DIETARY LIPID SOURCE AND VITAMIN E INFLUENCE ON CHICKEN MEAT QUALITY AND LIPID OXIDATION STABILITY

A Dissertation

by

CARLOS NARCISO GAYTAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Food Science and Technology

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ABSTRACT

Dietary Lipid Source and Vitamin E Influence on Chicken Meat Quality and Lipid Oxidation Stability. (May 2008)

Carlos Narciso Gaytán, B. A. G., Universidad Autonoma Chapingo;

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In the poultry industry, further processed meat products have the highest share in the market, and because there is a growing demand of food products with enriched amounts of unsaturated fatty acids, the objectives of this research were to assess lipid oxidation development and quality characteristics of chicken meat as affected by dietary fat and vitamin E levels. Broilers were fed during six weeks with diets containing animal/vegetable, lard, palm kernel, soybean, conjugated linoleic acid, flaxseed, or menhaden oil. Each lipid diet was supplemented with either a control (33 or 42 mg/kg) or a supranutritional level (200-400 or 200 mg/kg) of vitamin E. Breast and thigh meat, or skin, were processed, packaged, and refrigerated as raw meat, cooked patties, or cooked *sous vide* meat. The results showed that the chicken meat fatty acid composition reflected those from the dietary fats. In the meat or skin there was a higher lipid oxidation susceptibility as the proportion of unsaturated fatty acids increased, shown as malonaldehyde values, particularly in the treatments with low supplemented level of vitamin E ($P<0.05$). The relative lipid oxidative stability of the meat decreased in

consecutive order from raw, cooked *sous vide*, and cooked meat patties. *Sous vide* cooked meat developed lipid oxidation at a slow rate and showed not to be affected by nonheme iron values. Dietary fat and vitamin E level affected breast meat lightness (L* color space) values ($P<0.05$), but not muscle pH, Allo-Kramer shear force, or water holding capacity. In conclusion, the increment in the proportion of unsaturated fatty acids increases the susceptibility to lipid oxidation in the meat. Supranutritional supplementation levels of vitamin E are more effective at inhibiting the lipid oxidation development in chicken meat than some current levels used by the poultry industry. Neither dietary fat nor vitamin E level seems to affect the development of pale, soft, and exudative meat condition in chicken meat.

DEDICATION

To my wife and children:

Rebecca Gayle Bichsel

Nolan and Sofia Narciso-Bichsel

To my parents:

Said Narciso-Godinez and Teresa Gaytan-Morales

Thank you for all your love, support, and motivation.

You always will be my greatest inspiration. I love you all.

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CHAPTER I

INTRODUCTION

Lipid oxidation in muscle food, particularly in products with relative high content of unsaturated fatty acids, is considered one of the most important factors inducing spoilage, reducing the shelf-life, nutritional value and quality of meat (Pikul et al., 1987; Lin et al., 1987; Bou et al., 2001).

Throughout the years, the poultry industry has changed and adapted to meet the consumer demands of meat products, nowadays furthered processed meats have the highest market share, followed by chicken parts and whole carcasses with approximately 47%, 41% and 12%, respectively. Also, in order to assist in the consumer's health the poultry industry continuous to develop food products enriched with functional compounds, including those with omega-3 fatty acids and perhaps in the future with conjugated linoleic acid (CLA). These groups of polyunsaturated fatty acids have shown positive effects preventing and reducing the risks associated with cardiovascular diseases, rheumatoid arthritis, some types of cancer, obesity, and some other health problems.

However, the production of further processed meat products, especially from those with enhanced amounts of unsaturated fatty acids represent a great challenge for the poultry industry, which struggles to maintain their lipid oxidation stability and quality during prolonged storage and commercialization times, as they are prompt to

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This dissertation follows the style of *Poultry Science*.

develop lipid peroxidation (Gray et al, 1996; Jensen et al., 1998). Lipid peroxidation, commonly known as lipid oxidation, is considered one of the most important factors causing chemical spoilage of muscle food products, which occurs in raw and cooked meats due to exposure to oxidizing agents such as oxygen, heat, light, inorganic iron, enzymes, and other oxidizing initiators (Lin and Hultin, 1976; Asghar et al., 1988; Kanner et al., 1988a) that induce the formation of free radicals, intermediate oxygen species (Kubow, 1992; Nawar, 1996), and by-products such as malonaldehyde, during the development of the lipid oxidation chain reaction. Also, as consequece of the development of lipid peroxidation, the appearance of warmed-over flavors, off-odors, and discoloration result in reduced shelf-life of the meat and processed meat products (Love and Pearson, 1974; Rhee et al., 1996).

Because dietary fats influence the fatty acid composition of cell membranes and certain fatty acids have shown to affect the amount of calcium released from the sarcoplasmic reticulum (Messineo et al., 1984; Fletcher et al., 1990; Williams and Klug, 1995; Negretty et al., 2000), it is possible that dietary fats may also influence the glycolytic metabolism of muscle fibers and induce changes in the quality of the meat. In poultry and pigs, the quality of the meat is affected by human and environmental stressing conditions that induce the release of abnormally high amounts of calcium ions from the sarcoplasmic reticulum (Louis et al., 1993; Wang et a., 1999), particularly in stress susceptible animals that carrying the a genetic mutation in the Ryanodine receptor, known as Halothane gene (Lahucky et al., 1997; Chiang et al., 2004). Halothane gas positive animals' tend to exhibit muscle rigidity and fasten glycolytic metabolism that in

the live animal cause malignant hyperthermia while inducing higher carcass temperature and accelerated accumulation of lactic acid, with rapid drop in muscle pH in postmortem conditions. The combination of high muscle temperature and low pH leads to the development of pale, soft, and exudative (PSE) meat condition, which is characterized by a pale meat color, soft texture and reduced water holding capacity due to protein denaturation of the meat (Pietrzak et al. 1997; Wyenveen et al. 1999; Sandercock et al., 2001; Malheiros et al. 2003).

To prevent the development of lipid oxidation and PSE meat condition in poultry meat, it has been recommended that supranutritional supplementation levels of vitamin E should be included in the diet, approximately 200 mg/kg of vitamin E (Galvin et al., 1997; Lauridsen et al., 1997), in comparison to 10 mg/kg (NRC, 1994) and common commercial supplementing levels that range from about 25 to 45 mg/kg. Alpha-tocopherol, the most biologically active antioxidant isoform of vitamin E, has free radical scavenging properties that inhibit the lipid oxidation chain reaction (Burton and Traber, 1990); as well it has been shown to reduce the rate in muscle pH drop preventing the development of PSE meat condition in broilers (Olivo et al., 2002).

The objectives of the present research were to further elucidate the effect of dietary lipids and vitamin E on the lipid oxidation stability and quality of chicken meat, including raw and cooked meat in conventional and alternative packaging and cooking methods.

CHAPTER II

REVIEW OF LITERATURE

DIETARY FATTY ACIDS IN HUMAN HEALTH

Cardiovascular diseases (CD) and cancer are the leading causes of deaths among the adult population in the United States. Combined, they account for an annual direct and indirect medical cost of $583.3 billion and ~61% of deaths (American Heart Association, 2005; U.S. Cancer Statistics Working Group, 2005). Cardiovascular diseases in part may be increased by consumption of saturated and *trans* fatty acids (Kraus et al., 2000), which have been shown to raise serum triacylglycerols, LDL (low density lipoprotein) and reduced HDL (high density lipoprotein) cholesterol content (Hegsted et al., 1965; Gurr et al., 1989).

To prevent cardiovascular problems it has been recommended to substitute saturated lipid sources in the diet with monounsaturated and polyunsaturated fatty acids (PUFA). In particular, consumption of approximately 500 mg/day of EPA and DHA (Eicosapentaenoic and Docosahexaenoic, respectively) fatty acids has shown to improve human health (Gebauer et al., 2006). Omega-3 fatty acids have shown to provide additional health benefits to the consumers beyond their intrinsic nutritional value. In adults, ω-3 fatty acids have shown to significantly reduce the risks associated with cardio- and cerebrovascular problems, rheumatoid arthritis, depression and inflammatory problems, mainly by replacing araquidonic acid (n-6) in the synthesis of eicosanoids,

prostaglandins and thromboxanes. This has increased blood fluidity, reduced platelet aggregation, serum VLDL and LDL (very low and low density lipoproteins, respectively), and triacylglycerol concentrations (Beynen and Katan, 1985; Kinsella et al., 1990; Simopoulus, 1991; Nestel, 1990; Calder, 1996). Also, omega-3 fatty acids play an important biological role in children's brain, retina and cognitive development (Uauy-Dagach et al., 1994; Horrocks and Yeo, 1999). Additionally, omega-3 fatty acids individually or in combination with CLA (conjugated linoleic acid) modulate and decrease the development of tumors produced in certain types of cancers (Borek, 1994). CLA alone promotes health benefits by reducing the incidence of cardiovascular diseases, obesity and overweight problems by increasing the metabolic energy expenditure and inhibition of preadipocyte differentiation (Blankson et al., 2000).

INFLUENCE OF FAT AND FATTY ACIDS ON CHICKEN MUSCLE

Dietary fats and oils, when consumed by poultry in the proventriculus are partially emulsified and digested by gastric lipase and colipase-dependent lipase activity. Later, in the first portion of the intestine (duodenum) pancreatic lipases hydrolyze triacylglycerols into *sn*-1 and *sn*-3 free fatty acids and 2-monoacylglycerols. With the assistance of bile salts, they are further emulsified into micelles to facilitate their solubility in polar environment and increase intestinal absorption. Free fatty acids, 2-monoacylglycerols, phospholipids, lipid soluble vitamins, and other lipid compounds are absorbed in the intestinal lumen by enterocytes through passive diffusion. Free fatty

acids and monoacylglycerides are re-esterified in the enterocyte and along with other lipid compounds are packaged in chylomicrons to be transported and delivered to other body tissues (Freeman, 1984; Verkade and Tso, 2000).

In poultry, the fatty acid composition in muscle depends on fatty acid synthesis *de novo* in the liver (Leveille et al., 1975; Wakil et al., 1983; Hillgartner et al., 1995) and the source of fatty acids in the diet. Dietary fatty acids are digested, absorbed, transported, and deposited in the body without major structural and chemical changes (Hurtwitz et al., 1973; Sklan et al., 1973; Sklan, 1979; Doreau and Chilliard, 1997), which influences the fatty acid composition of the muscle. In general, chicken meat when compared to beef and pork, contains higher amounts of polyunsaturated fatty acids and fewer saturated fatty acids (Igene and Pearson, 1979). The type, proportion, and amount of either saturated, monounsaturated, or polyunsaturated fatty acids in chicken meat can be modified through inclusion of particular fats and oils in broiler diets (Machlin et al., 1962; Marion and Woodroof, 1963; Yau et al., 1991).

The predominant sources of saturated fatty acids include tallow, lard, and palm kernel oil, while olive and palm oil contain relatively high amounts of monounsaturated fatty acids. Plant derived (soybean, sunflower, flaxseed, linseed, canola oil, etc.) and marine oils (menhaden, tuna, salmon, mackerel oil, etc.) are the most important sources of PUFA (Wiseman, 1984). Incorporation of these lipid sources greatly influences the chicken muscle fatty acid composition, by modifying the neutral lipid and phospholipid fractions, and subcellular organels of the muscle fibers (Lin et al., 1989; Asghar et al., 1990). The particular type, amount, and proportion of fatty acids also influence the

nutritional value and lipid oxidation stability of the meat depending up on the chicken muscle type. It has been reported that dark chicken muscle contains higher amounts of total lipids and phospholipids than light muscle (Lin et al., 1989), as well as higher levels of vitamin E (Lin et al., 1989; Asghar et al., 1990; Monahan et al., 1992; Sheehy et al., 1993; Galvin et al., 1997).

LIPID PEROXIDATION IN MUSCLE FOODS

Lipid peroxidation, commonly known as lipid oxidation, is considered one the most important factors affecting the quality of muscle foods. As shown in Figure 1, lipid peroxidation is induced by the abstraction of a hydrogen atom from polyunsaturated fatty acids (LH), modulated by the presence of molecular oxygen (O_2) or other oxidizing agents such as light, and heat that lead to the formation of lipid radicals (L•), a process known as *initiation*. In the second stage of lipid peroxidation, *propagation*, lipid radicals react with molecular oxygen forming lipid peroxy radicals (LOO•) or hydroperoxyde (LOOH) and a free radical (L•), by the reaction with other fatty acids. The reaction of hydroperoxide with ferrous iron (Fe^{2+}) results in formation of alkoxyl (LO•) and hydroxyl radicals (OH•), and compounds with high reactivity that further extend the lipid oxidation chain reaction in meat systems. The third and last stage, *termination*, occurs when free radicals react with other free radicals forming non-reactive compounds (Asghar et al., 1988; Kubow, 1992; Nawar, 1996; Monahan, 2000).

***Initiation*:**

$$LH + O_2 \longrightarrow L\bullet + \bullet OOH$$

***Propagation*:**

$$L\bullet + O_2 \longrightarrow LOO\bullet$$

$$LH + LOO\bullet \longrightarrow LOOH + L\bullet$$

$$LOOH \longrightarrow LO\bullet + \bullet OH$$

***Termination*:**

$$L\bullet + L\bullet \longrightarrow L + L$$

$$L\bullet + LOO\bullet \longrightarrow LOOL$$

$$LOO\bullet + LOO\bullet \longrightarrow LOOL + O_2$$

Figure1. Lipid Oxidation Chain Reaction (adapted from Asghar et al., 1988).

In muscle foods, lipid peroxidation has been reported to initiate and propagate primarily in the phospholipid fraction of cell membranes, due to the high content of polyunsaturated fatty acids (Igene et al., 1980; Gray et al., 1996; Wagner et al., 1996). Unsaturated, particularly polyunsaturated fatty acids are prompt to undergo lipid peroxidation due to the high amount of double bonds in their carbon chain (Dahle et al., 1962). Rhee et al. (1996) compared different types of meats and observed that chicken meat was more susceptible to develop lipid peroxidation than beef or pork. The higher lipid peroxidation susceptibility of chicken meat is directly influenced by the relatively high amounts of polyunsaturated fatty acids. It has been reported that dark chicken meat

is more susceptible to peroxidation than white meat due to a higher content of phospholipids and unsaturated fatty acids (Igene and Pearson, 1979), as well as higher levels of nonheme iron (Kanner et al., 1988a).

Factors such as “free” iron, oxygen, heat, and light are important oxidizing agents in muscle foods (Wills, 1965; Kanner et al., 1988b). Rhee (1988) reported that in raw meat, lipid peroxidation is induced by both enzymatic and inorganic catalytic activity. Lin and Hultin (1976) showed that NADPH and NADH induced enzymatic lipid peroxidation in the chicken muscle microsomal fraction. The extent or susceptibility to lipid peroxidation seems to depend in great part on the fatty acid composition of the muscle. Asghar et al. (1990) observed that the NADPH-induced lipid peroxidation in microsomes and mitochondria was affected by the degree of unsaturation of fatty acids in the meat; higher lipid peroxidation was detected when linseed oil was included in the diet rather than coconut, olive, or hydrogenated soybean oil. Also, it has been observed that the extent of inorganic iron-induced lipid peroxidation is affected by the fatty acid composition and amount of α-tocopherol present in the muscle tissues. Monahan et al. (1992) observed that iron-induced lipid peroxidation in porcine muscle was higher when soybean oil and low levels of vitamin E (10-50 mg/kg) were included in the diet, compared to tallow and 200 mg/kg of vitamin E. In chicken meat, Lin et al. (1989) observed that feeding broilers with linseed oil as compared to coconut, olive, or hydrogenated soybean oil, increased the amount of polyunsaturated fatty acids in the muscle tissues, resulting in higher malonaldehyde values in chilled and frozen dark and

light meat. These authors also reported that supplementation of 100 mg/kg of α-tocopherol protected the lipid stability of the meat.

Great controversy remains about the pro-oxidant potency and capacity of protein-bound iron and inorganic iron in muscle foods. According to Kanner et al. (1988a), "free" iron is one of the most important initiators of lipid peroxidation. These researchers indicated that as the availability of "free" iron increased there was a higher lipid peroxidation rate in the meat. Love and Pearson (1974) reported that pure myoglobin had no prooxidant effect and that the lipid catalytic activity of iron was induced by the nonheme iron form. Sato and Hegarty (1971) determined that heme compounds had minimal effect on the development of warmed-over flavors in cooked meat and indicated that ferrous iron was the major catalyst of lipid peroxidation and warmed-over flavors. In a study conducted by Liu and Watts (1970) it was observed that both heme and nonheme iron were important lipid peroxidant catalysts. In either case, it is important to mention that iron-induced lipid peroxidation in subcellular fractions have showed that lipid peroxidation is catalyzed by both Fe^{3+} and Fe^{2+} in presence of hydrogen peroxide (H_2O_2) (Minotti and Aust, 1987).

In cooked meats and processed meat products, cooking releases higher amounts of nonheme iron, which has shown to accelerate the lipid peroxidation rate (Rhee et al., 1996). Nonheme iron has also been associated with the appearance of warmed-over flavors, off-odors, and low sensory scores in meat (Tarladgis et al., 1964; Sato and Hegarty, 1971; Chen et al., 1984; Igene et al., 1985; Monahan et al., 1993; Rhee et al., 1996). Igene et al. (1979) reported that phospholipids were the major contributors to the

development of warmed-over flavors in cooked meat model systems rather than triacylglycerols.

VITAMIN E IN POULTRY NUTRITION AND MEAT QUALITY

Vitamin E is a lipid soluble vitamin composed of 4 tocopherol isomers (α, β, γ, and δ) and 4 tocotrienols (α, β, γ, and δ) (Traber, 2000). Among those isomers, α-tocopherol is considered the most biologically potent antioxidant. It functions as a free radical scavenger inhibiting the propagation of the lipid oxidation chain reaction (Burton and Traber, 1990).

