PARTITIONING OF ETHANOLIC DELTA-TOCOPHEROL AND COOKING EFFECTS ON OXIDATIVE STABILITY AND QUALITY OF BEEF PATTIES DURING REFRIGERATED STORAGE

TODD MICHAEL WILLS

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Oklahoma State University

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Thesis approved:

Thesis Advisor

Dean of Graduate College

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"You gotta dance like nobody's watching, dream like you will live forever, live-like you're going to die tomorrow and love like it's never going to hurt" - Meme Grifsters. This quote pretty much sums up my college perspectives; however, many thanks are in order for those that kept me on track throughout my college days.

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FORMAT OF THESIS

This thesis is presented in the Journal of Agriculture and Food Chemistry style format, as outlined by the Oklahoma State University graduate college style manual. The use of this format allows for independent chapters to be suitably prepared for submission to scientific journals.

CHAPTER 1

INTRODUCTION

The past decade has seen an increased trend toward the consumption of convenient type items or heat and eat entrees. The meat industry has placed a tremendous amount of focus on establishing a niche in this marketing segment to capture the value associated with these consumer friendly foods. Many food companies today have expanded their operations to include processing facilities. This diversification has enabled producers to recapture a portion of the market value that could be otherwise lost. Processing meat is one such method that has been utilized to create the convenience items consumers now demand, while substantially increasing the variety and value of these products. This allows processors to utilize low demand cuts, and increase their value by the additional processing involved as well as the incorporation of other ingredients. In general, meat processing could be defined as all processes utilized in altering fresh meat except for simple mixing, grinding, or cutting.

Processed meat items are commonly manufactured from muscle tissue high in total fat to aid in the overall texture of the product or to act as a stabilizer in emulsion type products. The lipids of processed meat products are much more susceptible to oxidation than the lipids of whole muscle. This low oxidative stability is in large part due to incorporation of oxygen and release of heme catalysts and/or degradative enzymes due to cellular disruption during the grinding, mixing, or mincing process (Dawson &

Gartner, 1983). Furthermore, the phospholipids are generally considered more susceptible to oxidative deterioration than the neutral triacylglycerols in animal tissue (Dawson et al., 1990). This is primarily due to the higher degree of unsaturation of their fatty acids (Duckett & Wagner 1998). Oxidation in muscle foods can result in deterioration in many quality characteristics such as flavor, color, texture, nutritional value, and food safety (Buckley et al., 1996). For this reason, the oxidative stability of meat and precooked and restructured meat products is a problem for all those involved in the meat production chain, including the primary producers, processors, distributors, and retailers, and understanding and controlling the processes which lead to lipid oxidation is a major challenge for meat scientists (Morrissey et al., 1998).

Cooking of meat and meat products increases the rate and extent of oxidation. McCarthy et al. (2001) reported cooking of pork patties significantly increased TBARS values with a four-fold increase in oxidation levels being recorded in raw patties upon cooking. Kanner (1994) showed that high temperatures decreased the activation energy for oxidation, breaking down pre-formed hydroperoxides that propagate lipid peroxidation and the development of off-flavors. Cooking method and final internal temperature have an important effect on the formation and stability of the volatile compounds in meats.

Ohmic processing, sometimes described as resistive heating, consists of passing current directly through a conductive food, which in turn generates heat. Because heating accompanies the current, heat distribution throughout the product is far more rapid and even, which can result in better flavor retention and particulate integrity compared to conventional methods (Skudder, 1993). Ohmic heating has shown significant promise in

a number of food processes, including sterilization and pasteurization (Sastry & Li, 1996). Unlike conventional heating, which relies on heat transfer from the carrier medium to the particles, ohmic heating causes large food particles to heat at rates comparable to the surrounding liquid (Ruan et al., 1999). Products cooked in such a rapid manner could be less susceptible to oxidation; however, neither the oxidative stability nor the quality characteristics of such products have been investigated.

