler breast meat, which may greatly contribute to overall meat quality.

6.2. Introduction

In the United States, there is a continuous demand for high-quality, shelf-stable meat and meat products. Yet, meat quality is a complex concept since there are a variety of extrinsic and intrinsic factors that affect sensory attributes, such as tenderness, juiciness, and flavor. In the post-genomic era, proteomic tools such as two-dimensional gel electrophoresis are utilized to elucidate the biochemical mechanisms influencing muscle-to-meat conversion and meat tenderness (Mullen et al., 2006; Joseph et al, 2012; Paredi et al., 2013). Two-dimensional gel electrophoresis (2D gel) was first utilized to assess changes of porcine muscle in relation to meat tenderness. Lametsch et al. (2003) observed significant changes in 103 myofibrillar protein spots isolated from porcine longissimus dorsi muscle at time of slaughter and at 72 hour postmortem; twenty-seven of these spots were identified. This initial research led to more recent application of 2D gel in the study of beef quality, particularly tenderness.

Tenderness is one of the most important attributes influencing consumption of beef, yet there is a large variation in the rate and extent of post-mortem tenderization, which leads to inconsistency in meat tenderness. Variability in meat tenderness depends, in part, on differences in muscle fiber type (glycolytic vs. oxidative), glycogen content, collagen content, and protease activities. Therefore, muscle composition can greatly affect product quality. Recently, researchers have been exploiting proteomics to identify markers for meat tenderness in cattle (Bendixen, 2005; Bernard et al, 2007; Jia et al, 2009; Kee et al, 2008). Jia et al. (2009) reported that peroxiredoxin-6, an antioxidant enzyme that plays a role in protecting cells from oxidative stress, may be a potential protein marker for post-mortem meat tenderness.

As described above, the majority of proteomic research related to meat quality has primarily focused on beef and, to a lesser extent, pork. Despite the proven success of 2D gel proteomics in revealing some fundamental factors that influence red meat tenderness, it has not been utilized for poultry meat quality. For example, the differential abundance/expression of sarcoplasmic and myofibrillar proteins in broiler meat and their influence on meat quality have not been characterized. Although tenderness may not be a practical issue for most broiler meat, water-binding, drip loss, cooking loss, and flavor are common concerns that are all related to the quality of muscle proteins and metabolic enzymes. How the nutrition and quality of poultry diet affects the expression of muscle proteins and enzymes, as well as their metabolism during meat retail display must be studied.

Recently, nutrigenomic studies coupled with proteomic investigations have indicated a potential link between dietary nutrients and the expression of specific enzymes and metabolites in muscle (Hesketh, 2008). Li et al. (2009) reported that dietary supplementation with α-tocopherol improved meat tenderness and reduced lipid oxidation on broiler breast and thigh meat potentially through the modification of lipid metabolic enzymes. However, the genetic and regulatory mechanisms which define these metabolic physiological changes in muscle tissue are complex and poorly understood. Proteomic techniques are required to fully elucidate the biochemical mechanisms influencing muscle-to-meat conversion and water-holding capacity. Therefore, the objective of our study was to determine the impact of dietary feed, specifically oxidized oil and antioxidant supplementation with a commercial algae-based antioxidant containing Se

yeast and organic minerals, on muscle protein expression using 2D gel electrophoresis and mass spectrometry of broiler breast meat.

6.3 Materials and Methods

6.3.1. Materials

A commercial algae-based antioxidant containing Se yeast, EcoE, and organic minerals, Bioplex, was supplied by Alltech Inc. (Nicholasville, KY). Soybean oil was acquired from a local retailer with an initial peroxide value (POV) of 23 meq O_2/kg as determined according to AOCS (2007). To create oxidized oil, aluminum pans (41×13×4 cm) each containing 5 kg of the above oil were heated in a convection oven at 95 °C ± 5 °C for up to 7 d. The POV of the oxidized oil was monitored intermittently. When the POV reached the target level (120 meq O_2/kg), heating was discontinued and the oxidized oil was cooled to room temperature. The POV of the final pooled oil was 121 meq O_2/kg and was used immediately for diet preparation. All chemicals (reagent grade) were purchased from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