In poultry production, dietary supplementation with vitamin E is a common practice because it can not be synthesized by birds and is thus required in the diet. The NRC (1994) recommended supplementing a diet with 10 mg/kg of vitamin E, while in commercial broiler production systems levels of 30 to 45 mg/kg are commonly used. Vitamin E is usually supplemented in the form of d-l-α-tocopheryl acetate to protect its antioxidant activity (Jensen et al., 1998). In the intestinal lumen, d-l-α-tocopheryl acetate is hydrolyzed by pancreatic esterase into its α-tocopherol and acetate parts, during the digestive process (Muller et al., 1976; Jensen et al., 1999). Bjorneboe et al. (1990) reported that α-tocopherol is digested, absorbed, transported, and delivered in the body in the same manner as other lipid compounds, with the exception that no re-esterification between the acetate and the tocopherol parts occurs after the absorption process.

In the muscle, α-tocopherol is deposited and stored in cell membranes and adiposites where it excerpts its antioxidant activity maintaining the lipid stability. Asghar et al. (1990) found that vitamin E in cell membranes strongly protected the phospholipids from lipid peroxidation and that the antioxidant activity depends on the amount of α-tocopherol present in the muscle. Because dietary α-tocopherol is not deposited in muscle tissues as the main priority, supranutritional dietary supplementation levels are required for optimal lipid antioxidant activity. Yamauchi et al. (1991) found that in broilers, the major deposit sites of α-tocopherol are the liver and abdominal fat, and particularly dark muscle contained a 2-fold higher concentration than breast muscle. Sheehy et al. (1991) in a different study, reported that the order of deposition of α-tocopherol was in the heart ≈ lung > liver > thigh muscle > brain tissues, and that at least 180 mg/kg were required to reach muscle plateau level. Muscle deposition of α-tocopherol can be affected by multiple factors such as dietary vitamin E isomer type, supplementation level, feeding period (Sheehy et al., 1991; Bartov and Frigg, 1992; Jensen et al., 1995; Jensen et al., 1999), fatty acid composition (Bieri et al., 1978; Yamauchi et a., 1991) and oxidation level of the dietary oils (Sheehy et al., 1993), factors that could in turn affect the antioxidant activity of vitamin E in the live animal, muscle, and meat products.

Multiple studies have shown vitamin E to be effective at inhibiting lipid peroxidation in muscle foods. However, it has been generally recognized that the recommended level of vitamin E by the NRC (1994) and even the commercial levels used by the poultry industry do not maintain the lipid oxidation stability of chicken meat.

Therefore, dietary supranutritional supplementation levels of vitamin E are required for optimal preservation of the meat, 200 to 400 mg/kg of feed (Galvin et al., 1997). Yamauchi et al. (1991) indicated that supplementation of even 1000 mg/kg of α-tocopherol in broiler diets was not enough to prevent lipid oxidation in refrigerated ground cooked chicken meat. When Jensen et al. (1995) compared 100 and 500 mg of all-rac-α- and RRR-α-γ-δ-tocopheryl acetate, they observed that in raw frozen (6 months) and raw chill stored (8 days) breast and thigh chicken meat, there was no significant lipid oxidation development, and indicated that the antioxidant protection of the high level of vitamin E was negligible in these chicken parts. However, application of a precooking treatment resulted in rapid development of lipid oxidation, as determined by TBARS values. The authors concluded that dietary vitamin E supplementation resulting at 198 mg/kg feed was sufficient to maintain the lipid oxidation stability of precooked chicken meat.

Lauridsen et al. (1997) fed broilers diets containing tallow or olive oil and noted that lipid peroxidation developed faster in subcellular fractions of chicken muscle with dietary supplementation of 20 mg/kg of vitamin E, compared to 200 mg/kg. They concluded that dietary vitamin E promotes stability in the subcellular fractions and suggested that the vitamin E level is more determinant for the lipid stabilization of the meat, than the fatty acid composition. In a separate study, O'Neill et al. (1998) supplemented with 30 and 200 mg/kg of vitamin E in broiler diets and confirmed that the high supplementation level of vitamin E was required to protect refrigerated raw and cooked chicken patties from lipid oxidation. The authors reported significantly higher

values of malonaldehyde with a low vitamin E level on every storage day (0, 2, 4, and 7).

Galvin et al. (1998), evaluated cooked chicken meat from broilers supplemented with 100, 200, or 400 mg/kg of vitamin E and subjected to 0, 2.5, or 4.0 kGy of gamma irradiation, they observed that thigh meat tends to be more susceptible to lipid oxidation than breast meat during refrigerated storage. But in general, supplementation of 200 mg/kg of vitamin E was sufficient to prevent the lipid oxidation in the meat, and 400 mg/kg were required to protect cholesterol products. Also, supranutritional supplementation levels of vitamin E have been shown to have positive effects by preventing warmed-over flavors, rancid aromas, off-odors, and low sensory scores in muscle foods (De Winne and Dirinck, 1996; Bou et al., 2001; Carreras et al., 2004).

It is important to consider that there are additional factors or conditions that may affect vitamin E antioxidant activity for preventing the lipid oxidation of chicken meat. For instance, muscle type, fatty acid composition, total fat and myoglobin content, meat grinding, cooking, additives, packaging system, and storage condition should be taken into account to maximize the lipid oxidation stability of the meat (Bartov and Frigg, 1992; Sheehy et al., 1993; Ahn et al., 1995; King et al., 1995; Morrissey et al., 1998; O'Neil et al., 1999; Bou et al., 2001).

CHICKEN MEAT QUALITY AND PALE, SOFT, AND EXUDATIVE (PSE) MEAT CONDITION

Chicken meat quality is an important factor that drives consumer acceptance and influences the characteristics of further processed products. Meat texture, flavor, color, protein extractability, binding, and water holding capacity are some of the most important characteristics in meat quality (Lyon and Lyon, 2001; Smith, 2001).

The conditions for the transformation of muscle into meat have important implications in the quality of the meat. In normal post mortem metabolic conditions during the transformation of muscle into meat, rigor mortis (muscle death) develops due to the lack of oxygen and energy (ATP) availability. With the reduction of oxygen and the eventual termination of the respiration process, the muscle metabolism shifts from aerobic to anaerobic conditions. In an attempt to maintain homeostasis, the muscle breaks down glycogen to generate energy (ATP); being glycolysis the predominant source of energy. Under anaerobic conditions the end product of glycolysis is lactic acid and its accumulation drops muscle pH to approximately 5.9 to 6.1 units, in normal conditions (Aberle et al., 2001). However, when animals are subjected to antemortem stressing conditions, the glycolytic metabolism is enhanced, resulting in faster drop in muscle pH and increased carcass temperature that combined induce muscle protein denaturation. Denaturation of muscle proteins lowers the quality of the meat and its functional properties (Pietrzak et al. 1997; Wyenveen et al. 1999).

In broilers and turkeys, the selection for fast gain weight and lean meat has produced animals with higher muscle fiber hypertrophy and higher white:red muscle fiber ratio (Lengerken et al., 2002). The fast and high muscle mass production in the animal have shown to lower the muscle capillary density and capillary:muscle fiber ratio causing focal myopathy (Sosnicki and Wilson, 1991). Under stressing conditions, animals with high muscle density experience higher release of sarcoplasmic calcium that leads to the development of hyperthermia and subsequently PSE meat condition (Ferket and Foegeding 1994). Thus, broilers genetically selected for fast growing have higher glycolytic potential and are more likely to develop the PSE meat syndrome (ElRammouz et al., 2004).

Some genetically selected animals for fast growing carried a mutation in the muscle fiber sarcoplasmic calcium channels, a condition known as ryanodine receptor syndrome (Lahucky et al., 1997; Chiang et al., 2004). Animals carrying this gene are sensitive to Halotane gas (H+) and exhibit muscle rigidity as well as higher susceptibility to heat stress and higher sarcoplamic calcium release potential (Louis et al., 1993; Wang et al., 1999). Calcium is an important second messenger in muscle fibers that increases the glycolytic metabolism (Divet et al., 2005), through activation of myofibrillar ATPase and phospholipase-A enzymes (Ferket and Foegeding (1994). Thus, resulting in accelerated drop in muscle pH, 0.3 to 0.9 units, and lower than normal ultimate muscle pH values (Cheah et al., 1984). Also, Santiago (2002) reported that high meat yield lines broilers produced lighter meat (L*) with lower water holding capacity and higher expressible moisture.

PSE meat condition has been shown to develop year round in both turkeys and broilers influenced by genetic, human, and environmental factors (McKee and Sams, 1997; Barut, 1996; Barbut, 1998; Sams, 1999; Owens et al., 2000; Woelfel and Sams, 2001). Sams (1999) indicated that approximately 20-40% of the PSE problem is related to genetic defects and the remaining perhaps could be explained by environmental factors. Among the environmental factors, heat stress could be considered the most severe because it increases glycolytic activity, carcass temperature, protein catabolism and decreased muscle cell membrane stability (Sandercock et al., 2001; Malheiros et al. 2003). Santos et al. (1997) observed that ante mortem temperatures above 35^{o}C combined with high relative humidity (85%) induced PSE meat condition in pork. McCurdy et al. (1996) found that the incidence of PSE in the bird flocks is highest in the summer season, lowest in the winter and intermediate in spring and fall seasons. In a study with turkeys, McKee and Sams (1997) observed that heat-induced stress accelerated the drop in muscle pH and produced pale meat with increased drip and cooked loss.

Little has been reported about the nutritional effect on the incidence and development of PSE meat condition. However, it is possible that PSE meat could be also influenced by the lipid composition of muscle fibers. Under *in vitro* conditions, some experiments have shown that free fatty acids strongly influence the sarcoplasmic calcium exchange process. Messineo et al. (1984) reported that palmitic acid (16:0) significantly enhanced the calcium sequestration process, while oleic acid (18:1) exerted an inhibitory effect by increasing the sarcoplasmic membrane permeability to calcium. Additionally,

Shen and Du (2005), in a study with mice, reported that feeding dietary α-lipoid acid produced higher ultimate muscle pH, primarily by regulating the phosphorylation cascade (glycolysis) in the muscle fibers. Negretty et al. (2000) reported that polyunsaturated fatty acids reduced calcium availability and inhibited the calcium release process from the sarcoplasmic reticulum in cardiac myocytes. Changes in the amount of calcium in the sarcoplasm inhibited or stimulated the glycolytic activity of muscle fiber, due to its activity as a second messenger in muscle cells (Divet et al., 2005). Therefore, efforts to understand how the fatty acid composition is related to PSE development and incidence in poultry meat are needed.

Some other studies have also suggested that nutrition could be a way to control the PSE condition. Vitamin E is known to be a cellular membrane protector that decreases the damage of oxidative stress and global ischemia-reperfusion (Janero, 1991). Hoppe et al. (1998) increased the levels of vitamin E from 20 IU to 260 IU in the diet of pigs and observed that vitamin E lowered the incidence of PSE in stress susceptible animals, primarily by decreasing the glycolytic activity of the muscle, plasma levels of phosphocreatine kinase, preventing damage to cell membranes and reducing the lipid oxidation and deterioration of meat quality characteristics. Duthie et al. (1987) observed that the utilization of dietary supplementation with vitamin E decreased the occurrence of stress-susceptible syndrome in pigs. While Cheah et al. (1995) indicated that dietary supplementation of high levels of vitamin E improved the meat quality, mainly by suppressing the activity of phospholipase A_2, stabilizing the integrity of the cell membranes. Olivo et al. (2001) in a study supplementing broilers with supranutritional

levels of vitamin E (150 and 200 mg/kg during the 1-21 and 22-49 days, respectively) observed a reduction in the characteristics associated with PSE meat condition, including reduced rate in muscle pH drop, protein denaturation, and meat lightness (L*) in both non- and heat-stressed broilers, compared to those fed with commercial levels of vitamin E in the diet.

CHAPTER III

DIETARY FAT AND VITAMIN E EFFECT ON LIPID OXIDATION OF RAW AND COOKED CHICKEN MEAT

INTRODUCTION

In the poultry industry, the highest market segment is mainly composed of further processed meat products. This segment includes products that have been subjected to some degree of cooking or preparation that add convenience to consumers. In addition, there is also a growing demand for food products with enhanced amounts of unsaturated fatty acids, as alternatives for healthier eating, and this trend is currently influencing the production of poultry, as alternative foods rich in these fatty acid sources. Because of this, there is a trend towards enhancing the composition of chicken products by modifying their dietary practices, which in turn influences the deposition of fats in poultry products. However, subjecting the meat to further processing steps, particularly when it contains relatively high amounts of unsaturated fatty acids, may result in rapid lipid peroxidation and development of off-odors, off-flavors, and warmed-over flavors that affect the nutritive value, sensory attributes, and quality of the meat (Igene and Pearson, 1979; Igene et al., 1985).

In raw chicken meat, lipid oxidation development is known to be minimal (Bartov and Frigg, 1992) and considered of little practical relevance because bacterial spoilage appears earlier than noticeable oxidation by-products. However, several studies

have indicated that in raw meat lipid peroxidation could develop during storage time induced by enzymatic (Lin and Hultin 1976; Asghar et al., 1988) and inorganic iron activity (Kanner et al., 1988a). Because fatty acids are susceptible to undergo lipid peroxidation (Dahle et al., 1962), it is likely that the increment of unsaturated fatty acids in the meat lipid composition may result in higher susceptibility to lipid oxidation particularly when the meat is under prolonged storage and commercial display. In contrast, lipid oxidation development has been identified to occur rather rapidly in thermally processed chicken meat, influenced by further processing steps such as grinding and cooking (Rhee et al., 1996), that disrupt the meat compartmentalization and releases higher amounts of non-heme iron. "Free" iron has strong catalytic activity that promotes lipid peroxidation in meat (Kanner et al., 1988a).

One of the alternatives to prevent the lipid oxidation development in chicken meat has been through dietary supplementation of vitamin E in the broilers' diets, a practice that increases the muscle α-tocopherol content (Bartov and Frigg, 1992; Lauridsen et al., 1997; Morrissey et al., 1998; Bou et al., 2001). Alpha-tocopherol is considered the most biologically active antioxidant form of vitamin E, which functions as a free radical scavenger inhibiting the propagation of the lipid oxidation chain reaction (Burton and Traber, 1990; Jensen et al., 1998). Dietary α-tocopherol is deposited in muscle cell membranes (Asghar et al., 1990) where it protects phospholipids from free radical attack. In muscle foods, phospholipids are the primary target for the initiation and propagation of the lipid oxidation (Gray et al., 1996; Wagner et al., 1996). The lipid degree of lipid oxidation in muscle foods can be analyzed by

estimating the development of malonaldehyde values. Malonaldehyde is a group of secondary by-products derived from the degradation of fatty acids, formed by the reaction of aldehydes and ketones with 2-thiobarbituric acid that can be quantified by spectrophotometry (Guillen-Sans and Guzman-Chozas, 1998).

The objectives of the present study were to analyze the dietary fat (animal/vegetable blend, lard, palm kernel, and soybean oil) and vitamin E supplementation level effects on the lipid oxidation stability of commercially processed and tray-packed fresh chicken parts and minced-cooked breast and thigh meat after subjection to prolonged refrigerated storage.

MATERIALS AND METHODS

Six hundred Cobb x Ross broilers were raised during a six week feeding period at the Poultry Science Center at Texas A&M University. The birds were randomly assigned into 8 different treatments and 3 separate replications containing 25 broilers each. Broilers were fed with a basal corn-soybean meal diet formulated and pelleted to include 5% of either animal/vegetable (AV), lard (LA), palm kernel (PK), or soybean (SB) oil as lipid components. Each lipid type diet was supplemented with a low and a high supplementation level with *dl*-α-tocopheryl acetate[1] at 33 and 200-400 mg/kg, respectively. The high vitamin E combination level, 200-400 mg/kg, was supplemented during the starter and growing-finishing period, respectively (Table 1). At the end of the

[1] Rovimix™ 50% Abs. DSM, Inc. Parsippany, NJ

feeding period (42 days), broilers were slaughtered under simulated commercial conditions at the Poultry Science Center pilot processing plant.

Raw Chicken Parts

For the evaluation of fresh chicken samples, three half breasts, bone-in thigh muscles and harvested skin pieces from different carcasses were randomly selected and packaged independently on styrofoam trays over wrapped with PVC packaging shrink film[2]. Tray packages were stored in refrigeration and aerobic conditions at ~4.4^{o}C. The meat and skin packaged samples were subjected to ~1,100 lumens of direct fluorescent light exposure to simulate commercial retail display conditions. Samples were analyzed at 1, 5, 10, and 15 d of storage. For each determination, muscle samples were manually deboned and trimmed of visible connective and adipose tissue.

Cooked Patties

Breast and thigh muscle pieces were kept frozen at -20^{o}C until used to prepare cooked patties. Muscle pieces were allowed to thaw for at least 24 h at 4.4^{o}C before manually deboning and trimming of connective and adipose tissue. Samples were then ground twice through a 1/2" and 1/4" plates in a commercial meat grinder[3]. Patties of 150 g each were hand molded and then aerobically cooked to an internal temperature of

[2] SSD-330 packaging film, Cryovac Co. Duncan, SC
[3] model 4612 Hobart Corp. Troy, OH

74°C in a convection oven[4]. Internal temperature of the patties was monitored with an Omega Type-T thermometer[5]. After thermal processing, the patties were cooled and placed on styrofoam trays, wrapped with packaging film, and held aerobically under refrigerated conditions for 0, 1, 3, and 6 days for sampling.