Antioxidants are commonly added to food products to slow or inhibit oxidative deterioration. Muscle-based foods that contain high concentrations of endogenous tocopherol have demonstrated greater lipid and oxymyoglobin stability (Faustman et al., 1999). Dietary supplementation with vitamin E is generally regarded as an acceptable, "consumer friendly" supplement and when incorporated into animal diets is a highly effective lipid-soluble chain breaking antioxidant. When supplemented through feed, vitamin E partitions into membrane lipids and protects against oxidative attack. Furthermore, direct addition of tocopherol to processed meat products has previously been reported to have no significant antioxidant effect (Mitsumoto et al., 1993; Buckley et al., 1995; Kerry et al., 1998; Higgins et al., 1998). In these studies, the oxidative stability was not enhanced because antioxidants weren't incorporated into the membrane lipids.

Oxidation is considered to be a surface phenomenon, occurring at a lipid-water interface (Boyd et al., 1990). Determining the partitioning characteristics of antioxidants between different phases is an important parameter used to select antioxidants that favor distribution toward the microenvironment that is most susceptible to oxidation (Huang et al., 1997). Thus, directing the added antioxidant to or near the origin of the oxidation

initiation and/or propagation is of particular importance. The partitioning of antioxidants has generally been studied in various model or simple food systems (Cornell et al., 1970; Frankel et al., 1994; Huang et al., 1997). Results from these studies indicate partitioning is influenced by both the lipid and antioxidant surface (Porter et al., 1989). Recent studies with chicken muscle have shown that tocopherol partitioning between membrane lipids and triacylglycerols is significantly affected by the carrier used to deliver the antioxidant into the system (Sigfusson and Hultin 2002a,b). Using ethanol, significant amounts of tocopherol were incorporated into membrane lipids, whereas virtually no incorporation was observed when corn oil was used as a carrier.

The purpose of this study was to utilize an ethanol carrier to add vitamin E to meat and evaluate the oxidative stability of cooked beef patties. Specifically, the objectives were to 1) evaluate the effect of ohmic heating on quality of beef patties compared to cooking with an impingement oven, 2) evaluate the effectiveness of vitamin E in retarding oxidative deterioration in cooked beef patties, and 3) determine partitioning of vitamin E between membrane lipids and triacylglycerols as affected by cooking

CHAPTER 2

LITERATURE REVIEW

2.1 Beef Muscle

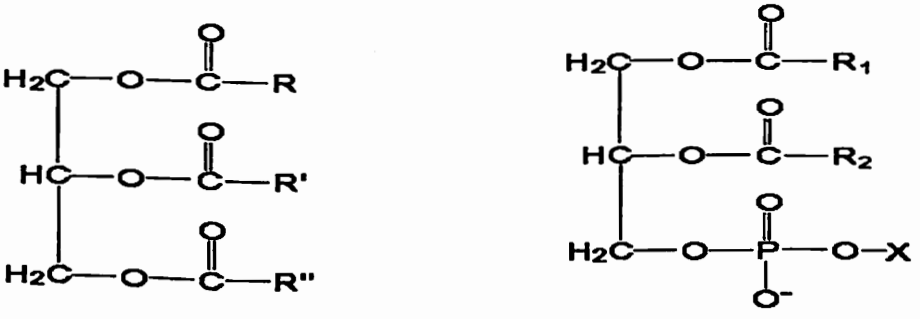
2.1.1 Chemical composition

The gross composition of beef muscle varies considerably due to differences in species, breed, sex, age, activity, and nutrition. On average, lean muscle contains 72% moisture, 21% protein, 5% lipids, and 1% ash (Lawrie, 1998). Variation in the composition is primarily influenced by the accumulation of lipids. In beef semitendinosus, longissimus and triceps brachii muscles, 9 to 14 % of total lipids consist of phospholipids and from 82 to 90% as neutral lipids (O'Keefe et al. 1968).