6.3.2. Broiler Production

Three independent feeding trials (n = 3) over a two-year period were performed. In each, 960 male broilers were raised from 1 to 42 d of age and randomly placed in 48 floor pens with 20 birds per pen. Each pen was randomly designated one of four dietary treatments consisting of feeding: (1) basal diet–low oxidized oil (**LO**); (2) basal diet–low

oxidized oil, supplemented with antioxidants (**ALO**); (3) basal diet–high oxidized oil (**HO**); (4) basal diet–high oxidized oil, supplemented with antioxidants (**AHO**). Broilers were randomly distributed into the 4 dietary groups with 12 replicate pens for each diet. Each pen was equipped with a feeder, a nipple drinker line, and a litter of soft wood shavings. Birds consumed feed in mash form and water on an *ad libitum* basis. A starter diet containing 22% crude protein (CP) and 3,120 kcal/kg was fed from 0–21 d of age and a grower diet containing 20% CP and 3,150 kcal/kg was fed from 21–42 d of age (Table 3.1). Photoperiod consisted of 22 h of light and 2 h of dark throughout the experiment.

6.3.3. Tissue Preparation and Isolation of Sarcoplasmic Proteome

After 42 d of feeding, six broilers per dietary treatment (total of 24 per trial) were randomly selected and humanely harvested. Immediately following exsanguination, aliquots of *Pectoralis major* muscle samples (approximately 5 g each) were removed from each broiler, frozen in liquid N_2 (–196 °C), and stored in a –80 °C freezer until use, within one year. Upon sarcoplasmic proteome extraction, partially thawed muscle samples were homogenized in cold extraction buffer (40 mM Tris, 5 mM EDTA, pH 8.0) at the ratio 1:4 (w/v) using a Model 51BL31 micro blender (Waring Commercial, Torrington, CT). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was filtered using a #1 Whatman filter paper.

6.3.4. Two-Dimensional Electrophoresis and Gel Image Analysis

The protein concentration of the sarcoplasmic proteins was determined using the Bradford assay (Bio-Rad). The sarcoplasmic proteome, 1200 µg, was mixed with rehydration buffer (Bio-Rad, Hercules, CA), optimized to 9 M urea, 4% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte, and 0.0001% Bromophenol Blue, loaded onto immobilized pH gradient (IPG) strips (pH 5–8, 17 cm), and subjected to passive rehydration for 16 h. First dimension isoelectric focusing (IEF) was conducted by applying a linear increase in voltage, initially, followed by a rapid voltage ramping to attain a total of 80 kVh, using a Protean IEF cell system (Bio-Rad). After IEF, the IPG strips were equilibrated in equilibration buffer I (6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% Glycerol, 2.5% (w/v) iodoacetamide), then in equilibration buffer II (6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% Glycerol, 2% (w/v) DTT), each for 15 min. The proteins were separated in the second dimension by 12% SDS-PAGE using a Protean XL system (Bio-Rad). The gels were stained with Colloidal Coomassie Blue for 5 days and de-stained for 1 day. Samples from each dietary treatment (LO, ALO, HO, AHO) were ran in duplicate. The stained gels were analyzed using PDQUEST software (Bio-rad) for image analysis.

6.3.5. Protein Identification by Mass Spectrometry

Significantly different ($P < 0.05$) spots were excised from the gel using a scalpel. Excised gel spots were minced and placed in a low-retention microcentrifuge tube and destained two times or until colorless with 50 mMNH_4HCO_3/50% CH_3CN (supernatants were discard) before it was dried in SpeedVac. After reduction with 10

mM DTT (Dithiothreitol) at 56°C for 30min and alkylation with 50 mM IAA (Iodoacetamide) at 25°C for 30min, the gel was dehydrated with ACN and dried with SpeedVac before digestion was performed with trypsin solution (Sigma, 20 ng/ul in 40 mM NH_4HCO_3, 9% CH_3CN) overnight at 37˚C. A solution of 50% ACN with 5% acetic acid was added to decrease the pH of the solution between 2 and 3 to stop the enzymatic reaction.