2-Thiobarbituric Acid Reactive Substances (TBARS)

TBARS analysis was conducted to determine the lipid oxidation stability of fresh and cooked chicken meat (Rhee, 1978). In duplicate, 30 g of meat samples were homogenized using a laboratory blender with the addition of EDTA-propyl gallate[6] to prevent further lipid oxidation of the meat. Fifty ml of malonaldehyde were extracted by distillation and a 5 ml aliquot was added to 5 ml of 2-thiobarbituric acid[7] solution followed by boiling in a water bath for 35 min. Samples were cooled for up to 10 min and then read using a spectrophotometer[8] at 530 nm wavelength by the use of a 1.5 ml UV-Visible light cuvettes[9]. Spectrophotometer values of malonaldehyde were adjusted by a correction factor (7.8) to calculate mg per kg of muscle (Tarladgis et al., 1960).

[4] model DN097, Hobart Corp. Troy, OH
[5] model HH501BT, Omega Engineering, Inc. Stamford, CT
[6] Sigma-Aldrich, Inc. St. Louis, MO
[7] Sigma-Aldrich, Inc. St. Louis, MO
[8] Cary 300 Bio UV-Visible Spectrophotometer,Varian Inc. Walnut Creek, CA
[9] VWR International

Table 1. Broilers Basal Experimental Diets

Ingredient	Starter (0-3 Wk)	Grower (4-5 wk)	Finisher (6 wk)
		%	
Corn	55.56	61.32	66.28
Soybean meal (48%)	35.45	29.88	29.89
Fat[1]	5.00	5.00	5.00
Biofos 16/21	1.56	1.35	1.44
Limestone	1.43	1.28	1.36
Salt	0.46	0.35	0.35
DL-Methionine 98	0.22	0.21	0.19
Lysine HCL	0.049	0.177	0.120
Choline CL 60	0.100	0.100	0.110
Coban 60	0.075	0.075	.
Mineral premix	0.050	0.050	0.050
Vitamin premix	0.025	0.025	0.025
Sodium bicarbonate	.	0.151	0.180
Calculated Nutrient Content			
Crude Protein (%)	22.10	20.00	19.82
ME energy (Kcal/lb)	3162.17	3224.50	3224.50
Calcium (%)	0.90	0.80	0.79
Available Phosphorous (%)	0.70	0.64	0.39
Methionine (%)	0.55	0.51	0.51
Methionine + Cystine (%)	0.92	0.85	0.83
Lysine (%)	1.23	1.18	1.17
Threonine	0.83	0.74	0.73
Sodium (%)	0.20	0.20	0.20

[1] 0.002% of sand was added to the soybean oil diet to make all diets isocaloric.
Mineral premix: Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8500 ppm, I 2100 ppm, Se 500 ppm, Mo 1670 ppm (Tyson Poultry 606 premix)
Vitamin premix (lb): A 14,000,000 I. U., D3 5,000,000 I. chick U., E 60,000 I. U., B12 24 mg, Riboflivin 12,000 mg, Niacin 80,000 mg, d-pantothenic acid 20,500 mg, K 2,700 mg, Folic acid 1,800 mg, B6 5,000 mg, Thiamine 4,000 mg, d-Biotin 150 mg (DSM Nutritional Products, Inc. Custom Premix, Sanderson Broiler Premix, Laurel, MO).

Fatty Acid Methyl Esters

Fat analysis of fresh breasts and thighs, and cooked patties made from both breast and thighs was performed by Nuclear Magnetic Resonance[10]. Dietary fats (Table 2) and muscle fatty acid methyl esters analysis were conducted by extracting total fat with methanol:chloroform. Fatty acid methyl esters were quantified by Gas Chromatography using a Varian Gas Chromatograph[11] fixed with a CP-8200 autosampler following the procedure established by Smith et al. (2002).

Statistical Analysis

The statistical analysis was performed using the General Linear Model Procedure of SAS (SAS Institute, 2002). A Completely Randomized Block Design with a 4 x 2 x 4 factorial arrangement, repetition was used as blocking factor. Factor A, B, and C were dietary fat, vitamin E level, and storage day, respectively.

[10] Smart Track System, CEM Co. Mathews, NC
[11] model CP-3800, Varian Inc. Walnut Creek, CA

Table 2. Fatty Acids Methyl Esters of Dietary Fats

Fatty acid	Fat			
	Animal Vegetable	Lard	Palm Kernel	Soybean Oil
	(% of Total Lipids)			
C12:0	.	.	46.73	.
C14:0	0.60	1.34	17.69	0.08
C16:0	24.37	23.91	9.72	10.95
C16:1	7.70	1.94	.	0.06
C18:0	5.85	16.80	2.60	4.06
C18:1	38.58	36.46	19.43	23.94
C18:1 c11	1.35	2.50	0.08	1.36
C18:2	17.93	12.05	3.08	52.01
C18:3	0.78	0.57	0.11	5.04
SFA[1]	30.81	42.04	76.74	15.08
MUFA[2]	47.62	40.89	19.50	25.35
PUFA[3]	18.71	12.62	3.19	57.05
PUFA/SFA	0.61	0.30	0.04	3.78

[1]SFA: saturated fatty acids (12:0, 14,0, 16:0, 18:0)
[2]MUFA: monounsaturated fatty acids (16:1, 18:1, 18:1c11)
[3]PUFA: polyunsaturated fatty acids (18:2, 18:3)

RESULTS

Chicken Muscle Total Fat and Moisture Content

In Table 3, the effect of dietary fats and vitamin E level on total chicken muscle fat and moisture contents is reported. In breast muscle, total fat content was affected by dietary fat ($P<0.05$), but no effect was observed from the supplemented vitamin E level. Palm kernel oil decreased muscle intramuscular fat content compared to the animal/vegetable and soybean oil, but not to the lard treatment. However, in thigh muscle no effect from dietary fat or vitamin E was detected in total fat content. Dietary vitamin E level did not influence the total fat content in neither type of chicken muscle ($P>0.05$). Breast and thigh muscle total moisture contents were not affected by either dietary fat or vitamin E level.

Chicken Muscle Fatty Acid Methyl Esters

The fatty acid methyl esters results from raw breast and thigh muscle tissues are summarized in Tables 4 and 5. The dietary fat significantly ($P<0.01$) affected the type and proportion of fatty acids deposited in both breast and thigh muscle tissues. Palm kernel oil induced the deposition of lauric acid (12:0) and raised the overall content of saturated fatty acids when compared to other lipid sources. On the contrary, soybean oil significantly increased the overall deposition of PUFA, particularly linoleic (18:2) and

linolenic (18:3) fatty acids compared to the other dietary fat treatments. On the other hand, animal/vegetable and lard increased the overall content of MUFA compared to palm kernel oil and soybean oil. In general, soybean oil increased the PUFA/SFA ratio compared to the other dietary fat treatments. Palm kernel oil decreased the PUFA/SFA ratio only in thigh muscle.

Table 3. Raw Chicken Muscle Total Fat and Moisture Content Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Muscle	**Breast**		**Thigh**	
	Fat (%)	**Moisture (%)**	**Fat (%)**	**Moisture (%)**
Dietary Fat				
Animal/ Vegetable	1.72 [a]	75.19	1.95	77.52
Lard	1.68 [ab]	75.13	2.13	77.00
Palm Kernel	1.53 [b]	75.17	2.26	77.10
Soybean Oil	1.86 [a]	74.94	2.31	77.22
P-value	0.0141	0.5781	0.1864	0.2401
Vitamin E (mg/kg)				
Low	1.66	75.17	2.19	77.10
High	1.73	75.05	2.14	77.32
P-value	0.2301	0.3967	0.6367	0.2405
Root MSE[1]	0.14	0.33	0.29	0.44

a, b: least squares means between rows with different letters are significantly different
[1]Root mean square error

Table 4. Fatty Acids Methyl Esters of Raw Breast Chicken Muscle Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Fatty acid	Fat				Vitamin E		Root MSE[1]
	Animal/ Vegetable	Lard	Palm Kernel	Soybean Oil	Low	High	
	(% of Total Fat)						
C12:0	.	.	1.43	.	.	.	
C14:0	0.40 [b]	0.56 [b]	2.83 [a]	0.30 [b]	1.12	0.93	0.47
C16:0	20.10 [a]	20.95 [a]	20.22 [a]	18.11 [b]	19.73	19.96	0.78
C16:1	4.09 [a]	2.98 [b]	2.94 [b]	2.08 [c]	3.00	3.00	0.29
C18:0	8.65 [b]	9.60 [a]	9.02 [ab]	8.87 [ab]	9.19	8.88	0.66
C18:1	32.88 [a]	33.48 [a]	30.04 [b]	27.27 [c]	30.90	30.94	1.49
C18:1*c*11	2.64 [a]	2.63 [a]	2.12 [b]	1.84 [c]	2.38	2.23	0.20
C18:2	18.04 [b]	17.70 [b]	18.24 [b]	28.88 [a]	20.48	21.00	1.50
C18:3	0.73 [b]	0.67 [b]	0.69 [b]	2.00 [a]	1.00	1.04	0.09
C20:4	3.77	3.59	3.90	3.54	3.68	3.72	0.97
SFA[2]	29.15 [c]	31.11 [b]	33.51 [a]	27.24 [d]	30.03	30.48	1.67
MUFA[3]	39.54 [a]	39.09 [a]	35.10 [b]	31.18 [c]	36.28	36.17	1.82
PUFA[4]	22.54 [b]	21.96 [b]	22.85 [b]	34.54 [a]	25.16	25.77	3.55
PUFA/ SFA	0.77 [b]	0.70 [b]	0.69 [b]	1.27 [a]	0.85	0.87	0.01

a, b, c, d: least squares means between columns with different letters are different ($P<0.01$)

[1]Root mean squares error

[2]SFA: saturated fatty acids (12:0, 14,0, 16:0, 18:0)

[3]MUFA: monounsaturated fatty acids (16:1, 18:1, 18:1c11)

[4]PUFA: polyunsaturated fatty acids (18:2, 18:3, 20:4).

Table 5. Fatty Acids Methyl Esters of Raw Thigh Chicken Muscle Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Fatty Acid (%)	Fat				Vitamin E (mg/kg)		Root MSE[1]
	Animal Vegetable	Lard	Palm Kernel	Soybean Oil	Low	High	
	(% of Total Fat)						
C12:0	.	.	4.11	.	.	.	
C14:0	0.76 [b]	0.81 [b]	3.98 [a]	0.62 [b]	1.51	1.57	0.18
C16:0	21.12 [a]	21.07 [a]	21.01 [a]	18.82 [b]	20.48	20.55	0.43
C16:1	4.29 [a]	3.68 [a]	3.68 [a]	2.62 [b]	3.59	3.55	0.27
C18:0	9.78	9.49	8.52	8.71	9.12	9.13	1.61
C18:1	31.01 [a]	31.63 [a]	28.35 [b]	25.64 [c]	29.20	29.11	2.23
C18:1 c11	2.17 [a]	2.26 [a]	1.64 [b]	1.63 [b]	1.88	1.97	0.02
C18:2	17.87 [b]	17.84 [b]	16.81 [b]	26.55 [a]	19.81	19.72	4.72
C18:3	0.64 [b]	0.66 [b]	0.66 [b]	1.96 [a]	1.00	0.95	0.02
C20:4	3.60	3.80	3.13	3.69	3.49	3.61	0.54
SFA[2]	31.66 [b]	31.37 [b]	37.62 [a]	28.16 [c]	32.04	32.36	3.88
MUFA[3]	37.63 [a]	37.72 [a]	34.19 [b]	30.00 [c]	34.87	34.90	3.76
PUFA[4]	22.11 [b]	22.31 [b]	20.60 [b]	39.19 [a]	24.29	24.31	7.59
PUFA/ SFA	0.70 [b]	0.72 [b]	0.55 [c]	1.15 [a]	0.77	0.79	0.01

a, b, c: least squares means between columns with different letters are different ($P<0.01$)

[1]Root root mean squares error

[2]SFA: saturated fatty acids (12:0, 14,0, 16:0, 18:0)

[3]MUFA: monounsaturated fatty acids (16:1, 18:1, 18:1c11)

[4]PUFA: polyunsaturated fatty acids (18:2, 18:3, 20:4).

It is important to indicate that among the fatty acids identified, stearic (18:0) and araquidonic (20:4) fatty acids were the only ones not affected by the dietary lipid composition. Similarly, dietary vitamin E level did not influence the deposition of fatty acids in chicken muscles and no interactions between dietary fat and vitamin E levels were observed in any of the fatty acids identified.

Lipid Oxidation Stability of Raw Chicken Meat and Skin

Raw breast meat lipid oxidation stability was no significantly (P>0.05) affected by dietary fat (Table 6). However, there was an interaction between vitamin E level and storage day, showing higher values of malonaldehyde in meat samples from chicken fed with low levels of vitamin E sampled at day 10 of refrigerated storage (Table 7).

Table 6. Malonaldehyde Values (mg/kg) of Raw Chicken Breast Meat Affected by Dietary Fat (Least Squares Means)

Dietary Fat	Animal/ Vegetable	Lard	Palm Kernel	Soybean Oil	Root MSE[1]	*P*-value
	0.13	0.12	0.12	0.13	0.06	0.7400

[1] Root mean square error.

Table 7. Malonaldehyde Values (mg/kg) of Raw Chicken Breast Meat Affected by the Interaction of Vitamin E Level and Storage Day (Least Squares Means)

Vitamin E/ Storage Day	Low[1]	High[2]	Root MSE[3]	*P*-value
1	0.14 $^{b\,y}$	0.15 $^{a\,x}$	0.06	0.0001
5	0.11 $^{b\,x}$	0.10 $^{b\,x}$		
10	0.16 $^{a\,x}$	0.09 $^{b\,y}$		
15	0.18 $^{a\,x}$	0.07 $^{b\,y}$		

a, b/ x, y least squares means between rows/columns with different letters are significantly different.
[1]33 mg/kg 6 week feeding period
[2]200 and 400 mg/kg during 0-3 and 4-6 weeks, respectively
[3]Root mean square error.

In raw thigh meat (Table 8) and skin (Table 9) samples, the lipid oxidation stability was affected by the interaction of dietary fat and storage day. Higher malonaldehyde values were detected in the treatments with low level of vitamin E at day 10 of storage, compared to the other treatments. In thigh meat the lipid oxidation stability of the meat from the animal/vegetable, lard, and palm kernel oil treatments did not significantly ($P>0.05$) changed during the entire storage time. An interaction between dietary fat and storage day was observed at day 5 and 10 of refrigerated storage, in thigh meat and skin, respectively, compared to the supranutritional dietary level.

Lipid Oxidation Stability of Cooked Chicken Patties

The lipid oxidation stability of cooked breast meat patties was affected by the interaction of dietary fat and vitamin E level, fat and storage day, as well as vitamin E level and storage day (Table 10). With the low level of vitamin E, patties from the soybean oil and animal/vegetable blend treatments were the most susceptible to develop lipid oxidation, while patties from the palm kernel oil treatment showed the lowest lipid oxidation among the fat treatments. At the high level of vitamin E, patties from the soybean oil treatment also showed the highest malonaldehyde values.

With respect to dietary vitamin E levels, patties from the supranutritional supplemented level showed higher lipid oxidation stability than the control level, at day 1, 3, and 6. Patties from the low dietary level of vitamin E had 2.9, 3.8, and 3.3-fold higher levels of malonaldehyde than the supranutritional level treatment.

In cooked thigh meat patties, the lipid oxidation development was affected by dietary fat effect, as well as the interaction of vitamin E level with storage day. Patties from the soybean oil treatment showed higher malonaldehyde values than the other fat treatments (Table 11). With respect to vitamin E level, over storage time patties from the low level of vitamin E had higher lipid oxidation development, at day 1, 3, and 6 the malonaldehyde values were 1.8, 2.0, and 2.4-fold higher, respectively, than in the supranutritional supplemented level treatment patties.

Table 8. Malonaldehyde Values (mg/kg) of Raw Chicken Thigh Meat Affected by the Interaction of Dietary Fat or Vitamin E Level with Storage Day (Least Squares Means)

Dietary Fat x Storage Day

Day	Animal/ Vegetable	Lard	Palm Kernel	Soybean Oil
1	0.13 $^{a\,x}$	0.11 $^{a\,x}$	0.10 $^{a\,x}$	0.10 $^{c\,x}$
5	0.14 $^{a\,x}$	0.16 $^{a\,x}$	0.11 $^{a\,x}$	0.16 $^{c\,x}$
10	0.20 $^{a\,y}$	0.25 $^{a\,y}$	0.16 $^{a\,y}$	0.31 $^{b\,x}$
15	0.27 $^{a\,y}$	0.26 $^{a\,y}$	0.19 $^{a\,y}$	0.53 $^{a\,x}$
P-value	0.0003			

Vitamin E Level x Storage Day

Day	Low[1]	High[2]
1	0.12 $^{c\,x}$	0.10 $^{b\,x}$
5	0.18 $^{c\,x}$	0.10 $^{b\,y}$
10	0.31 $^{b\,x}$	0.15 $^{b\,y}$
15	0.40 $^{a\,x}$	0.22 $^{a\,y}$
P-value	0.0038	
Root MSE[3]	0.11	

a, b, c/ x, y least squares means between rows/columns with different letters are significantly different.
[1]33 mg/kg 6 week feeding period
[2]200 and 400 mg/kg during 0-3 and 4-6 weeks, respectively
[1]Root mean square error.