2.1.2 Neutral lipids (triacylglycerols) vs polar membrane lipids (phospholipids)

Lipids are a diverse group of biological substances made up primarily of nonpolar groups. As a result of their nonpolar character, lipids typically dissolve more readily in nonpolar solvents such as ether, chloroform, and benzene, than in water. Neutral lipids are characterized as so, because at cellular pH they contain no charged groups, are completely non-polar, and have no affinity for water. Neutral lipids are comprised of fatty acids esterified to either glycerol or a fatty alcohol and are commonly found in cells as storage fats and oils. Triacylglycerols (TAG) contain three fatty acids esterified to

glycerol and are primarily found as intermuscular deposits in adipose tissue. A group of phosphate-containing molecules with structures related to the triglycerides are phospholipids. Phospholipids are the primary lipids associated with biological membranes. The polar nature of the phospholipids arises from their phosphate containing headgroup linked to the 3-carbon. This group confers the greatest variation in the physical properties of the phospholipids (Sigfusson, 2000). Furthermore, phospholipids play an important role in governing the quality of meat during both raw processing and cooking and they are important flavor precursors because of their high content of long-chain polyunsaturated fatty acids (Caboni et al., 1994). Structures of lipids are shown in Figure 1.



Triacylglycerols (TAG) **Phospholipids (PL)**

Figure 1. Structures of triacylglycerols (TAG) and phospholipids (PL). R_i represents fatty acids of varying chain length and unsaturation. X represents a number of different substituents. (Darnell et al., 1990).

2.1.3 Membrane structure and composition

Biological membranes are sheetlike structures that are composed of protein and lipid molecules held together by noncovalent interactions. Membranes are highly selective permeable barriers and enable the formation of cytoplasmic compartments. Because all phospholipids are amphipathic, hydrophobic interactions between the fatty

acyl chains of glycolipid and phospholipid molecules create a sheet containing two layers of phospholipid molecules whose polar head groups face the surrounding water and the fatty acyl chains form a continuous hydrophobic interior (Darnell et al., 1990). A model illustrating the membrane bilayer structure is shown in Figure 2.

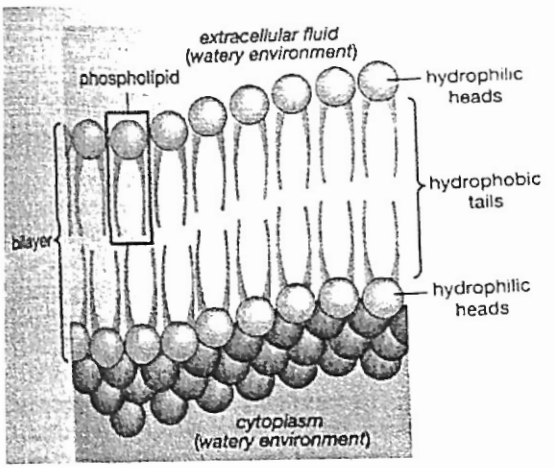


Figure 2. Fluid mosaic model of phospholipid bilayer.
http://sun.menloschool.org/%7Ecweaver/cells/c/cell_membrane/

The composition of membranes differs significantly between muscles and species. The phospholipid classes generally constitute between 60-90% of the total lipids of the membranes, with the remaining 10-40% being neutral lipids (Alberts et al., 1989). The neutral lipid components are cholesterol, steroids, tocopherols, ubiquinols, and triacylglycerols. Triacylglycerols have often been reported to be present in "traces" or, at best, derived from calculated differences between more sizeable lipid classes. However, previous observations indicate TAG as an intrinsic membrane component that seems to play a specific role in metabolic events such as cell stimulation or transformation (Lerique et al., 1994). Lerique and others also indicate the TAG content of biomembranes decreases with the age of the organism.