LC-MS/MS analysis was performed using an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with an Eksigent Nanoflex cHiPLC™ system (Eksigent , Dublin, CA) through a nano-electrospray ionization source. The peptide samples were separated with a reversed phase cHiPLC column (75 μm x 150 mm) at a flow rate of 300 nL/min. Mobile phase A was water with 0.1% (*v*/*v*) formic acid while B was acetonitrile with 0.1% (*v*/*v*) formic acid. A 50 min gradient condition was applied: initial 3% mobile phase B was increased linearly to 50% in 24 min and further to 85% and 95% for 5 min each before it was decreased to 3% and re-equilibrated. The mass analysis method consisted of one segment with eight scan events. The 1st scan event was an Orbitrap MS scan (100−1600 m/z) with 60,000 resolution for parent ions followed by data dependent MS/MS for fragmentation of the 7 most intense ions with collision induced dissociation (CID) method.

The LC-MS/MS data were submitted to a local mascot server for MS/MS protein identification via Proteome Discoverer (version 1.3, Thermo Fisher Scientific, Waltham, MA) against other lobe-finned fish and tetrapod clade taxonomy subset of Swissprot database. Typical parameters used in the MASCOT MS/MS ion search were: trypsin digest with maximum of two miscleavages, cysteine carbamidomethylation, methionine

oxidation, a maximum of 10 ppm MS error tolerance, and a maximum of 0.8 Da MS/MS error tolerance. A decoy database was built and searched. Filter settings that determine false discovery rates (FDR) are used to distribute the confidence indicators for the peptide matches. Peptide matches that pass the filter associated with the strict FDR (with target setting of 0.01) are assigned as high confidence. For MS/MS ion search, proteins with two or more high confidence peptides were considered unambiguous identifications without manual inspection. Proteins identified with one high confidence peptide were manually inspected and confirmed.

6.3.6. Statistical Analysis

Three independent trials (n = 3) each with duplicate gel analyses per dietary treatment (total of 8 per trial) were conducted. PDQUEST software (Bio-rad) was used to detect and match spots from each dietary group which were normalized by expressing the relative quantity of each spot (ppm) as the ratio of individual spot quantity to the total quantity of valid spots. For each spot in a given dietary sample, spot quantity values in triplicate gels were averaged for statistical analysis. A spot was considered to be significant in differential abundance when it was associated with $P < 0.05$ in a pairwise Student t-test.

6.4 Results and Discussion

6.4.1. Sarcoplasmic Proteome Analysis

Colloidal Coomassie blue staining of 2-DE gels was used to determine the effects of dietary feeding regimen (oxidized oil and/or antioxidants) on protein expression (Figure 6.1). Statistical analysis indicated that 4 protein spots were differentially abundant ($P < 0.05$) between dietary treatments with spot intensity difference of 1.5-fold or more (Figure 6.2). The differently abundant protein spots were excised from the gels, subjected to in-gel tryptic digestion, and analyzed by tandem MS. The resulting mass spectra were used to identify proteins through MS/MS ion search using MASCOT software, where all proteins were matched to *Gallus gallus* in the Swissprot database. Table 6.1 details the proteins identified by tandem MS along with their Swissprot accession number, species, functional category, overabundance in diet, molecular weight, isoelectric pH, and sequence coverage. The identified proteins include glyceraldehyde 3-phosphate dehydrogenase, triose phosphate isomerase, creatine kinase, and heat shock protein beta-1.

6.4.2. Metabolic Enzymes

Chicken breast muscle is fairly homogenous and is comprised of predominately fast twitch type IIx, glycolytic fibers. These fibers contain low myoglobin content, few mitochondria, large amount of creatine phosphate, and can utilize ATP quickly. Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) and triose phosphate isomerase are important enzymes in the glycolytic pathway. In the present study, GAPDH was more abundant in ALO compared with LO and AHO. Joseph et al. (2012) reported that