Table 9. Malonaldehyde Values (mg/kg) of Raw Chicken Skin Affected by the Interaction of Dietary Fat or Vitamin E Level with Storage Day (Least Squares Means)

Dietary Fat x Storage Day

Day	Animal/ Vegetable	Lard	Palm Kernel	Soybean Oil
1	0.08 $^{b\,x}$	0.16 $^{a\,x}$	0.08 $^{a\,x}$	0.10 $^{c\,x}$
5	0.09 $^{b\,x}$	0.05 $^{b\,x}$	0.06 $^{a\,x}$	0.07 $^{c\,x}$
10	0.14 $^{b\,xy}$	0.14 $^{a\,xy}$	0.07 $^{a\,y}$	0.19 $^{b\,x}$
15	0.19 $^{a\,y}$	0.19 $^{a\,y}$	0.11 $^{a\,y}$	0.39 $^{a\,x}$
P-value	0.006			

Vitamin E Level x Storage Day

Day	Low	High
1	0.13 $^{c\,x}$	0.09 $^{a\,x}$
5	0.09 $^{c\,x}$	0.05 $^{a\,x}$
10	0.19 $^{b\,x}$	0.08 $^{a\,y}$
15	0.33 $^{a\,x}$	0.11 $^{a\,y}$
P-value	0.002	
Root MSE[1]	0.12	

a, b, c/ x, y least squares means between rows/columns with different letters are significantly different.
[1]Root mean square error.

Table 10. Malonaldehyde Values (mg/kg) of Cooked Breast Meat Patties Affected by Dietary Fat and Vitamin E Level, Fat and Storage Day, and Vitamin E and Storage Day (Least Squares Means)

Fat x Vitamin E Level

	Animal/ Vegetable	Lard	Palm Kernel	Soybean Oil
Low	4.24 $^{a\,xy}$	3.99 $^{a\,y}$	2.82 $^{a\,z}$	4. 62 $^{a\,x}$
High	0.85 $^{b\,y}$	1.02 $^{b\,y}$	0.65 $^{b\,y}$	2.30 $^{b\,x}$
P-value	0.0125			

Fat x Storage Day

0	0.46 $^{d\,x}$	0.52 $^{d\,x}$	0.37 $^{d\,x}$	0.84 $^{d\,x}$
1	1.48 $^{c\,y}$	1.46 $^{c\,y}$	1.09 $^{c\,y}$	2.32 $^{c\,x}$
3	3.46 $^{b\,x}$	3.40 $^{b\,x}$	2.27 $^{b\,y}$	4.13 $^{b\,x}$
6	4.78 $^{a\,y}$	4.64 $^{a\,y}$	3.32 $^{a\,z}$	6.56 $^{a\,x}$
P-value	0.0028			

Vitamin E Level x Storage Day

Day	Low	High
0	0.68 $^{d\,x}$	0.41 $^{c\,x}$
1	2.35 $^{c\,x}$	0.82 $^{c\,y}$
3	5.24 $^{b\,x}$	1.39 $^{b\,y}$
6	7.39 $^{a\,x}$	2.21 $^{a\,y}$
P-value	0.0001	
Root MSE[1]	0.49	

a, b, c, d/ x, y, z least squares means between rows/columns with different letters are significantly different.
[1]Root mean square error.

Table 11. Malonaldehyde Values (mg/kg) of Cooked Thigh Meat Patties Affected by Interaction of Dietary Fat or Vitamin E Level with Storage Day (Least Squares Means)

Dietary Fat				
Fat	Animal Vegetable	Lard	Palm Kernel	Soybean Oil
	3.48 [b]	3.52 [b]	3.32 [b]	5.97 [a]
P-value	0.0001			
Vitamin E Level x Storage Day				
Day	Low	High		
0	1.20 [d x]	0.68 [c y]		
1	3.72 [c x]	2.07 [b y]		
3	6.71 [b x]	3.41 [a y]		
6	10.39 [a x]	4.37 [a y]		
P-value	0.0001			
Root MSE[1]	1.38			

a, b, c, d/ x, y, z least squares means between rows/columns with different letters are significantly different.
[1]Root mean square error.

DISCUSSION

The dietary fat effect on total fat content in chicken muscle was found to be muscle dependent. In breast muscle, total fat was lower in the palm kernel oil treatment compared to the animal/vegetable and soybean oil counterparts, but not significantly

different from lard treatment. These results suggest that feeding broilers with lipid sources containing relatively high amounts of saturated fatty acids such as in the case of palm kernel oil and lard reduced breast muscle total fat content. Analysis of the fatty acid methyl esters of the fats used for the preparation of the diets showed that palm kernel oil had the highest percent of saturated fatty acid (76.74), followed by lard (42.04), animal/vegetable (30.81), and soybean oil (15.08). In previous experiments, the feeding of broilers with coconut, olive, or linseed oil showed no significant effects on the in intramuscular fat content (Lin et al., 1989). However, even though contrary to our results, it has been reported that the accumulation of lipids could be influenced by different dietary fatty acids sources, some studies indicate that the deposition of adipose tissue in the abdominal cavity of broilers is increased by unsaturated rather than saturated fatty acids in the diet (Sanz et al., 1999; Crespo and Steve-Garcia, 2002). It is unclear what the factors are triggering a higher lipid accumulation in the muscle tissues, hence further research is required to elucidate this issue.

The fatty acid composition of chicken muscle samples reflected those from the dietary oils, confirming that dietary fats influence the lipid composition of chicken muscle (Yau et al., 1991). In general, inclusion of soybean oil and palm kernel oil significantly ($P<0.01$) increased the proportion of PUFA and SFA, respectively, while animal vegetable oil and lard induced higher deposition of MUFA than the other treatments. These changes in proportion of fatty acids resulted in the highest and lowest PUFA/SFA ratio in the soybean and palm kernel oil treatment, respectively. Valencia et al. (1993) in a similar study in broilers, also reported that palm kernel oil induced

deposition of lauric fatty acid, increased the proportion of saturated fatty acids, and reduced the mono- and polyunsaturated fatty acids in muscle when, compared to palm or poultry oil used as lipid source in the diet.

In raw meat, breast meat showed no lipid oxidation susceptibility associated with the different dietary fats during the entire storage time of the samples; however, the supranutritional level of vitamin E lowered malonaldehyde values over storage time, showing its high antioxidant activity. On the other hand, raw thigh meat and raw skin were susceptible to develop lipid oxidation, especially in samples from chicken fed with soybean oil and low vitamin E supplementation levels. This indicates that over storage time this type of chicken meat is prompt to develop lipid oxidation. Thus, when broilers are fed with unsaturated sources of fatty acids, the supranutritional supplementation of vitamin E would be required to prevent lipid oxidation development in meat and skin.

Igene and Pearson (1979) earlier reported that increased amounts of unsaturated fatty acids in the muscle cell membranes increased the susceptibility to develop lipid peroxidation in the meat. Asghar et al. (1988) observed that the inclusion of linseed oil in broilers' diets increased the amount of unsaturated fatty acids in the muscle microsomal fraction and increased the lipid oxidation development when compared to coconut and olive oil. Lauridsen et al. (1997) also reported that chicken muscle mitochondria and microsome fractions were more susceptible to lipid oxidation, showing higher malonaldehyde values with olive oil samples as compared to tallow. Morrissey et al. (1998) estimated that approximately the oxidation rate of fatty acids containing 1, 2, 3, 4, 5, or 6 double-bonds developed 0.025, 1, 2, 4, 6, and 8-times faster, respectively.

Also, Wood et al. (2003) indicated that α-linolenic fatty acid (18:3) contents close to 3% in the muscle tissues is determinant to cause negative effects on the lipid oxidation stability of the meat.

In cooked chicken patties, for both breast and thigh samples, there as a significantly faster development of lipid oxidation than in raw product as expected. This confirms that further processing steps such as meat grinding and thermal processing accelerate the development of lipid oxidation in chicken meat. As well, patties from the soybean oil treatment and those from the low dietary level of vitamin E showed higher lipid oxidation development compared to all the other treatments. These results indicate that in cooked chicken meat the deposition of higher proportion of unsaturated fatty acids also reduces the lipid oxidation stability, particularly when relatively low levels of vitamin E are supplemented in the broilers' diets. Therefore, results indicated that 200 mg/kg of vitamin E were effective at inhibiting the lipid oxidation in the cooked chicken meat when compared to the current commercial level (33 mg/kg) used by the poultry industry.

After cooking and storage, the degradation of total lipids and unsaturated fatty acids has been shown to play an important role in the development of lipid oxidation and warmed-over flavors of poultry meat (Igene and Pearson, 1979). The action of oxidizing agents such as heat, oxygen, and inorganic iron (nonheme), has been shown to further accelerate the peroxidation of unsaturated fatty acids in muscle foods (Igene et al., 1985; Kanner et al., 1988a; Kanner et al., 1988b; Rhee et al., 1996). Similar results were observed in these experiments.

The dietary supplementation levels of vitamin E appear to be important in inhibiting the lipid oxidation development in muscle foods, especially in further processed meat samples. Supranutritional supplementation of vitamin E has been shown to be more effective at inhibiting the meat lipid peroxidation in raw, cooked meat, and lipid peroxidation models. Lauridsen et al. (1997) reported that 200 mg/kg of vitamin E in the diet was more effective at stabilizing the lipid integrity of breast and thigh muscle mitochondria and microsomes, than 20 mg/kg, as shown by lower values of malonaldehyde. These authors also indicated that the α-tocopherol content is more determinant for the lipid oxidation stability of chicken meat than the actual fatty acid composition of the meat. The results obtained in the present experiment support these observations because lower malonaldehyde values were detected in the treatments with supranutritional supplementation of vitamin E, regardless of the type of fat included in the diet.

In raw chicken meat, Jensen et al. (1995) indicated that supplementation with 100 mg/kg of vitamin E provided minor benefits for the oxidative stability of the meat because raw meat lipid oxidation development in breast and thigh meat samples up to 8 days of refrigerated storage was not significant. However, in our study, we observed that in thigh meat and skin samples the lipid oxidation was significant ($P<0.05$) when soybean oil and the low vitamin E levels were included in the diet, at day 5 and 10 of refrigerated storage, respectively. These results indicate that the lipid oxidation in raw chicken meat is also influenced by the inherent composition of the muscle or skin. Kanner et al. (1988a) indicated that dark chicken meat contained higher amounts of total

lipids and nonheme iron, which turn increased the susceptibility of lipid oxidation in this type of chicken meat.

The antioxidant activity of vitamin E depends on the amount deposited in the cell membranes, where it functions as a free radical scavenger by inhibiting the propagation of the lipid oxidation chain reaction (Asghar et al., 1988). In poultry, the amount of α-tocopherol deposited in the muscle cell membranes is directly related to the amount of vitamin E included in the diet and the length of the feeding period (Sheehy, 1991). It has been indicated that in broilers that approximately 200 mg/kg of α-tocopheryl acetate during at least 24 days (Sheehy, 1991) or 4 weeks (Morrissey et al., 1997) of feeding period are needed to reach muscle α-tocopherol plateau levels in broilers. This suggests that approximately 200 mg/kg of vitamin E in the diet may be needed to reach the highest antioxidant potential of vitamin E. This is in agreement with the statement that in order to stabilize the lipid oxidation development in cooked chicken meat, 200 mg/kg of vitamin E should be include in broilers' diets, particularly when the birds are fed with polyunsaturated fatty acid sources (Jensen et al., 1995; Galvin et al., 1998). Our results also support these observations in both raw and cooked breast, thigh, and skin chicken samples.

In conclusion, the dietary fat source influences the total fat content and fatty acid composition of chicken meat. Changes in the fatty acid composition of chicken muscles affect the lipid oxidation stability of both raw and cooked chicken meat over prolonged storage times at refrigerated conditions. In addition, dietary supranutritional supplementation of vitamin E is more effective at preventing the lipid oxidation

development in chicken meat than a commercial dietary level currently used in feeding broilers by the poultry industry.

CHAPTER IV

DIETARY FAT AND VITAMIN E EFFECT ON CHICKEN MEAT QUALITY

INTRODUCTION

Chicken meat quality is one of the most important factors in the poultry food industry that influences consumer acceptance and preference. Meat quality properties are directly related to the ultimate muscle pH and the conditions under which rigor mortis develops in the muscle. Rapid development of rigor mortis and low muscle pH are associated with the incidence of pale, soft, and exudative (PSE) meat condition. PSE meat is characterized for having light color, soft texture, and low water holding capacity that translates into negative changes in tenderness, purge, drip loss, cooked yield, and functionality of the meat (Camou and Sebranek, 1991; McCurdy et al., 1996; McKee and Sams, 1997).

The PSE condition occurs in poultry meat all year round in both turkeys and broilers (Barbut, 1998; Owens et al., 2000), particularly in birds carrying a genetic mutation that alters the sarcoplasmic reticulum calcium channels, the ryanodine receptor gene (Lahucky et al., 1997), and those subjected to acute antemortem heat stress (McKee and Sams, 1997). It has been shown that heat stressed birds have increased body temperature (Altan et al., 2000; Sandercock et al., 2001) and stress susceptible animals show higher release of calcium into the sarcoplasm (Louis et al., 1993; Wang et a., 1999). Calcium is an important second messenger in muscle cells that causes accelerated

glycolytic activity in the muscle (Divet et al., 2005). Elevated and prolonged glycolytic activity speeds up the rigor mortis development dropping the muscle pH (accumulation of lactic acid) and elevating carcass temperature (Cheah et al., 1984). The interaction of these factors induces muscle protein denaturation negatively affecting the inherent functional and quality properties of the harvested meat (Pietrzak et al., 1997; Sandercock et al., 1999).

Most research to date intended to reduce the incidence of PSE meat has overlooked the aspect of animal nutrition. However, it is possible that dietary lipids and vitamin E may influence the incidence of PSE meat in poultry. Olivo et al. (2001) observed that supranutritional supplementation of vitamin E reduced the incidence of PSE meat and improved its functionality. On the other hand, it has been indicated that different lipid sources or fatty acids may influence Ca^{2+} release from the sarcoplasmic reticulum (Fletcher et al., 1990; Williams and Klug, 1995). Experiments *in vitro* with muscle fibers have shown that free fatty acids strongly influenced the sarcoplasmic Ca^{2+} exchange process and may result in higher or lower calcium concentration in the sarcoplasm. It is possible that this effect could be more severe during heat stress conditions considering that heat has a direct effect on the amount of Ca^{2+} released from the sarcoplasmic reticulum. The combination of dietary lipids and environmental factors may result in a higher incidence of PSE in poultry meat. The effect of dietary fats and oils, and supranutritional supplementation of vitamin E needs to be studied to determine their effect or contribution to the incidence of PSE in poultry meat.

Therefore, the objectives of the present study were to determine the effects of dietary lipid fat source, vitamin E supplementation, and antemortem heat stress on chicken meat quality in broilers.

MATERIALS AND METHODS

Six hundred Cobb x Ross broilers were raised during a 6 week feeding period under commercial-like conditions at the Poultry Science Research Center at Texas A&M University. The broilers were fed with a basal corn-soybean meal diet including 5% of animal/vegetable (AV), palm kernel (PK), or soybean (SB) oil as lipid sources. Each oil type diet was supplemented with either 33 or 200 mg/kg of *dl*-α-tocopheryl acetate[12], during the 6 week feeding period. Feed and water were provided *ad libitum*. The experimental diets were formulated to be isocaloric and isoproteic (Table 12).

At 42 days of age, broilers were subjected to simulated environmental heat stress for 3 days and 2 nights in order to induce the development of PSE meat condition. Chicken house temperature was elevated by using thermostatically controlled gas heaters. The heat and relative humidity achieved was recorded with portable data loggers, and ranged between 28 to 31^{o}C. For the slaughtering process, broilers were stunned with an electric knife and bled to death through a ventral cut to the carotid and jugular arteries. After manual evisceration, the carcasses were pre-chilled for 15 min (7.2^{o}C) and chilled for 45 min (0^{o}C), then stored under refrigerated conditions during 5 h

[12] Rovimix 50% Abs. DSM, Inc. Parsippany, NJ

of an aging period. Upon completion of the aging period, the carcasses were hand-deboned and breast fillets were collected for analysis by Allo-Kramer shear force and expressible moisture as later described.

Muscle pH

To determine rigor mortis development, breast muscle samples were collected at 15 min, 2 h, and 24 h postmortem. Breast muscle samples from 3 different carcasses were collected postmortem at 15 min (prior scalding), 2 h, and 24 h. Muscle samples were placed in labeled aluminum foil pouches and inmediatelly frozen in liquid nitrogen to stop metabolic process, followed by frozen storage at -20^{o}C for further analysis. Muscle pH values were determined by the iodoacetate procedure (Marsh, 1954).

Muscle Color

After carcass deboning, left side breast fillets were collected and analyzed for L*, a*, and b* color space values (lightness, redness, and yellowness, respectively) in triplicate on the internal surface of the breast fillets with a Minolta Chroma Meter[13]. Calibration of the colorimeter was conducted with a white tile following manufacturer's specifications.

[13] model CR-200, Minolta Corp., Ramsey, NJ

Table 12. Broiler Basal Experimental Diets

Ingredient	Starter (0-3 Wk)	Grower (4-5 wk)	Finisher (6 wk)
		%	
Corn	55.56	61.32	66.28
Soybean meal (48%)	35.45	29.88	29.89
Fat/oil[1]	5.00	5.00	5.00
Biofos 16/21	1.56	1.35	1.44
Limestone	1.43	1.28	1.36
Salt	0.46	0.35	0.35
DL-Methionine 98	0.22	0.21	0.19
Lysine HCL	0.049	0.177	0.120
Choline CL 60	0.100	0.100	0.110
Coban 60	0.075	0.075	.
Mineral premix	0.050	0.050	0.050
Vitamin premix	0.025	0.025	0.025
Sodium bicarbonate	.	0.151	0.180
Calculated nutrient content			
Crude Protein (%)	22.10	20.00	19.82
ME energy (Kcal/lb)	3162.17	3224.50	3224.50
Calcium (%)	0.90	0.80	0.79
Available Phosphorous (%)	0.70	0.64	0.39
Methionine (%)	0.55	0.51	0.51
Methionine + Cystine (%)	0.92	0.85	0.83
Lysine (%)	1.23	1.18	1.17
Threonine	0.83	0.74	0.73
Sodium (%)	0.20	0.20	0.20

[1] 0.002% of sand was added to the soybean oil diet to make all diets isocaloric.
Mineral premix: Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8500 ppm, I 2100 ppm, Se 500 ppm, Mo 1670 ppm (Tyson Poultry 606 premix)
Vitamin premix (lb): A 14,000,000 I. U., D3 5,000,000 I. chick U., E 60,000 I. U., B12 24 mg, Riboflivin 12,000 mg, Niacin 80,000 mg, d-pantothenic acid 20,500 mg, K 2,700 mg, Folic acid 1,800 mg, B6 5,000 mg, Thiamine 4,000 mg, d-Biotin 150 mg. Sanderson, DSM Nutritional Products, Inc. Parsippany, NJ.