Proteins may constitute up to three-quarters of the total dry weight of the membranes (Stanley, 1991). All biological membranes, no matter how carefully purified, are found to contain proteins. The percentage and exact nature of the adhering proteins vary considerably with membrane type (Stryer, 1988). Membrane proteins are generally classified according to the type of interactions that associates them to the membrane. Integral membrane proteins (intrinsic proteins) contain amino acid residues with hydrophobic side chains that interact with the fatty acyl groups of the membrane phospholipids. Intrinsic proteins are partially embedded in the membrane and completely traverse the bilayer. Removal of such proteins requires the action of detergents to displace the lipids bound to the hydrophobic side chains. Peripheral proteins (extrinsic proteins) are usually loosely bound to the membrane surface indirectly through interactions with integral membrane proteins or directly by interacting with polar lipid head groups. Extrinsic proteins can be removed from the membrane with high ionic strength solutions, which disrupt ionic bonds (Darnell et al., 1990). Cellular membrane proteins further associate with the cytoskeleton and determine cell shape, tissue integrity, and play a role in cell organelle structure (Luna &Hitt, 1992).

2.1.4 Ultracentrifugation techniques for isolation of membrane lipids

Differential ultracentrifugation methods are most widely employed for the isolation of membranes or subcellular particles from tissue homogenates (Fleischer & Rouser, 1965). When isolating membranes, the muscle must be suspended in several volumes of buffer and homogenized to form a suspension. The membrane suspension should maintain a net negative charge (pH 7.2-7.5) at the membrane surface to enable repulsive forces to keep the membranes suspended during centrifugation steps

(Sigfusson, 2000). The negatively charged suspension then undergoes multiple ultracentrifugation steps where cell separation occurs due to differing sedimentation rates of the particles. The initial centrifugation step requires low speeds to force sedimentation of large muscle components to the bottom of the tube to form a pellet. Additional centrifugation steps are utilized at progressively higher forces to separate further bound components. Membrane isolation may appear to be a relatively straightforward laboratory operation, but, in practice, this is rarely the case. Occasionally, membranes can be isolated intact, such as erthyrocyte "ghosts", but most often tissue is homogenized, and the disrupted membrane fragments self-associate to form sealed vesicles that are then harvested by differential centrifugation (Stanley, 1991).

2.2 Lipid extraction methods

Total lipid content of biological samples is an important component used in many studies. No single standard method is employable for lipid extraction; however, the method used should depend on the type of material being analyzed and the nature of the subsequent analytical problems being studied. Isolation, or extraction, of lipid from tissues is performed with the use of various organic solvents. In principle, the solvent or solvent mixture used must be adequately polar to remove lipids from their association with cell membranes and tissue constituents but also not so polar that the solvent does not readily dissolve all triacylglycerols and other non-polar lipids (Iverson et al., 2001). Generally, successful extraction requires that bonds between lipids and other compounds be broken so that the lipids are freed and solubilized. Solubility is achieved when polarities of the lipid and solvent are similar. Non-polar triglycerides are dissolved in

non-polar solvents such as hexane and petroleum ether, whereas polar compounds, such as glycolipids, are soluble in alcohols (Pomeranz & Meloan, 1994).

A rapid method for lipid extraction was originally proposed by Folch et al. (1957) for isolation and purification of total lipids from animal tissue by means of phase partition of a ternary mixture of chloroform-methanol-water. Bligh and Dyer (1959) simplified the method. In the simplified procedure, the sample is homogenized with a mixture of chloroform and methanol in such proportions that a miscible system is formed with the water in the sample. Dilution with chloroform and methanol separates the homogenate into two layers, the chloroform layer containing all the lipids and the methanol layer containing all the non-lipids (Pomeranz & Meloan, 1994; Lee et al., 1996).