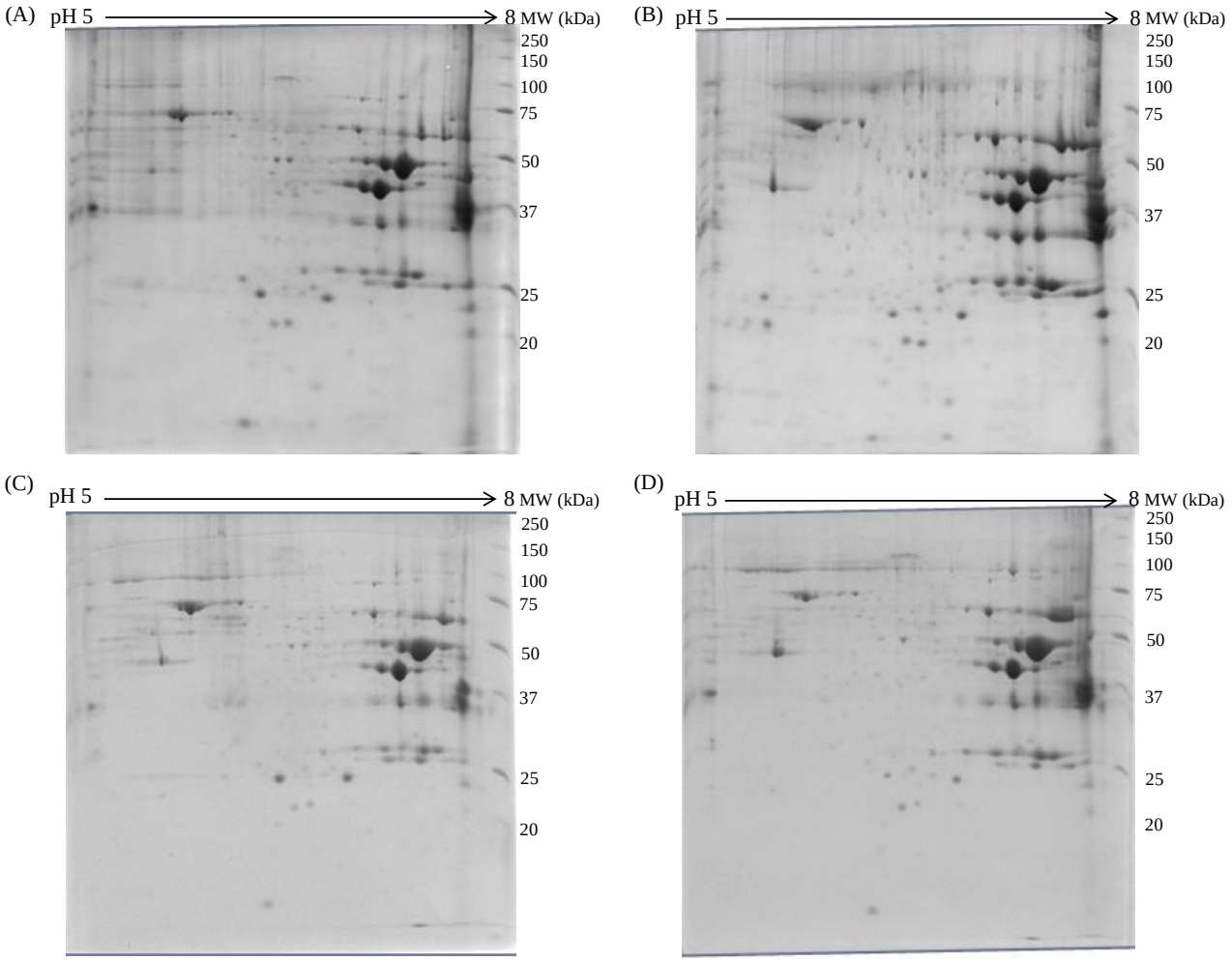

Figure 6.1. Effect of dietary treatments on the Coomassie-stained two-dimensional gels of the sarcoplasmic proteins extracted from fresh chicken breast (A–D). Dietary treatments LO: A; ALO: B; HO: C; AHO: D.