Meat Texture

Fresh breast fillets, deboned after 5 h of aging period and refrigerated storage (4°C) for 24 h, were cooked in a convection oven[14] on wire racks to an internal temperature of 74°C. Wire thermocouples were inserted to the geometric center of the breast and the internal temperature was recorded with an Omega Type-t thermometer[15]. Cooked breast filets were immediately wrapped in aluminum foil, cooled at room temperature and held overnight under refrigeration (4°C). Tenderness of cooked breast meat samples (40 x 20 x 5 mm) was determined after weighing and subjecting to a constant shearing in a 10-blade Allo-Kramer shear compression cell at a crosshead speed of 500 mm/min. The 500 kg load cell was set to a 200 kg full load range adapted to an Instron Universal Testing Machine[16] as previously reported (Sams, 1990). Allo-Kramer shear values were corrected by meat sample weight and reported in kg/g of meat.

Water Holding Capacity

Expressible moisture of cooked breast fillets was determined using the press method modified from Urbin et al. (1962). Meat samples of 0.5 g were weighed and placed on dried filter paper[17] between metal plates and subjected to 500 lb compression for 1 min (Sams, 1990). Total moisture content was determined in triplicate for each

[14] model DN097Hobart Corp. Troy, OH
[15] model HH501BT, Omega Engineering, Inc. Stamford, CT
[16] Instron Corp. Canton, MA
[17] Whatman filter papers 541

sample by weighing 3 g of meat into labeled pans and drying in an oven at 105^{o}C for 24 h.

RESULTS

Postmortem Muscle pH

Table 13 shows the rigor mortis development in breast chicken muscle, shown as postmortem muscle pH. Neither dietary fat nor vitamin E level influenced the drop in muscle pH (P>0.05). Muscle pH was only affected by postmortem time, lower pH values were observed as the postmortem time increased, consecutively from 15 min, 2 h, and 24 h (P>0.0001).

Meat Color

Dietary fat source and vitamin E level both influenced meat color (Table, 14). Higher L* color space (lightness) values (P<0.0138) were detected in breast fillets from the animal/vegetable oil treatment (55.29) compared to palm kernel and soybean oil treatments (52.99 and 53.39, respectively). With respect to vitamin E supplementation, breast fillets from the low supplemented level were lighter (54.72) than the high level of vitamin E (53.06).

Table 13. Breast Muscle pH Affected by Dietary Fat, Vitamin E Level, and Postmortem Time (Least Squares Means)

Fat	**Animal/ Vegetable**	**Palm Kernel**	**Soybean Oil**
	6.35	6.36	6.36
P-value	0.4808		
Vitamin E (mg/kg)	**33**	**200**	
	6.35	6.37	
P-value	0.3517		
Time	**15 min**	**2 h**	**24 h**
	6.68 [a]	6.46 [b]	5.93 [c]
P-value	0.0001		
Root MSE[1]	0.1768		

a, b, c: least squares means with different letter are significantly different .
[1]Root mean square error.

Redness (a* color space) in the meat was only affected by dietary fat source with the animal/vegetable oil having lower a* color space value compared to palm kernel oil, but neither were different from the soybean oil treatment. The lower a* color space value in animal/vegetable corresponded to its high L* value. In contrast, yellowness (b*) of the meat was only affected by the dietary level of vitamin E. Fillets from the low supplemented level had a higher b* color space value (3.88) compared to other treatments (3.20).

Table 14. Breast Muscle Color Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Factor	Lightness (L*)	Redness (a*)	Yellowness (b*)
Fat			
Animal/Vegetable	55.29 [a]	2.15 [b]	3.82
Palm Kernel	52.99 [b]	3.19 [a]	3.57
Soybean Oil	53.39 [b]	3.0 [ab]	3.23
P-value	0.0365	0.1429	0.1902
Vitamin E (mg/kg)			
33	54.72 [a]	2.55	3.88 [a]
200	53.06 [b]	2.73	3.20 [b]
P-value	0.0138	0.4950	0.0129
Root MSE[1]	3.34	1.77	1.29

a, b: least squares means between rows with different letters are significantly different.
[1]Root mean square error.

Meat Texture Water Holding Capacity

Neither dietary fat nor vitamin E influenced the tenderness and water holding capacity of chicken breast meat. No significant ($P>0.05$) differences were observed in Allo-Kramer shear force, expressible moisture, and total moisture content in cooked breast fillets (Table 15).

Table 15. Breast Meat Tenderness and Water Holding Capacity Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Factor	Allo-Kramer Shear Force (kg/g)	Expressible Moisture (%)	Total Moisture (%)
Fat			
Animal/Vegetable	5.57	44. 07	80.42
Palm Kernel	5.80	41.97	78.68
Soybean Oil	5.38	42.14	79.44
P-value	0.4008	0.3836	0.2683
Vitamin E (mg/kg)			
33	5.41	42.57	79.17
200	5.76	42.88	79.85
P-value	0.1198	0.8237	0.4234
Root MSE[1]	1.27	6.48	2.07

[1]Root mean square error
n=24

DISCUSSION

The results of the present study indicate that dietary fat and vitamin E level may not directly affect the rigor mortis development in chicken breast meat, as shown by no significant differences in drop of muscle pH at 15 min, 2 h, and 24h, between the treatments. These results are in agreement with those reported by Hoving-Bolink et al. (1998) who indicated that in pork supplementation of 200 mg/kg of vitamin E did not

affect the post mortem pH decline, when compared to 8 mg/kg. In contrast, Olivo et al. (2001) indicated that supranutritional supplementation of vitamin E, about 150 to 200 mg/kg, significantly reduced the drop in muscle pH in both non- and heat-stressed and broilers, and suggested that high dietary levels of vitamin E could reduced the incidence of PSE meat condition.

The results from this study also indicate that the rigor mortis developed in broilers from this experiment followed the pattern of "normal" (non-PSE) chicken meat. Alvarado and Sams (2000) reported muscle pH values, at 15 min, 2 h, and 24 h postmortem were approximately 6.54, 6.41, and 6.02, respectively. Sams et al. (1990) as well observed an ultimate muscle pH (24 h) in aged carcasses of about 5.80 units; while Sandercock et al. (2001) reported the ultimate muscle pH (24 h) in broilers of 35 d of age, with and without antemortem heat stress to be 5.68 and 5.74, respectively. Meat from heat stressed birds showed negative effects for water holding capacity, which is associated with PSE development in meat.

Meat color values were influenced by either dietary fat or vitamin E level. Breast fillets from the animal/vegetable treatment was lighter in color (L* color space) compared to palm kernel and soybean oil. Because dietary fat did not affect the drop in muscle pH, it is unclear to determine the cause of this variation in color of breast meat between fat treatments. Light meat color is commonly associated with the development of PSE meat condition, due to the denaturation of the myofibrillar proteins that results in poor water holding capacity in muscle, which in turn increases the reflection of the light making the meat look pale. Regarding the dietary vitamin E, Olivo et al. (2001) reported

that L* values with supranutritional supplementation of vitamin would be expected to be lower than a low dietary level; however, in the present study the effect of vitamin E on L* values was not observed.

It is known that high L* color space values are correlated with the PSE condition. Previous studies have indicated that in chicken meat L* color space values of 49-50 units and above are indicative of the PSE condition in which reduced water holding capacity of the meat has been observed (McCurdy et al., 1996; Barbut, 1997). However, according to Woelfel et al. (2002), a L* color space value of 54 is the threshold for differentiating pale from normal meat in broilers while in turkeys, this value has been suggested to be 50 (Barbut, 1996) or 53 units (Owens et al., 2000).

The results showed of this study showed that neither dietary fat nor vitamin E level affected the texture, expressible moisture and total moisture of the meat ($P>0.05$) under the conditions evaluated. These suggest that changes in the fatty acid composition or amount of α-tocopherol in the muscle tissues do not necessarily influence the quality of the meat and its physicochemical properties. The Allo-Kramer shear force values obtained in this experiment resembled those reported by Alvarado and Sams (2000) who obtained values of 4.94 and 4.56 kg/g from broilers subjected to non- and electrical stimulation, respectively. It has been reported that Allo-Kramer shear force values in the range of 3.5 to 6.6 kg/g are considered “slightly” to “moderately” tender (Lyon and Lyon, 1991); while shear values above 8 kg/g would be considered “tough” by consumers (Simpson and Goodwin, 1974).

The fact that no effect on the from vitamin E was observed in the meat water holding capacity by dietary fat or vitamin E supplementation was observed is in agreement with the literature. Previously, it have been reported that supranutritional supplementation of vitamin E did not have an effect on the water holding capacity of the meat (Hoving-Bolink et al., 1998; Olivo et al., 2001). Expressible moisture refers to the amount of water that is not bound to the muscle proteins and upon the application of pressure forces the water is expelled from the meat system.

In conclusion, neither dietary fat nor vitamin E level directly influence the quality of chicken meat, which suggests that the development of PSE meat condition in chicken meat is not likely to occur due to changes in the type and proportion of fatty acids and α-tocopherol in the muscle tissue.

CHAPTER V

DIETARY FAT AND VITAMIN E EFFECT ON LIPID OXIDATION STABILITY OF *SOUS VIDE* COOKED CHICKEN MEAT

INTRODUCTION

As the popularity for precooked chicken meat products continuous to rise, new technologies are needed to meet consumer expectations for meat quality, nutritional value and sensory characteristics. *Sous vide* processed meat consists of minimally processed meat vacuum packaged and cooked in heat resistant bags. This type of processing is one of the most preferred presentations of precooked meats by the food industry, restaurants, and catering services (Anon, 1987; Gorris, 1996). *Sous vide* cooking presents several advantages over conventional cooking and packaging methods. The pasteurization and stabilization processes of the meat occur in heat resistant high oxygen barrier boilable pouches which facilitate the storage, distribution, and commercialization of the product (Bertelsen and Juncher, 1996). Also, *sous vide* meat has been shown to exhibit longer shelf-life, maintain desirable sensory attributes (Creed, 1995; Armstrong and McIlveen, 2000), nutritional value and lipid oxidation stability for prolonged periods of storage (Smith and Alvarez, 1988; Vaudagna et al., 2003; Wang et al., 2004). Low malonaldehyde values in *sous vide* meat can be expected during prolonged refrigerated storage as long as the meat is kept under vacuum conditions (Smith and Alvarez, 1988). However, when a high level of unsaturated fatty acids is

present in chicken muscle (Lin et al., 1989; Lauridsen et al., 1997), the cooked chicken meat is susceptible to develop lipid oxidation (Rhee et al., 1996) and low sensory scores (Bou et al., 2001). Thus, susceptibility of *sous vide* meat to lipid oxidation could be important when a relatively high content of unsaturated fatty acids has been deposited in the muscle tissue. Current market trends towards healthier diets are pressuring the poultry industry to incorporate healthier lipids into dietary regimens to modify the lipid content of poultry products. Enriching for unsaturated fatty acids in chicken meat is seen as an opportunity to improve the acceptability of these products by the health conscious consumer. However, changes in the lipid composition can led to problems associated with faster lipid oxidation rates for these lipids which are more susceptible to lipid peroxidation processes due to the presence of double bonds in fatty acids increases, their susceptibility to lipid peroxidation also increases (Dahle et al., 1962).

In meat, lipid peroxidation is initiated by the abstraction of hydrogen radicals from unsaturated fatty acids, induced by light (Boselli et al., 2005), heat, metal ions (Kanner et al., 1988), or other oxidizing agents. The reaction of oxygen with preformed free radicals results in accelerated lipid peroxidation (Frankel, 1984) which leads to the formation of secondary by-products from polyunsaturated fatty acids such as malonaldehyde and the potential appearance of warmed-over flavors, off-flavors, off-odors, or lower sensory scores (Tarladgis et al., 1964; Sato and Hegarty, 1971; Igene et al., 1979; Igene et al., 1985).

Supplementation of vitamin E in animal diets has been shown to be successful to enhance the lipid oxidation stability of chicken meat. Alpha tocopherol, the most

biologically active form of vitamin E, decreases free radical formation (Gatellier et al., 2000) as well as scavenges existing free radicals, thus breaking the lipid oxidation chain reaction (Burton and Traber, 1990). In muscle foods, phospholipids are considered to be the location where the initiation of lipid peroxidation takes place (Asghar et al., 1988). In muscle fibers, α-tocopherol is deposited in the membranes where it protects the phospholipids from lipid peroxidation (Fang et al., 2002).

The objective of this study was to asses the lipid oxidation stability of *sous vide* breast and thigh chicken meat as affected by different dietary fats and the use of conventional or supranutritional levels of dietary vitamin E during prolonged refrigerated storage of the meat.

MATERIALS AND METHODS

Breast and thigh meat samples from Cobb x Ross broilers raised during a 6 week feeding period under commercial-like conditions at the Poultry Science Research Center at Texas A&M University were used. The broilers were fed with a basal corn-soybean meal diet including 5% of animal/vegetable (AV), palm kernel (PK), or soybean (SB) oil. Each oil type diet was supplemented with 33 or 200 mg/kg of *dl*-α-tocopheryl acetate[18]. The fatty acid composition of the diets is reported in Table 16.

Prior to the collection of the meat, broilers were subjected to simulated environmental heat stress for 3 days and 2 nights, at 42 days of age. The chicken house

[18] Rovimix 50% Abs. DSM, Inc. Parsippany, NJ

internal temperature was monitored by thermostatically controlled gas heaters. The heat and relative humidity achieved was recorded with portable data loggers and ranged between 28 to 31^{o}C. Broilers were slaughtered in commercial-like conditions using an electric knife. After manual evisceration, carcasses were pre-chilled for 15 min (7.2^{o}C) and chilled for 45 min (0^{o}C), then stored under refrigeration for 5 h of aging period. Upon completion of the aging period, the carcasses were hand-deboned and breast and thigh muscle samples were collected.

Sous Vide Preparation and Cooking

Breast and thigh muscles were skinned, deboned, trimmed of visible adipose and connective tissues, and cut into 5 cm^2 cubes. From a pool of muscle pieces from each experimental unit, 3 breast or thigh meat samples from independent carcasses were randomly placed in high oxygen barrier boilable pouches[19], vacuum-packaged[20] and thermally processed as *sous vide* product. Packages were cooked by complete submersion in a water bath[21] to an internal temperature of 74^{o}C. The final cooking temperature was continuously monitored using a meat sample with a wired Omega Type-T thermometer[22]. After the target internal temperature was reached, the *sous vide*

[19] 4 MIL Boil Vac Pouch, Ultravac Solutions, Kansas City, MO
[20] model C200, Multivac Inc. Kansas City, MO
[21] model GP-400, Neslab Instruments Inc. Newington, NH
[22] model HH501BT, Omega Engineering, Inc. Stamford, CT

packages were immediately chilled in ice-water to less than 4.4°C and subsequently refrigerated stored [23] at 4.4°C for 1, 5, 10, 25, and 40 days.

Table 16. Fatty Acid Methyl Esters of Broiler Diets

Fatty Acid (%)	Basal Feed	Animal Vegetable	Palm Kernel	Soybean Oil
		(% of Total Fat)		
C12:0	.	1.15	22.22	0.31
C14:0	.	0.81	9.67	0.21
C16:0	10.6	20.02	12.29	12.13
C16:1	.	4.55	0.36	0.41
C18:0	2.315	4.78	3.07	3.74
C18:1	21.09	32.13	22.84	23.70
C18:1 c11	0.75	1.61	0.53	1.24
C18:2	48.01	29.15	27.42	51.69
C18:3	1.98	1.59	1.35	4.23
SFA[1]	6.46	6.69	11.81	4.10
MUFA[2]	10.92	12.76	7.91	8.45
PUFA[3]	24.99	15.37	14.38	27.96
PUFA/SFA	3.87	2.30	1.22	6.82

[1]SFA: saturated fatty acids (12:0, 14,0, 16:0, 18:0)
[2]MUFA: monounsaturated fatty acids (16:1, 18:1, 18:1c11)
[3]PUFA: polyunsaturated fatty acids (18:2, 18:3).

[23] model 2005, VWR, Cornelius, OR

Chemical Analysis

2-Thiobarbituric acid reactive substances (TBARS) analysis by distillation method was conducted to determine the lipid oxidation development of the meat according to Rhee (1978). Thirty g of meat, in duplicate, from each package were added with 15 ml of EDTA:propyl gallate solution[24], blended for 2 min, and 2 subsamples of 30 g were added with 2 mL hydrochloric acid 4 N and boiling chips each. Upon the boiling process in the Kjeldahl apparatus, 50 mL of malonaldehyde were distilled and 5 mL of it were added with 5 ml of TBA solution. The mixture was boiled in a water bath for 30 min, followed cooling in water at room temperature for 10 min. TBARS values were measured in a spectrophotometer[25] at 530 nm, using 1.5 ml UV-Visible light cuvettes[26]. Values were multiplied by a correction factor (7.8) and reported in mg of malonaldehyde per kg of muscle (Tarladgis et al., 1960).