Extensive literature is available on the comparison of the extraction efficiencies of various lipid classes from muscle tissues (Iverson et al., 2001; Phillips et al., 1997). Lee and others (1996) developed a simple and rapid solvent extraction method for determining total lipids in fish tissue. This method is less labor intensive, uses lower solvent quantities, and is more precise than earlier methods. The procedure also eliminates problems associated with laborious filtration and does not require an exact reading of chloroform volume; instead it utilizes a theoretical volume which depends on solvent volume and ratio used. Phase separation carried out under refrigerated conditions, will also minimize oxidative deterioration of the lipid extract. Furthermore, Sigfusson (2000) adapted this procedure to extract membrane lipids and tocopherols from chicken muscle.

2.3 Lipid oxidation

2.3.1 General

Lipid oxidation is one of the major causes of quality deterioration in raw and cooked meat products (Hui et al., 2001). Lipid oxidation proceeds via a complex, radical chain reaction, which is generally separated into initiation, propagation and termination stages (Coupland & McClements, 1996). Because the activation energy required for the oxidation reaction is somewhat high (30 to 45 kcal/mol), the formation of free radicals is thermodynamically unfavorable and therefore requires catalytic means, e.g. light, temperature, enzymes, metals, metalloproteins, or radiation (St. Angelo, 1996). Oxidation steps are described below:

Initiation, i.e., the formation of free-radicals:

[1] $LH \xrightarrow{k1,\ \text{initiator}} L\cdot$

Propagation of the chain reaction by abstraction of hydrogen atom at a position α to double bonds of fatty acids, addition of oxygen and the production of peroxy radicals. The peroxy radicals extract another hydrogen atom to form a hydroperoxide, which propagates the radical reaction. Most food oils naturally contain enough lipid peroxides to promote lipid oxidation, even if other sources of radical generators are rigorously eliminated.

[2] $L\cdot + O_2 \xrightarrow{k2} LOO\cdot$

[3] $LOO\cdot + LH \xrightarrow{k3} LOOH + L\cdot$

Termination, i.e., the formation of non-radical products:

[4] $L\cdot + L\cdot \xrightarrow{k4}$

[5] $L\cdot + LOO\cdot \xrightarrow{k5}$ } non-radical + O_2

[6] $LOO\cdot + LOO\cdot \xrightarrow{k6}$

During lipid oxidation, a number of decomposition reactions occur simultaneously, eventually leading to the formation of a complex mixture of reaction products, including aldehydes, ketones, alcohols and hydrocarbons. These compounds are partly responsible for the development of off-odors and flavors in meat products.

2.3.2 Oxidation in muscle foods

The low oxidative stability of meat and precooked and restructured meat products is a problem for all those involved in the meat production chain, including the primary producers, processors, distributors, and retailers, and understanding and controlling the processes which lead to lipid oxidation is a major challenge for meat scientists (Morrissey et al., 1998). Oxidation of lipids can occur in vivo, during the conversion of muscle to meat, and in meat and meat products. Lipid oxidation in vivo does not possess such a significant problem because animals have evolved several mechanisms that limit inappropriate exposure to reactive oxygen species (Yu, 1994). However, the biochemical changes that accompany the conversion of muscle to meat give rise to conditions where oxidation in the highly unsaturated phospholipid fraction in subcellular membranes is no longer tightly controlled and the balance between prooxidative factors and antioxidative capacity favors oxidation (Morrissey et al., 1998). The most significant phase of lipid

oxidation occurs during handling, processing, storage and cooking of meat and meat products. These processes lead to cellular disruption, release of heme-catalysts, enzymes and incorporation of oxygen, which in turn promote oxidation.

The phospholipids are generally considered more susceptible to oxidative deterioration than the neutral triacylglycerols in animal tissue (Dawson et al., 1990). This is primarily due to the higher degree of unsaturation of their fatty acids (Duckett & Wagner 1998), and the phospholipid surface area is much larger than the triacylglycerols (Hultin, 1981). The rate and extent of membrane oxidation varies depending on muscle types. Asghar et al. (1989) found the subcellular fractions of chicken muscle isolated from the thigh muscles were more susceptible to oxidation than compared to membranes of breast muscles. This effect is proposed to be due to the differences in lipid content between the two tissues. The locomotive muscles generally contain a greater concentration of phospholipids than the support muscles (Asghar et al., 1989).