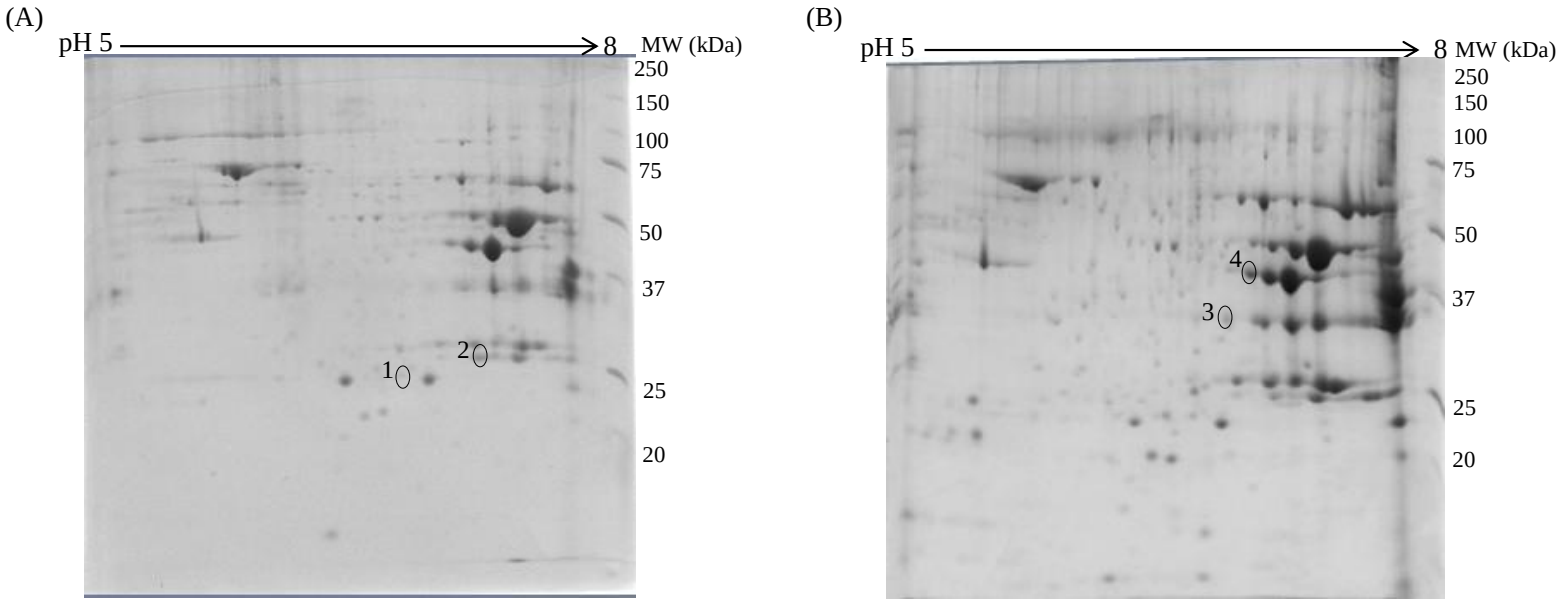

Figure 6.2. Coomassie-stained two-dimensional gels of the sarcoplasmic proteins extracted from fresh chicken breast muscle. Four spots, differentially abundant in LO and ALO are numbered. Dietary treatments LO: A; ALO: B

Table 6.1. Spot number refers to the numbered spots in gel images (Figure 6.2). For each spot, different parameters related to protein identification are provided (Swissprot accession number; species; sequence coverage of peptides in tandem mass spectrometry; theoretical protein mass (kDa) and isoelectric pH (pI)). For each protein, functional category of the protein, diet with greater abundance of the protein, and spot ratio are indicated.

Spot	Protein	Accession Number	Species	Functional Category	Overabundant in Diet	Sequence Coverage	Spot Ratio	Molecular Weight	pI
1	Heat shock protein beta-1	Q00649	*Gallus gallus*	Chaperone	LO	64.77	1.75	21.7	6.1
2	Triosephosphate isomerase	P00940	*Gallus gallus*	Enzyme	HO	78.63	2.00	26.6	7.2
3	Glyceraldehyde-3-phosphate dehydrogenase	P00356	*Gallus gallus*	Enzyme	ALO	56.16	2.00	35.7	8.5
4	Creatine Kinase	P00565	*Gallus gallus*	Enzyme	ALO	71.39	1.61	43.3	7.0