Fat and Moisture of Raw and Cooked Meat

After thawing of the meat, raw meat samples were trimmed of visible connective and adipose tissues, ground in a meat processor[27], placed in sampling bags, held in refrigeration, and in duplicate 3 to 5 g subsamples were analyzed for fat and moisture by

[24] Sigma Aldrich, St. Louis, MO
[25] Varian, Cary 300 Bio UV-Visible Spectrophotometer, Walnut Creek, CA
[26] VWR International, Cornelius, OR
[27] model HC3000, Black & Decker Corporation, Towson, MD

Nuclear Magnetic Resonance[28], and fatty acid methyl esters by Gas Chromatography using a Varian Gas chromatograph[29], following the procedure established by Smith et al. (2002). To quantify fat and moisture values of cooked meat, meat samples were collected after opening of cooked *sous vide* meat packages and processed as previously described.

Cooked Meat Nonheme Iron

Determination of nonheme iron in cooked *sous vide* meat was performed in samples from day 1 and 25 of refrigerated storage. Meat was ground in a food processor[30] and a 4 g sample was placed in a 50 mL test tube, adding 12 mL of double distilled water and then homogenized. An aliquot of 1.5 mL of the mixture were obtained and 0.5 mL of 2% ascorbic acid[31] solution was added, after 5 min holding at room temperature 1 mL of 11.3% TCA was added followed by centrifugation[32] at 4000 rpm for 15 min. Samples were read at 562 nm in a spectrophotometer[33]. Nonheme iron analysis and standard curve preparation were performed following the procedure established by Ahn et al. (1993).

[28] Smart Track System, CEM Co. Matthews, NC
[29] model CP-3800 fixed with a CP-8200 autosampler Varian Inc. Walnut Creek, CA
[30] model HC3000, Black & Decker Corporation, Towson, MD
[31] Sigma-Aldrich, St. Louis, MO
[32] model RT6000B Sorvall, Dupont Company, Wilmington, Delaware
[33] model DU64, Beckman Instruments Inc. Fullerton , CA

Muscle Tocopherol Content

Alpha-tocopherol was extracted applying a modified procedure from Liu et al. (1996). One gram of muscle was placed in 50 mL test tube completely wrapped with aluminum foil, added with 250 mg of L-ascorbic acid and 7.3 ml of Potassium Hydroxide[34] solution (11% KOH in ethanol:water, 55:45%), and mixed with a homogenizer[35]. Mixed samples were incubated in a water bath[36] for 20 min at 70°C. Samples were cooled in tab water and 1.5 mL of Hydrochloric acid (6 N) to neutralize the pH and 4 mL of hexane to separate the non-polar fraction from the polar phase were added. After the mixture was vortexed[37] for 1 min, the upper phase was recovered with a separating funnel and dried in a water bath with Nitrogen flush at 40°C in 25 mL amber test tubes. Samples were reconstituted with 0.5 mL of Methanol:n-propanol (1:1) and 20 µl were injected in a HPLC equipment[38] for separation of tocopherol isomers, using a gradient reverse mobile phase (methanol:n-propanol:water,78:17:5, respectively; water added with .025M acetic acid, C18 15 cm x 4.6 mm, 5 µm column[39], equipped with a C18 5u guard column[40]. A flow rate of 1.4 ml/min was applied at 35°C and 22 min run time. Detection of isomers was conducted with a photodiode array detector at 210 and 295 nm. Alpha-tocopherol[41] was identified with a standard curve.

[34] Sigma-Aldrich, St. Louis, MO
[35] model 21-4221, Cincinnati, OH
[36] model 1157P, VWR International, West Chester, PA
[37] model Vortex-Genie 2, VWR International, West Chester, PA
[38] model 510 Water Millipore, Franklin, MA
[39] Supelcosil, Sigma-Aldrich/Supelco, St. Louis, MO
[40] Alltech Inc. Nicholasville, KY
[41] Sigma-Aldrich/Fluka, St. Louis, MO

Statistical Analysis

The statistical analysis was conducted using the General Linear Model procedure of SAS (SAS Institute, 2000) using a Completely Randomized Block Design with 3 x 2 x 4 factorial arrangements. Factor A, B, and C were dietary lipid source, supplemented vitamin E level, and storage day, respectively. Repetition was used as blocking factor and least squares means of the variables are reported.

RESULTS

Chicken Muscle Fatty Acid Composition

Table 17 shows the dietary fat effect on the fatty acid composition of chicken muscles. Breast and thigh muscle fatty acids were significantly ($P<0.01$) affected by dietary fat, but not ($P>0.05$) by vitamin E level. Palm kernel oil significantly increased the deposition of lauric (12:0), myristic (14:0), myristoleic (14:1), and reduced the deposition of linoleic (18:2), linolenic (18:3), and araquidonic (20:4) fatty acids, compared to animal/vegetable and soybean oil. Contrary, soybean oil increased the proportion of linoleic (18:2) and linolenic (18:3) fatty acids, and reduced the deposition of myristoleic (14:1), palmitoleic (16:1), and oleic cis11 (18:1cis11) fatty acids, compared to other dietary treatments. Stearic (18:0) and araquidonic (20:4) fatty acids were not affected by dietary fats in neither muscle type.

Chicken Muscle α-Tocopherol Content

Muscle α-tocopherol content was significantly affected by dietary level of vitamin E ($P<0.0001$), but not by dietary fat treatment ($P>0.05$). In both breast and thigh muscle dietary supplementation of 200 mg/kg induced higher deposition of α-tocopherol than the control level (33 mg/kg), approximately 1.95 and 2.13-fold higher in breast and thigh muscle respectively (Table 18).

Raw and Cooked Meat Fat and Moisture Content and Cooked Yields

The fat and moisture content of raw meat samples are shown in Table 19. Dietary fat affected the moisture content in thigh meat ($P<0.05$), but not in breast meat. Meat samples from the soybean oil treatment had significantly lower moisture content than the other treatments. Vitamin E level also influenced the total moisture content but only in breast meat samples, the dietary high level of vitamin E significantly increased ($P<0.05$) the total moisture percent compared to the low level counterparts.

Cooked *sous vide* meat total fat content was affected by dietary fat, but not by vitamin E supplementation. Only breast meat samples from the soybean oil treatment showed higher total ($P<0.05$), when compared to the palm kernel oil treatment. In thigh meat samples, neither dietary fat nor vitamin E influenced the total fat or moisture contents. It is important to indicate that cooked yield of *sous vide* meat, breast and thigh, was not affected by dietary factors (Table 20).

Table 17. Fatty Acid Methyl Esters of Breast and Thigh Muscles Affected by Main Effect of Dietary Animal/Vegetable (AV), Palm Kernel (PK), and Soybean (SB) oil (Least Squares Means)

Fatty	Breast			Root	Thigh			Root
Acid	AV	PK	SB	MSE[1]	AV	PK	SB	MSE
				(% of Total Fat)				
12:0	0.90 [b]	6.11 [a]	1.01 [b]	0.91	1.46 [b]	8.10 [a]	0.87 b	1.10
14:0	1.11 [b]	4.37 [a]	1.10 [b]	0.41	1.34 [b]	5.31 [a]	0.70 [c]	0.46
14:1	0.14 [b]	0.43 [a]	.	0.10	0.32 [ab]	0.66 [a]	0.11 [b]	0.14
16:0	21.15	20.52	21.73	2.03	23.96 [a]	21.62 [ab]	20.90 [b]	2.49
16:1	4.40 [a]	3.15 [b]	2.33 [c]	1.38	5.89 [a]	4.10 [b]	2.90 [c]	0.81
18:0	7.81	7.77	8.13	3.66	6.55	6.74	7.13	0.83
18:1	29.71 [a]	26.16 [b]	27.02 [b]	5.24	33.44 [a]	28.24 [b]	27.49 [b]	1.51
18:1c11	2.74 [a]	2.35 [ab]	2.11 [b]	4.79	2.28 [a]	1.94 [b]	1.69 [c]	0.12
18:2	17.90 [b]	15.13 [c]	26.15 [a]	5.22	18.90 [b]	15.40 [c]	29.88 [a]	1.12
18:3	0.62 [b]	0.48 [c]	1.35 [a]	0.52	0.71 [b]	0.49 [c]	1.78 [a]	0.10
20:4	4.36	4.22	4.45	1.10	2.44	2.90	2.88	0.86
SFA[2]	30.99 [b]	38.82 [a]	31.88 [b]	2.40	33.15 [b]	41.77 [a]	29.61 [c]	3.10
MUFA[3]	34.26 [a]	29.74 [b]	28.89 [b]	2.44	39.49 [a]	32.81 [b]	30.46 [c]	2.24
PUFA[4]	22.99 [b]	19.76 [c]	31.54 [a]	2.04	21.70 [b]	18.70 [c]	34.03 [a]	1.89
PUFA/ SFA	0.75 [b]	0.51 [c]	1.00 [a]	0.10	0.66 [b]	0.45 [c]	1.16 [a]	0.10

a, b, c: least squares means between columns with different letters are significantly different ($P<0.01$)

[1]Root mean square error; [2]SFA: saturated fatty acids (12:0, 14:0, 16:0, and 18.0)

[3]MUFA: monounsaturated fatty acids (14:1, 16:1, 18:1, 18:1cis)

[4]PUFA: polyunsaturated fatty acids (18:2, 18:3, and 20:4).

Table 18. Muscle α-Tocopherol Content Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Muscle	Breast	Thigh
Fat		
Animal/Vegetable	6.75	9.88
Palm Kernel	6.38	8.66
Soybean Oil	7.28	9.69
P-value	0.4310	0.7052
Vitamin E (mg/kg)		
33	4.61 [b]	6.02 [b]
200	9.00 [a]	12.81 [a]
P-value	0.0001	0.0001
Root MSE[1]	1.89	3.91

a, b: least squares means between rows with different letters are significantly different.
[1]Root mean square error.

Lipid Oxidation Stability of Cooked *Sous Vide* Chicken Meat

The results showed (Table 21) that the lipid oxidation stability of *sous vide* meat was affected independently by dietary vitamin E level and storage day ($P<0.05$), but not significant effects were observed by dietary fat. Both breast and thigh meat from broilers supplemented with 200 mg/kg of vitamin E had lower malonaldehyde values compared to the 33 mg/kg treatment; meat samples from the low vitamin E level had

approximately 1.4- and 1.5-fold higher of malonaldehyde in breast and thigh, respectively. During storage, at day 10, in both breast and thigh meat higher ($P<0.05$) malonaldehyde values were detected, but the meat lipid oxidation remained relatively stable throughout the rest of the storage period; up to 40 days, none of the meat samples showed malonaldehyde values above 1 mg/kg.

Table 19. Raw Muscle Total Fat and Moisture Content Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Muscle	**Breast**		**Thigh**	
	Fat (%)	**Moisture (%)**	**Fat (%)**	**Moisture (%)**
Dietary Fat				
Animal/Vegetable	1.44 [b]	72.59	3.66	74.36 [a]
Palm Kernel	1.43 [b]	72.89	3.71	74.38 [a]
Soybean Oil	1.77 [a]	72.42	3.92	73.55 [b]
P-value	0.0038	0.3640	0.7461	0.0468
Vitamin E (mg/kg)				
33	1.50	72.25 [b]	3.77	74.12
200	1.59	72.97 [a]	3.75	75.04
P-value	0.3003	0.0164	0.9458	0.7953
Root MSE[1]	0.29	0.93	0.93	0.94

a, b: Least squares means between rows with different letters are significantly different.
[1]Root mean square error.

Table 20. Cooked *Sous Vide* Total Fat, Moisture Content, and Cooked Yield Affected by Dietary Fat and Vitamin E Level (Least Squares Means)

Dietary Fat	Fat (%)	Moisture (%)	Cooked Yield (%)
Breast			
Dietary Fat			
Animal/Vegetable	1.08 [ab]	72.02	86.62
Palm Kernel	0.92 [b]	72.60	84.40
Soybean Oil	1.32 [a]	71.48	84.02
P-value	0.0301	0.1265	0.2256
Vitamin E (mg/kg)			
33	1.12	72.13	85.91
200	1.09	71.94	84.12
P-value	0.8376	0.6599	0.1796
Root MSE[1]	0.40	1.51	6.43
Thigh			
Dietary Fat			
Animal/Vegetable	6.81	71.84	88.23
Palm Kernel	7.07	71.63	89.13
Soybean Oil	6.44	73.36	87.80
P-value	0.5541	0.2164	0.2023
Vitamin E (mg/kg)			
33	6.95	72.55	88.80
200	6.59	72.00	87.98
P-value	0.4529	0.5076	0.1865
Root MSE	1.36	2.37	2.30

a, b: least squares means between columns with different letters are significantly different.
[1]Root mean squares error.

Nonheme Iron Values of Cooked *Sous Vide* Meat

Nonheme iron values in cooked meat showed to be affected by the interaction of dietary fat and vitamin E level and the interaction of vitamin E level and storage day in breast meat. And in thigh meat, nonheme iron values were affected only by the interaction of vitamin E level and storage day (Table 22).

Table 21. Cooked *Sous Vide* Chicken Meat Malonaldehyde Values Affected by Dietary Fat, Vitamin E Level, and Storage Day (Least Squares Means)

Meat	Breast Meat	Thigh Meat
	(mg/kg)	
Fat		
Animal/Vegetable	0.47	0.52
Palm kernel oil	0.44	0.48
Soybean oil	0.49	0.53
P-value	0.20	0.39
Vitamin E (mg/kg)		
33	0.55 [a]	0.61 [a]
200	0.39 [b]	0.41 [b]
P-value	0.0001	0.0001
Storage Day		
1	0.30 [b]	0.16 [c]
5	0.36 [b]	0.41 [c]
10	0.55 [a]	0.58 [b]
25	0.58 [a]	0.66 [ab]
40	0.55 [a]	0.74 [a]
P-value	0.0001	0.0001
Root MSE[1]	0.13	0.15

a, b, c, d: least squares means between rows are significantly different.
[1]Root mean squares error.

Table 22. Nonheme Iron Values of Cooked *Sous Vide* Chicken Meat Affected by Dietary Fat, Vitamin E Level, and Storage Day (Least Squares Means)

Meat	Fat			Vitamin E (mg/kg)		Root MSE[1]
	Animal/ Vegetable	Palm Kernel	Soybean Oil	33	200	
Breast			(µg/g)			
Dietary fat x vitamin E level (P<0.0048)						
33	0.21 $^{b\,y}$	0.24 $^{a\,x}$	0.20 $^{a\,y}$			0.03
200	0.26 $^{a\,x}$	0.22 $^{a\,y}$	0.23 $^{a\,xy}$			
Storage Day x Vitamin E (P<0.0249)						
1				0.21 $^{a\,y}$	0.25 $^{a\,x}$	
25				0.22 $^{a\,x}$	0.22 $^{b\,x}$	
Thigh						
Storage Day x Fat (P<0.0133)						
1	0.63 $^{a\,x}$	0.51 $^{a\,x}$	0.51 $^{a\,x}$			0.12
25	0.32 $^{b\,y}$	0.47 $^{a\,x}$	0.40 $^{a\,xy}$			

a, b/x, y: least squares means between rows or columns with different letters are significantly different (P<0.05).
[1]Root mean square error.

DISCUSSION

The amount and type of fatty acids found in the muscle tissues depended on the dietary source of fatty acids included in the broiler's diets. Thus, inclusion of saturated, unsaturated, or polyunsaturated sources of fatty acids would directly result in higher

content of these fatty acids in the meat, as previously reported in other studies (Yua et a., 1991; Sanz et a., 1999).

Up to 40 days refrigerated storage in breast and thigh meat samples, extended lipid oxidation stability was found in *sous vide* cooked chicken meat, as shown by the relatively low levels of malonaldehyde, below 1 mg/kg of meat. The lipid oxidation rate in the meat developed slowly regardless of the differences in the composition and proportion of fatty acids in the meat induced by animal/vegetable, palm kernel, and soybean oil. This suggests that increasing the proportions of linoleic (18:2) and linolenic (19:3) fatty acids in the meat, as in the case of soybean oil, does not affect the lipid oxidation stability of chicken meat when processed as *sous vide* meat. It has been reported that in meat under vacuum conditions, the lipid oxidation develops at a low rate due to the lack of available oxygen as initiator of lipid peroxidation. Kanner et al. (1988) reported that the lipid peroxidation in meat is oxygen dependent, they observed that canned meat maintained low levels of malonaldehyde during prolonged storage time, however when opening the cans and exposing the meat to air, it resulted in rapid production of malonaldehyde in the meat, as an indication of lipid oxidation development in the meat. Smith and Alvarez (1988) also reported that in refrigerated cooked-in-bag turkey rolls the malonaldehyde values remained low during 82 days of storage under vacuum, with maximum values around 1.0 mg/kg of meat. However, opening of the bags resulted in rapid development of lipid oxidation within hours, due to the exposure to oxygen.

Supranutritional supplementation of vitamin E resulted in higher lipid oxidation stability of the meat, lower malonaldehyde values were found in meat samples compared to the control level, due to the higher content of α-tocopherol in the muscle tissues. Approximately 1.95- and 2.13-fold higher of α-tocopherol were detected in breast and thigh muscle, respectively, from the supranutritional supplemented treatment compared to the control level. Previously, supranutritional supplementation of vitamin E has been shown to be necessary to maintain the lipid oxidation stability (Yamahuchi et al., 1991; Galvin et al., 1997) and sensory characteristics of the meat (De Winne and Dirinck, 1996). However, due to the relatively low levels of malonaldehyde detected in cooked *sous vide* meat with the control level (33 mg/kg) of vitamin E used, the authors of the present study recommend sensory evaluation to be conducted to justify supranutritional supplementation levels of vitamin E when the intended use of the meat is for *sous vide* product. Though, it should not be overlooked that *sous vide* meat has the potential to develop sour off-odors and metal-like off-flavors even with low malonaldehyde levels, less than 10 μmoles/kg (Hansen et al., 1995). Tarladgis et al. (1964) indicated that the threshold of malonaldehyde to detect off-odors in cooked meat ranges between 0.1 and 0.2 mg.