muscles with pronounced glycolytic metabolism had improved color stability. In our previous study (Chapter 4), the *a** value remained unchanged for ALO samples packaged in HiOx and PVC compared with all other dietary treatments, potentially indicating a more stable myoglobin structure due to a greater abundance of GADPH in the tissue. Supplementation with an algae-based antioxidant containing Se yeast may be incorporated into the cellular membrane and reduce lipid and myoglobin oxidation, also improving color stability. However, cattle with more abundant fast gylcolytic type proteins, such as GAPDH and triphosphate isomerase, had less tender meat (Picard et al., 2012), which disagreed with our previous study (Chapter 4). This may be attributed to the difference in species. Furthermore, Anderson (2011) stated that glyceraldehyde-3-phosphate dehydrogenase was not a robust indicator of tenderness across muscles because alterations to the relative abundance of these proteins were not directly related to the amount of tenderization that occurred during postmortem storage. Similarly, Triose 3-phosphate isomerase, another glycolytic enzyme, was higher in HO compared with AHO.

Creatine kinase (**CK**) regenerates ATP by catalyzing the reversible transfer of an N-phosphoryl group from phosphocreatine to ADP (Kenyon and Reed, 1983). There are 5 different isoforms of CK, 3 cytosolic (brain, muscle, and heterodimer) and 2 mitochondrial (ubiquitous and sarcomeric). Muscle type CK has been found to contribute to the M-line, indicating a structural role in the sarcomere (Turner et al., 1973). Upon the conversion of muscle to meat, muscle CK utilizes phosphocreatine to generate ATP through the re-phosphorylation of ADP, which may delay the onset of rigor mortis. In the present study, CK was overabundant in ALO samples compared with AHO. The greater abundance of CK in the ALO samples may have contributed to the overall meat quality

through a delayed onset of rigor mortis. In our previous studies, ALO breast samples had lower lipid and protein oxidation (Chapter 3) and improved tenderness and juiciness (Chapter 4) which may be influenced, in part, by a slower fall in pH during rigor mortis. Thus, the lower carcass temperature and a slower fall in pH could reduce the incidence of pale, soft and exudate (PSE) meat and protein denaturation. Finally, other studies have also shown that mitochondrial CK can act as an antioxidant by reducing the rate of mitochondrial reactive oxygen species (ROS) generation through an ADP re-cycling mechanism (Meyer et al., 2006; Santiago et al., 2008). Therefore, feeding an algae-based antioxidant containing Se yeast and organic minerals to broilers may increase the expression of creatine kinase, acting as an antioxidant against oxidative stress thereby reducing ROS generation, ultimately improving the oxidative stability of lipids and proteins in chicken breast meat.

6.4.3. Small Heat Shock Proteins

Small Heat shock proteins (**sHSPs**) have a molecular mass ranging from 12 to 43 kDa and are comprised of a large and diverse family of proteins that act as molecular chaperones, which assist in the non-covalent folding or unfolding of proteins. The expression of sHSPs is increased in response to different kinds of injury or stress, such as heat shock. Specifically, sHSPs play an important role in the prevention of the formation of insoluble actin aggregates, which can be induced upon heat shock or other stressors (Pivovarova et al., 2007). In the present study, HSP beta-1 was more abundant in LO compared with HO. The higher abundance in the basal dietary group (LO) could be due to a lower degree of oxidative stress *in vivo*. Whitam and Fortes (2008) reported that HSP

expression reduces ROS generation through the activation of antioxidants. In our previous study, broilers fed a high-oxidized diet without antioxidant supplementation had a higher amount of carbonyl formation (protein oxidation) and lower antioxidant enzyme activity in the breast muscle (Figure 3.2). Therefore, the lower amount of HSP beta-1 in HO samples may be due to long term stress lowering the expression of genes encoding for sHSPs, ultimately reducing tissue antioxidant enzymatic activity. Overexpression of sHSPs proportionally increased the level of intracellular glutathione and decreased the level of ROS (Mehlen et al., 1996). Jammes et al. (2009) reported that patients suffering from chronic fatigue syndrome had higher levels of TBARS and lower levels of HSP-27 kDa and HSP-70 kDa compared to healthy individuals.

HSP-27 kDa, specifically, plays an important role in the stabilization of myofibrillar proteins by protecting denatured actin from aggregation (Pivovarova et al., 2005). Actin is an important myofibrillar protein and a primary constituent of the cell cytoskeleton for muscle contraction. HSP-27 kDa forms soluble complexes with denatured actin, thereby protecting the cytoskeleton from damage caused by the accumulation of large insoluble aggregates under heat shock conditions (Pivovarova et al., 2007). Thus, meat samples with higher amounts of HSP-27 kDa may result in better consumption quality (i.e. improved juiciness and tenderness) due to a lower degree of insoluble aggregates present within the myofibrillar matrix. Previous studies have mainly focused on the correlation between protein oxidation and meat quality (Kim et al., 2010; Lund et al., 2011; Rowe et al., 2004). Beef steaks packaged in HiOx had a greater degree of myosin heavy chain cross-linking, due to protein oxidation, and significantly lower tenderness and juiciness scores compared to steaks packaged under vacuum (Kim et al.,

2010). Similarly, Liu et al. (2010) reported increased carbonyl content, higher degree of myofibrillar and sarcoplasmic protein cross-linking and reduced water-holding capacity in pork muscle exposed to a strong oxidizing environment. Therefore, the larger abundance of chaperone proteins present may reduce protein denaturation and aggregation and ultimately improve tenderness and juiciness of meat throughout storage.

6.5. Conclusion

In conclusion, 2-DE and mass spectrometry were utilized to interpret the effects of dietary oxidized oil and antioxidant supplementation on specific protein expression in chicken broiler breast meat. The overabundance proteins functioning as metabolic enzymes and chaperone proteins in the low-oxidized dietary regimen may positively correlate with greater oxidative stability based on previous studies. Thus, more work is needed to elucidate the interaction between dietary regimen and protein expression in order to improve the oxidative stability of meat. Utilizing proteomics to examine the biochemical mechanisms influencing meat quality during the farm to food conversion could lead to the development of more consistent meat products, increased consumer confidence, and cost-effective feed formulations.

CHAPTER 7

OVERALL CONCLUSIONS

In conclusion, feeding diets with poor quality oil increased the vulnerability of lipids and proteins to oxidative processes in broiler breast and thigh meat during refrigerated and/ or frozen storage in various packaging conditions, yet these effects were alleviated upon dietary supplementation with a commercial algae-based antioxidant pack containing Se yeast (EcoE) and organic minerals (Bioplex). The protective barrier imparted by dietary antioxidant supplementation may be attributed to enhanced cellular antioxidant enzymatic activity and reduced ROS propagation *in vivo*. In fresh chicken breast meat, samples from birds fed an antioxidant supplemented diet (ALO, AHO) showed higher catalase, superoxide dismutase, and glutathione peroxidase enzymatic activity, compared to their respective controls, which may have contributed to their greater oxidative stability throughout storage. Furthermore, the reduced lipid and protein oxidation improved the water-holding capacity and tenderness of fresh chicken breast meat, potentially through the minimization of disulfide bond cross-linking of the myosin heavy chain. Although the negative effects of poor quality oil on broiler meat quality were exacerbated in pre-frozen chicken thigh meat, dietary supplementation with antioxidants thwarted these properties.

Proteomic data revealed enzymatic and antioxidative proteins overabundant in the dietary group fed low-oxidized oil, regardless of antioxidant supplementation. Feeding broilers poor quality oil for an extended period appeared to correlate with long term stress, which may lower the expression of genes encoding for antioxidative proteins,

ultimately reducing oxidative stability, meat quality, and storage stability of fresh and pre-frozen chicken breast meat.

The results show that feeding regimen influences gene and protein expression. Therefore, utilizing nutrigenomics to upregulate genes that encode for proteins that favor specific meat quality traits and improve oxidative stability can be done through programmed nutrition. Feeding broilers a diet supplemented with EconomasE–Bioplex can improve chicken meat quality through the upregulation of specific antioxidant proteins, such as glyceraldehyde 3 phosphate, creatine kinase and heat shock protein β-1, and antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase.

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VITA

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