It is important to point out that in *sous vide* meat, the lipid oxidation development showed not to be influenced by nonheme iron values in the meat system. Statistical analysis showed that the correlation coefficient in breast meat was not significant ($P>0.05$) and in thigh meat, though significant ($P<0.02$) it showed to be negative (-0.43).

In conclusion, *sous vide* cooked chicken meat lipid oxidation stability is not affected by dietary fats when animal/vegetable, palm kernel, or soybean oil is included in broilers' diets. Supranutritional supplementation of vitamin E increases the amount of α-tocopherol in chicken muscles and enhances the lipid oxidation stability of the meat processed by the packaging-cooking system. This experiment also indicates that the lipid oxidation stability of cooked *sous vide* chicken meat is not influenced by relative high amounts of unsaturated fatty acids or nonheme iron values in the meat.

CHAPTER VI

CONJUGATED LINOLEIC ACID, FLAXSEED, AND MENHADEN FISH OIL, AND VITAMIN E EFFECT ON LIPID OXIDATION STABILITY OF *SOUS VIDE* CHICKEN MEAT

INTRODUCTION

Poultry products enriched with omega-3 fatty acids have been developed in an attempt to meet the growing consumer demand for functional food products, those that promote health benefits beyond their nutritional value (Milner, 2000). Omega-3 fatty acids, particularly EPA (eicosapentaenoic, 20:5) and DHA (docosahexaenoic, 22:6) have shown multiple health benefits in humans, including reduction of risk associated with heart and cerebrovascular problems, rheumatoid arthritis, depression, inflammation, and some types of cancers (Tamura et al., 1986; Nestel, 1990, Horrocks and Yeo, 1999; Meydani, 1994). Also, conjugated linoleic acid (CLA), another group of fatty acids, has shown to promote health benefits in humans and animal models by reducing overweight, obesity, and some types of cancers (Blankson et al., 2000; Kraus et al., 2000; Evans et al., 2002; Wang and Jones, 2004). Even though CLA isomers can be naturally found in several food products such as beef, cheese, and milk, it is not yet approved to be included in human food products or animal feeds because scientific research is still required to understand their effects on consumers a well as in food products. Therefore CLA is yet to be approved as a GRAS compound (FDA, 2007).

Enrichment of chicken meat with omega-3 and CLA fatty acids has been successfully demonstrated in multiple studies through the dietary inclusion of primarily marine (tuna, menhaden, salmon, red fish and algae) and plant lipid sources (flaxseed, canola, and sunflower) (Marion and Woodroof, 1963; Hulan et al., 1989; Ajuyah et al., 1991; Mooney et al., 1998; Lopez-Ferrer et al., 2001; Milinsk et al., 2003; Schreiner et al., 2005), and commercial concentrates of CLA (Szymczyk et al., 2001). However, development of chicken meat and meat products enhanced with PUFA's represents a great challenge for the food industry to preserve their lipid oxidative stability during prolonged storage time, mainly in aerobic conditions. Ajuyah et al. (1993) reported that the increment of omega-3 fatty acids in chicken muscle resulted in an accelerated lipid oxidation development in cooked chicken meat, and despite the fact that supplementation of natural antioxidants reduced the lipid oxidation rate, relatively high malonaldehyde values were reported at day 5 of storage in refrigerated conditions, indicating signs of lipid spoilage. In contrast, addition of CLA oil in ground raw and cooked beef patties reduced the lipid oxidation development, extending the shelf-life of the product (Chae et al., 2004).

Because the lipid oxidation stability of the meat can be negatively affected by PUFA, particularly in conventional cooking and packaging systems, alternative cooking methods such as *sous vide* should be explored in order to enhanced the lipid stability of the meat. *Sous vide* food products are thermally processed and stored in vacuum conditions, and the easy handling and convenient preparation methods are in growing demand by restaurants, catering, retail, and food service establishments (Bertensen,

1996; Gorris, 1996). In our previous study, we observed that *sous vide* chicken meat had low lipid oxidation development during 40 days of refrigerated storage, regardless of differences in the type and amount of fatty acids deposited in the meat induced by animal/vegetable, palm kernel, or soybean oil. Because these dietary fats could be consider of relatively low degree of unsaturation, it is necessary to determine the lipid oxidation stability of chicken meat when sources of high unsaturated fatty acids are fed to broilers.

The objective of the present study was to assess the lipid oxidation stability of precooked chicken meat affected by dietary oils rich in unsaturated fatty acids and supranutritional supplementation of vitamin E.

MATERIALS AND METHODS

Six hundred and twenty four Cobb x Ross one day old chicks were raised under commercial-like conditions during a 6-week period at the Poultry Science Research Center, Texas A&M University. Broilers were randomly assigned into 6 treatments, 4 replications, with 26 broilers each, and fed with diets including 2% of Conjugated Linoleic Acid[42], pressed flaxseed[43], or menhaden fish[44] oil as source of polyunsaturated fatty acids (Table 23), each oil type diet was supplemented with 42 or 200 mg/kg of α-tocopheryl acetate[45]. Feed and water were provided *ad libitum*; a basal corn-soybean

[42] Luta-CLA® 60, BASF, Florham Park, NJ
[43] Pizzey's Milling Co. Gurnee, IL
[44] Virginia Prime Silver™, Omega Protein, Inc. Hammond, LA
[45] Rovimix 50% Abs™. DSM, Inc. Parsippany, NJ

meal diet was used (Table 24). All experimental diets were kept under refrigeration without light prior to feeding to the broilers so to prevent the lipid oxidation development of the lipid components of the feed.

Table 23. Fatty Acid Composition of Dietary Oils

Fatty Acid (%)	CLA	Flaxseed Oil	Menhaden Oil
		(% of Total Fat)	
12:0	.	.	0.25
14:0	0.05	0.06	10.92
16:0	5.36	5.72	20.96
16:1	.	.	13.10
18:0	4.35	3.28	3.53
18:1	22.64	20.55	8.21
18:1 *c*11	0.57	0.60	3.68
*c*9*t*11CLA	30.04	.	.
*t*10*c*12CLA	30.24	.	.
18:2	0.33	15.12	1.25
18:3	0.28	53.03	0.81
20:4	0.58	0.14	0.91
20:5	.	.	7.49
22:6	.	.	9.72

Table 24. Broilers Basal Experimental Diets According to Growing Period

Ingredient (%)	Starter (0-3 wks)	Grower (4-5 wks)	Finisher (6 wk)
Corn	58.81	63.97	68.84
Soybean meal	34.81	29.94	25.32
Biophos	1.67	1.59	29.93
Limestone	1.52	1.45	27.81
Oil	2.00	2.00	2.00
Salt	0.51	0.45	0.31
Vitamin Premix[1]	0.25	0.25	0.25
DL-Methionine	0.20	0.07	.
Choline 60	0.10	0.10	0.10
Coban 60	0.08	0.08	.
Mineral Premix[2]	0.05	0.05	0.05
Sodium bicarbonate	.	0.05	0.21
Calculated nutrient content			
Crude Protein (%)	22.00	20.00	18.15
ME energy (Kcal/lb)	3007.00	3056.22	3105.14
Calcium (%)	0.95	0.90	0.85
Available Phosphorous (%)	0.47	0.45	0.42
Methionine (%)	0.53	0.38	0.32
Methionine + Cystine (%)	0.90	0.72	0.63
Lysine (%)	1.18	1.05	0.92
Threonine (%)	0.82	0.75	0.68
Sodium (%)	0.22	0.21	0.20

[1]Vitamin premix (lb): A 2,000,000 I.U, D3 700,000, E 8,333 I.U., B12 3.0 mg, riboflavin 1,083 mg, niacin 8,333 mg, d-pantothenic acid 3,667 mg, choline 86,667 mg, K 267 mg, folic acid 317 mg, B6 1,300 mg, thiamine 533 mg, d-biotin 100. Breeder turkey, DSM Nutritional Products, Inc., Parsippany, NJ.
[2]Mineral premix: Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8500 ppm, I 2100 ppm, Se 500 ppm, Mo 1670 ppm (Tyson Poultry 606 premix).

The fatty acid composition of the experimental diets is reported in Table 25.

Table 25. Fatty Acid Composition of Broilers' Experimental Diets

Fatty Acid (%)	Basal	CLA	Flaxseed	Menhaden
		(% of Total Fat)		
12:0	0.96	0.52	0.56	1.14
14:0	0.26	0.38	0.52	3.03
16:0	14.64	10.89	11.56	15.58
16:1	0.22	0.33	0.48	3.73
18:0	3.22	3.53	3.08	3.04
18:1	28.24	24.41	23.82	20.26
18:1 *c*11	0.82	0.76	0.84	1.60
*c*9*t*11CLA	.	11.09	.	.
*t*10*c*12CLA	.	11.01	.	.
18:2	47.03	29.41	36.80	32.99
18:3	2.36	3.50	20.03	1.91
20:4	0.25	0.38	0.26	0.51
20:5	.	.	.	4.17
22:6	.	.	.	3.22

At the end of the feeding period, broilers were withdrawn from feed for 8 h and transported to the pilot processing plant. The birds were processed under commercial-like conditions and after a 5 h aging period, breast and thigh meat samples were

collected, skinned, deboned, trimmed of connective and adipose tissues, and dissected into 5 cm^2 cubes. From both muscle types, 3 muscle pieces were vacuum-packed[46] in heat resistant boilable pouches[47], and cooked in a water bath[48] up to an internal temperature of 74°C. Internal temperature of the meat was recorded with an Omega Type-T thermometer[49]. After reaching the target internal temperature, cooked meat packages were immediately chilled in ice-water, and later stored under refrigeration[50] at 4.4°C during 0, 5, 10, 15, and 30 days.

At each storage day, TBARS (2-thiobarbituric acid reactive substances) analysis was conducted to estimate the lipid oxidation development, through the distillation process (Rhee, 1978). Per meat package (total of 4 packages), two 30 g meat samples were blended with 15 mL of 0.5% EDTA-propyl gallate[51] solution and 45 mL of double distilled water at 50°C, during 2 min. In duplicate, meat slurry subsamples of 30 g each were placed in 500 mL flasks, adding Slipicon spray, boiling chips, 2.5 mL of hydrochloric acid 4 N and 76.5 mL of double distilled water at 50°C. Upon distillation, 50 mL of malonaldehyde were extracted and 5 mL were collected in 25 mL test tube and added 5 mL of TBA, the solution was boiled in a water bath during 35 min and cooled for 10 min before quantification of malonaldehyde in a spectrophotometer[52].

Fatty acid methyl esters in raw meat samples were determined using the method established by Smith et al. (2002). In cooked meat, nonheme iron values were analyzed

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[46] model C200, Multivac Inc. Kansas City, MO
[47] 4 MIL Boil Vac Pouch, Ultravac Solutions, Kansas City, MO
[48] model GP-400, Neslab Instruments Inc. Newington, NH
[49] model HH501BT, Omega Engineering, Inc. Stamford, CT
[50] model 2005 VWR, Cornelious, OR
[51] Sigma-Aldrich, St. Louis, MO
[52] Varian, Cary 300 Bio UV-Visible Spectrophotometer, Walnut Creek, CA

following the procedure established by Ahn et al. (1993), 4 g of ground meat were placed in a 50 mL test tube, adding 12 mL of double distilled water and then homogenized. Aliquots of 1.5 mL of the mixture were obtained and 0.5 mL of 2% ascorbic acid[53] solution was added, after 5 min rest at room temperature 1 mL of 11.3% TCA was added followed by centrifugation[54] at 4000 rpm for 15 min. Samples were read at 562 nm in a spectrophotometer[55]. Muscle total fat and moisture analysis were performed using Microwave drying and Nuclear Magnetic Resonance[56] through the use of the CEM Smart Track System. Meat were thoroughly ground and analyzed in duplicate, weighing approximately 3-5 g of meat.

Statistical Analysis

The statistical analysis was performed in each muscle type using the General Lineal Model (SAS, 2002). The data was analyzed by a Completely Randomized Block Design, replication as a blocking factor, with 3 x 2 x 5 factorial arrangement: factors A, B, and C were dietary oil, vitamin E level, and storage day, respectively.

[53] Sigma-Aldrich, St. Louis, MO
[54] model RT6000B, Sorvall, Dupont Comp, Wilmington, Delaware
[55] model DU64, Beckman Instruments Inc. Fullerton, CA
[56] Smart Track System, CEM, Matthews, NC

RESULTS

The fatty acid composition of breast and thigh muscles were influenced by dietary oils ($P<0.05$), but not by vitamin E level (Table 26). CLA oil induced deposition of *cis*9,*trans*11 and *trans*10,*cis*12 CLA fatty acids, as well it significantly increased the proportion of saturated fatty acids (16:0, and 18:0) and decreased the ones from mono- (16:1, 18:1, 18:1*c*11) and polyunsaturated fatty acids. In contrast, flaxseed oil induced higher deposition of oleic (18:1), linoleic (18:2), linolenic (18:3), and araquidonic (20:4) fatty acids, particularly in thigh muscle; while menhaden fish oil induced higher deposition of EPA (20:5) and DHA (22:6) fatty acids compared to the other oil treatments.

Table 27 shows the dietary oil and vitamin E level effect on raw muscle total fat and total moisture content. Total fat in breast and thigh muscle was not affected by neither dietary oil nor vitamin E level effect ($P>0.05$). And total moisture was only affected in breast muscle, by the interaction between dietary oil and vitamin E level ($P<0.0206$). Meat samples from the flaxseed oil at the low vitamin E level showed lower moisture content than the other treatments.

Table 26. Fatty Acid Methyl Esters of Chicken Muscle Affected by Main Effect of Dietary Oils (Least Squares Means)

Fatty Acid	Breast			Root MSE[2]	Thigh			Root MSE
	CLA	Fish[1] Oil	Flaxseed Oil		CLA	Fish Oil	Flaxseed Oil	
				(% of Total Fat)				
14:0	1.19 [a]	1.35 [a]	0.54 [b]	0.47	1.08 [b]	1.53 [a]	0.47 [c]	0.30
16:0	31.34 [a]	24.67 [b]	21.33 [b]	4.48	29.92 [a]	24.65 [b]	20.11 [c]	2.76
16:1	1.90 [b]	3.18 [a]	3.72 [a]	1.22	2.16 [c]	5.13 [a]	4.03 [b]	0.84
18:0	13.06 [a]	9.38 [b]	7.83 [c]	0.96	13.76 [a]	8.27 [b]	7.23 [c]	0.96
18:1	20.07 [c]	25.10 [b]	28.45 [a]	1.58	21.13 [c]	28.03 [b]	31.09 [a]	1.80
18:1 *c*11	1.16 [b]	2.31 [a]	2.07 [a]	0.22	1.17 [c]	2.05 [a]	1.82 b	0.18
18:2	16.99	16.47	17.49	1.30	17.01 [b]	17.21 [b]	18.62 [a]	1.30
*c*9*t*11 CLA	3.28	.	.	0.67	3.36	.	.	0.38
*t*10*c*12 CLA	2.05	.	.	0.53	2.08	.	.	0.32
18:3	0.94 [b]	1.81 [b]	5.46 [a]	1.10	0.99 [c]	1.86 [b]	7.31 [a]	0.41
20:4	1.17	1.76	1.87	0.57	1.08 [b]	1.35 [b]	1.89 [a]	0.44
EPA	0.56 [c]	1.69 [a]	1.07 [b]	0.27	0.53 [b]	1.68 [a]	0.66 [b]	0.19
DHA	0.84 [b]	3.93 [a]	1.40 [b]	0.83	0.88 [b]	2.49 [a]	0.79 [b]	0.33
SFA[3]	44.23 [a]	34.73 [b]	29.38 [c]	3.62	44.76 [a]	34.35 [b]	27.72 [c]	2.70
MUFA[4]	23.75 [c]	30.55 [b]	33.95 [a]	2.49	24.46 [b]	35.21 [a]	36.95 [a]	2.38
PUFA[5]	20.12 [c]	25.37 [b]	27.53 [a]	1.77	20.31 [c]	24.59 [b]	29.28 [a]	1.97
SFA/ PUFA	2.22 [a]	1.35 [b]	1.08 [c]	0.23	2.21 [a]	1.41 [b]	0.96 [c]	0.16
Total n-3[6]	2.07 [b]	7.67 [a]	7.80 [a]	0.98	2.40 [c]	6.03 [b]	8.76 [a]	0.57

a, b, c: least squares means with different superscripts are significantly different ($P<0.05$).
[1]Menhaden fish oil.
[2]Root mean square error
[3]SFA: saturated fatty acids (14:0, 16:0, and 18:0)
[4]MUFA: monounsaturated fatty acids (16:1, 18:1, and 18c11)
[5]PUFA: polyunsaturated fatty acids (18:2, 18:3, 20:4, 20:5, and 22:6)
[6]Total omega-3 fatty acids (18:3, 20:5, and 22:6).

Table 27. Total Fat and Moisture Content in Raw Breast and Thigh Muscle Affected by Dietary Oil and Vitamin E Level (Least Squares Means)

Meat	Oil			Vitamin E (mg/kg)		Root
	CLA	Flaxseed	Menhaden	42	200	MSE[1]
Breast (%)						
Fat	1.08	1.20	1.06	1.12	1.10	0.27
Moisture (Oil x Vitamin E $P<0.0206$)						
42 mg/kg	74.07 $^{a\,x}$	73.92 $^{a\,x}$	74.09 $^{a\,x}$			0.57
200 mg/kg	74.45 $^{a\,x}$	73.20 $^{b\,y}$	74.04 $^{a\,x}$			
Thigh (%)						
Fat	2.67	2.81	2.62	2.67	2.72	0.47
Moisture	75.73	75.48	75.74	75.58	75.72	0.79

a,b/x,y least squares means with different letters between columns and rows, respectively, are significantly different.
[1]Root mean square error.

In both breast and thigh cooked *sous vide* meat total fat, total moisture, and cooked yield were not significantly ($P>0.05$) different between treatments, neither dietary oil nor vitamin E level effected these meat components (Table 28).

Table 29 shows that nonheme iron values of cooked *sous vide* meat were significantly ($P<0.0001$) affected by dietary oils, but not by vitamin E level ($P>0.05$). Breast meat samples from the CLA treatment showed higher nonheme iron values compared to the flaxseed and menhaden oil treatments. However, in thigh meat both CLA and menhaden oil treatments showed higher values than the flaxseed oil treatment. No effect from vitamin E level was detected in either breast or thigh meat ($P>0.05$).

Table 28. Total Fat, Moisture, and Cooked Yield of Cooked *Sous Vide* Chicken Meat Affected by Dietary Oil and Vitamin E Level (Least Squares Means)

Meat	Breast			Thigh		
	Fat (%)	Moisture (%)	Cooked Yield (%)	Fat (%)	Moisture (%)	Cooked Yield (%)
Dietary Oil						
CLA	1.57	70.71	83.50	3.63	72.80	84.55
Flaxseed	1.55	71.02	83.94	3.70	72.28	83.94
Menhaden	1.43	71.17	84.99	3.25	72.35	84.99
P-value	0.4375	0.4614	0.3727	0.2351	0.1003	0.3874
Vitamin E (mg/kg)						
42	1.58	71.25	84.34	3.38	72.49	84.81
200	1.45	70.69	83.95	3.67	72.46	84.17
P-value	0.3400	0.0776	0.6618	0.7800	0.8907	0.3079
Root MSE[1]	0.34	1.07	4.82	0.78	0.72	3.32

[1]Root mean square error.

Table 29. Nonheme Iron Values of *Sous Vide* Meat Affected by Dietary Oil and Vitamin E Level (Least Squares Means)

Meat	Breast	Thigh
	(µg/g)	
Dietary Oil		
CLA	0.28 [a]	0.37 [a]
Flaxseed	0.22 [b]	0.26 [b]
Menhaden	0.23 [b]	0.33 [a]
P-value	0.0001	0.0001
Vitamin E (mg/kg)		
42	0.25	0.31
200	0.24	0.32
P-value	0.6049	0.5066
Root MSE[1]	0.04	0.04

a b: least squares means between rows with different letters are significantly different.
[1]Root mean square error.

Lipid Oxidation Stability of *Sous Vide* Chicken Meat

The lipid oxidation stability in both breast and thigh meat was affected independently by the interaction of dietary fat or vitamin E level with storage day (Table 30). Significantly ($P<0.05$) higher values of malonaldehyde were found in meat samples from the menhaden and flaxseed oils compared to the CLA treatment, starting at day 5

of storage in breast and thigh meat, and remained throughout the rest of the storage time. At day 30, the maximum malonaldehyde values in CLA, flaxseed, and menhaden fish oil treatments in breast meat were 2.51, 3.52, and 3.53 mg/kg, respectively, and in thigh meat 2.16, 3.54, and 3.70 mg/kg, respectively.

Regarding vitamin E level, higher ($P<0.05$) malonaldehyde values were detected in meat samples from the low dietary level of vitamin E, starting at day 5 and 10 in breast and thigh meat, respectively. At day 30 of refrigerated storage, meat samples from the low level of vitamin E had approximately 1.25- and 1.23-fold higher of malonaldehyde than in the treatment with the high supplemented level in breast and thigh meat, respectively.

Table 30. Malonaldehyde Values (mg/kg) of Cooked *Sous Vide* Chicken Meat Affected by the Interaction of Dietary Oil or Vitamin E Level with Storage Day (Least Squares Means)

Meat	Dietary Oil			Vitamin E (mg/kg)		Root MSE[1]
	CLA	Flaxseed Oil	Menhaden Oil	42	200	
Breast						
0	0.24 ^{dx}	0.28 ^{ex}	0.26 ^{ex}	0.29 ^{ex}	0.23 ^{ex}	0.43
5	0.55 ^{cdy}	0.99 ^{dx}	0.88 ^{dxy}	0.90 ^{dx}	0.72 ^{cy}	
10	0.91 ^{bcy}	1.90 ^{cx}	1.57 ^{cx}	1.55 ^{cx}	1.37 ^{by}	
15	1.19 ^{by}	2.36 ^{bx}	2.17 ^{bx}	2.12 ^{bx}	1.69 ^{by}	
30	2.51 ^{ay}	3.53 ^{ax}	3.53 ^{ax}	3.55 ^{ax}	2.83 ^{ay}	
P-value	0.0067			0.0667		
Thigh						
0	0.19 ^{ex}	0.42 ^{ex}	0.30 ^{ex}	0.31 ^{ex}	0.28 ^{ex}	0.33
5	0.65 ^{dy}	1.16 ^{dx}	1.01 ^{dx}	1.03 ^{dx}	0.85 ^{dx}	
10	1.06 ^{cy}	1.66 ^{cx}	1.61 ^{cx}	1.59 ^{cx}	1.30 ^{cy}	
15	1.46 ^{by}	2.33 ^{bx}	2.40 ^{bx}	2.17 ^{bx}	1.97 ^{bx}	
30	2.17 ^{ay}	3.70 ^{ax}	3.54 ^{ax}	3.46 ^{ax}	2.81 ^{ay}	
P-value	0.0001			0.0223		

a, b, c, d, e: least squares means between rows are significantly different ($P<0.05$)
x, y: least squares means between columns are significantly different ($P<0.05$)
[1]Root mean square error.

DISCUSSION

As expected, the fatty acid composition of chicken of chicken muscles reflected the fatty acid composition of the dietary oils and experimental diets. Dietary CLA induced the highest proportion of SFA and the lowest proportions of MUFA and PUFA, resulting in the highest SFA:PUFA ratio, in both breast and thigh muscles. In addition, it was clear that feeding broilers with CLA induced deposition of *cis*9*trans*11 and *trans*10*cis*12 CLA fatty acid isomers, approximately 3.3% and 2.1%, respectively, in both breast and thigh meat. Du and Ahn (2002) reported that inclusion of 2% of CLA in broiler diets induced deposition of *cis*9*trans*11 and *trans*10*cis*12 fatty acids in breast chicken meat, at approximately 3.48% and 4.10%, respectively. Also, previously it had been reported that dietary CLA induced deposition of *cis*9*trans*11 and *trans*10*cis*12, increased SFA, and decreased MUFA and PUFA in broilers (Szymczyk et al., 2001; Badinga et al., 2003) and egg yolk (Cherian et al., 2002), when compared to other types dietary oils such as linseed oil, corn oil, or menhaden oil, respectively.

Flaxseed oil induced the lowest proportion of SFA and SFA:PUFA ratio, and the highest proportion of MUFA and PUFA, particularly linolenic (18:3) fatty acid that compared to CLA and menhaden oil had 5.9- and 3.0-fold higher in breast and 7.4- and 3.9-fold higher in thigh muscle, respectively. Menhaden oil induced the highest deposition EPA (20:5) and DHA (22:6) fatty acids. These results indicate that including these lipid sources in the broilers' diets the meat would be enhanced with omega-3 fatty acids, as previously reported by other studies feeding broilers with menhaden or flaxseed

oil (Marion and Woodroof, 1963; Gonzalez-Esquerra and Leeson, 2000; Lopez-Ferrer et al., 2001).

Previously, it had been reported that dietary CLA reduced broilers' carcass fat deposition (Du and Ahn, 2002). However, our results showed that dietary CLA oil had no effect on neither breast nor thigh muscle total fat deposition, which suggests that CLA may reduce the adipose tissue deposition only in the abdominal cavity and not in the muscle tissue. Sirri et al (2003) also did not observed changes in total lipid content in chicken breast or drumstick meat when feeding different dietary levels of CLA, compared to a soybean oil control treatment. According to Pariza (2004), reduction of body fat would be expected by dietary CLA due to its inhibitory effect on adipocyte differentiation and lipid accretion, by decreasing the adipocyte lipoprotein lipase activity, for which the *trans*10*cis*12 CLA isomer has shown the strongest physiological effects.

Regarding the lipid oxidation stability of the meat, the results of the present study indicate that as the proportions of MUFA and PUFA in breast and thigh muscle increase, the lipid oxidation susceptibility of *sous vide* cooked meat also increases over storage time. Meat samples from broilers fed with menhaden or flaxseed oil were more susceptible to lipid oxidation than to their counterparts from the CLA treatment. Higher peroxidation susceptibility of unsaturated fatty acids has been previously describe by Dahle et al. (1962), who showed that as the amount of double bonds increased in the fatty acids carbon chain so did the production of malonaldehyde and peroxide values. The enhancement of chicken meat with unsaturated and especially polyunsaturated fatty

acids has shown to drastically reduce the lipid oxidation stability of the meat, particularly with low amounts of an active antioxidant in the diet (Ajuyah et al., 1993; Morrissey et al., 1998).

It is important to point out that previous research has indicated that in aerobic conditions faster lipid peroxidation occurred in cooked thigh meat, rather than breast meat (Ajuyah et al., 1993), attributed to a higher catalytic "free" iron activity (Kanner et al., 1988). However in the present study, when tested, no significant differences in malonaldehyde content were detected between thigh and breast meat, despite the fact that thigh meat contained higher fat and nonheme iron values than breast meat. Due to the anaerobic conditions in *sous vide* meat, the results suggest that the lipid peroxidation in cooked *sous vide* meat is not dependent of nonheme iron values, as also reported in our previous study with *sous vide* meat (chapter IV). Analysis of the correlation coefficient between malonaldehyde and nonheme iron values was not significant ($P>0.05$) in breast meat and low and negative (-0.27) in thigh meat. The lipid oxidation stability of *sous vide* meat seems to be prolonged due to the anaerobic conditions the packaging system. Studies in canned turkey meat (Kanner et al., 1988) and *sous vide* turkey rolls (Smith and Alvarez, 1988) showed low malonaldehyde values during the vacuum conditions during prolong storage time, but rapid lipid peroxidation was detected soon after exposure of the meat to the ambient.

Meat samples from the CLA treatment showed higher nonheme iron values at all sampling days, but lower malonaldehyde values than those from the flaxseed and menhaden oil treatments. The authors of the present study have no explanation about the

reason why the meat CLA meat samples showed higher amounts of nonheme iron. In our previous study with *sous vide* meat no effect from dietary oils was observed and it is considered that the release process of nonheme iron is more affected by cooking temperatures (Bochowski et al., 1988). Because the meat packages were cooked in batches including the samples from all the treatments, the cooking effect is ruled out in this situation.

Supranutritional supplementation (200 mg/kg) of vitamin E was more effective at inhibiting the lipid oxidation development than the control level (44 mg/kg), particularly in thigh meat. Thus, it could be inferred that the higher deposition of α-tocopherol in the muscle tissues, induced by the supranutritional level, had a higher antioxidant activity in the meat. In chicken muscle tissues, the accumulation α-tocopherol is dependent of the amount of vitamin E supplemented in the diet and feeding period, which directly influences the lipid oxidation stability of the meat and processed meat products (Asghar et al., 1990; Sheehy et al., 1991; Bartov and Frigg, 1992; Jensen et al., 1999). Hence, higher antioxidant activity in the meat would be expected as the supplementation level is increased in the broilers' diets, disregarding the aerobic or anaerobic conditions in which the meat is maintained.

In conclusion, dietary CLA, flaxseed, and menhaden oil induced deposition of their predominant fatty acids into the chicken muscles. Changes in the composition, amount, and degree of unsaturation of fatty acid in the meat affects the lipid oxidation development in *sous vide* cooked chicken meat. Supranutritional supplementation of

vitamin E is more effective at inhibiting the lipid oxidation development than a current commercial level.

CHAPTER VII

SUMMARY AND CONCLUSIONS

SUMMARY

In recent years there has been an increased in demand of further processed meat products and food products enhanced with unsaturated and polyunsaturated fatty acids such as those from the omega-3 and CLA groups, so as to provide convenience as well as health benefits to the consumers. Unfortunately, unsaturated fatty acids and those muscle foods that contain them are susceptible to develop lipid oxidation, which reduces the quality, shelf-life, and nutritional value of the meat.

This situation is of great economical importance for the food industry because the development of lipid oxidation is associated with the appearance of objectionable off-odors, off-flavors, warmed-over flavors, and discoloration of the meat that decrease the consumer acceptance of the product. As well and perhaps more importantly, the development of lipid peroxidation results in accumulation of free radicals and chemical by-products that may affect the consumer health status. Thus, as important as to enhance the proportion of unsaturated and polyunsaturated fatty acids in muscle foods, is the preservation of the nutritional value and quality of the meat through the inhibition of the lipid oxidation development.

In the first experiment, it was observed dietary fat excerpted and important effect on the accumulation of the type and proportion of saturated, mono-, and unsaturated

fatty acids in the muscle tissues. In general, soybean oil and palm kernel oil increased and reduced the proportion of unsaturated and saturated fatty acids, respectively. Changes in the proportions of these fatty acids resulted in differentiated lipid oxidation stability in raw chicken meat and skin; while breast meat showed not to be susceptible to lipid oxidation, thigh meat and skin developed higher lipid oxidation during refrigerated storage with the soybean oil, compared to the animal/vegetable, lard, and palm kernel oil. Cooked chicken meat showed rapid development of lipid oxidation over storage time, particularly in meat samples from the soybean oil treatment. Supranutritional supplementation of vitamin E showed to be more effective at inhibiting the lipid oxidation development in both raw and cooked chicken meat over storage time, than the commercial (33 mg/kg) control level used in this experiment.

In the second experiment, it was observed that dietary fats and vitamin E did not affect the some physicochemical properties and quality characteristics of chicken meat, regardless of the differences between treatments in the fatty acid composition and α-tocopherol amount in the meat. Neither drop in muscle pH, meat tenderness, expressible moisture, nor total moisture showed to be affected by the diet. Color of the meat was affected by either dietary fat or vitamin E; however the differences observed between the treatments seem not to be associated with the development of pale, soft, and exudative (PSE) meat condition in chicken meat.

In the third experiment, because in the previous experiment the lipid oxidation of cooked chicken meat patties, stored in aerobic refrigerated conditions, developed relatively fast in a short period of time, even in the treatments with supranutritional

supplementation of vitamin E, it was necessary to analyze the meat lipid oxidation stability using an alternative packaging-cooking method such as *sous vide*. *Sous vide* is meat that is processed and stored in vacuumed conditions and it is relevant importance for the restaurant, catering, and food services. It was observed that *sous vide* has prolonged refrigerated shelf-life, shown as relatively low values of malonaldehyde, up to 40 days of storage. As well, the lipid oxidation development in this type of meat is not affected by dietary fats or nonheme iron values when animal/vegetable, palm kernel, or soybean oil is fed to the broilers. Even though lipid oxidation development in *sous vide* meat is relatively slow, supranutritional supplementation of vitamin E (200 mg/kg) provides higher lipid stability than the commercial level (33 mg/kg) used.

In the fourth experiment, based on the results found in the third experiment where *sous vide* meat had prolonged shelf-life under refrigeration, regardless of the differences in the proportion of fatty acids in the muscle tissues. The goal of the experiment was to enhance the meat with polyunsaturated fatty acids such as those from the omega-3 and CLA group, and at the same time maintain the lipid oxidation stability of cooked chicken meat. It was observed that dietary CLA increased the proportion of saturated fatty acids, induced deposition of CLA isomers, and decreased the proportion of mono- and polyunsaturated fatty acids in the meat; while menhaden and flaxseed oils increased the overall deposition of mon- and polyunsaturated fatty acids, particularly those from the omega-3 group (18:3, 20:5, and 22:6). Cooked *sous vide* meat from the CLA treatment showed lower lipid oxidation development, compared to the menhaden and flaxseed oil treatments. As well, supranutritional supplementation of vitamin E (200

mg/kg) exhibited a more effective antioxidant activity than the commercial level (33 mg/kg) used. Lipid peroxidation in cooked *sous vide* meat does not depend on nonheme iron values.

CONCLUSIONS

Both raw and cooked chicken meats are susceptible to develop lipid oxidation under refrigerated conditions, primarily affected by the accumulation of unsaturated fatty acids in the meat, induced through the diet.

Neither dietary fat nor vitamin E level seem to affect physicochemical properties and quality attributes of chicken meat such as postmortem muscle pH drop, tenderness and water holding capacity. Because of these, the dietary effects of meat color may not be associated with development of PSE meat condition in chicken meat.

Sous vide meat has prolonged shelf-life under refrigerated conditions, as shown by the relative low lipid oxidation development in the meat. Thus, relative high proportions of unsaturated fatty acids can be increased without affecting the meat lipid oxidation stability. Nonheme iron is not an important factor inducing lipid oxidation in *sous vide* meat.

Chicken meat through the diet can be also enhanced with functional fatty acids such as omega-3 and CLA. Chicken meat with enriched amounts of polyunsaturated fatty acids presents relatively long lipid oxidation stability when processed under the

sous vide method. Nonheme iron does not affect the lipid oxidation development in *sous vide* meat.

Supranutritional supplementation of vitamin E is more effective at enhancing the lipid oxidation stability of either raw, cooked, and *sous vide* meat regardless on the type and proportion of fatty acids in the muscle tissues, than the commercial level used in the present experiments. Higher muscle accumulation of α-tocopherol is induced by supranutritional levels.

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