A consequence of membrane deterioration is the loss of ability to act as a semipermeable membrane or diffusion barrier. The practical consequence of such deterioration directly affects water-holding capacity of meat and leads to drip loss. The modification of membrane functionality as a result of lipid oxidation results in: uncoupling of oxidative phosphorylation in mitochondria, alteration of endoplasmic reticulum function, increased permeability, loss of fluidity, inactivation of membrane-bound enzymes, and polymerization, cross-linking, and covalent binding of proteins (Stanley, 1991).

2.3.3 Warmed over flavor in cooked meat

The rapid development of off-flavors in cooked meats was initially described as "warmed-over-flavor" (WOF) by Tims and Watts (1958). However, the term "meat flavor deterioration" (MFD) has been suggested to better describe the complex series of chemical reactions that contribute to an overall increase in off-flavor notes and a loss in desirable meat flavor quality (Drumm and Spanier, 1991). WOF has commonly been described as displaying odors and flavors in meat products described as "stale", "cardboard-like", "painty", "musty", or "rancid". The accelerating effect of heating on the development of oxidative rancidity in meat products has been observed by a number of researchers (Su et al., 1991). WOF is generally considered to be due to the oxidation of phospholipids located in the cell membranes; heat breaks the lipoprotein complex, releasing and liquefying the lipid fraction, exposing its unsaturated lipids to oxygen and catalysts, resulting in lipid oxidation (Allen & Foegeding, 1981). Lipid hydroperoxides, the initial products of lipid oxidation, are unstable and undergo further degradation through free-radical mechanisms to form aliphatic aldehydes, alcohols, ketones, and hydrocarbons. These secondary products, referred to as 2-thiobarbituric acid reactive substance (TBARS), are the major contributors to off-flavor in meats. However, WOF is not only the result of peroxidation of unsaturated lipids, but is also caused by loss of desirable odorants involved in the pleasant flavor of freshly cooked meat (Kerler and Grosch, 1996).

Hexanal has been used as a successful marker for the oxidative decomposition of oxidized n-6 polyunsaturated fatty acids (Kim et al., 2003). Hexanal, as well as total volatiles, show a highly significant correlation with the sensory evaluation scores and

TBARS values in cooked meat systems (Lee and Lillard, 1997). Stabilizing the membranes can inhibit WOF development in meat and meat products.

2.4 Antioxidants

2.4.1 General

According to the USDA Code of Federal Regulations [21, CFR 170.3 (0) (3)] "antioxidants are substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation". The main justification for using antioxidants is to extend the shelf life of foodstuffs and to reduce wastage and nutritional losses by inhibiting and delaying oxidation. Furthermore, it has been suggested that an ideal food-grade antioxidant should be safe, not to impart color, odor or flavor, be effective at low concentrations, be easy to incorporate, to survive after processing, and to be stable in the finished product as well as be available at a low cost (Christen & Smith, 2000). Wide varieties of natural and synthetic antioxidants are utilized in processed foods; however, the concentration of use is strictly controlled.

Antioxidants are typically classified according to their mode of action; antioxidants may be classified as free radical terminators, chelators of metal ions, or as oxygen scavengers that react with oxygen in closed systems (Shahidi & Wanasundara, 1992). Phenolic antioxidants, which are free radical terminators, interfere with lipid oxidation by rapidly donating a hydrogen atom to a lipid radical, forming a relatively stable antioxidant radical. Current synthetic phenolic antioxidants permitted for use in foods are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), dodecyl gallate (DG) and tertiary-butylhydroquinone (TBHQ). The use of

synthetic antioxidants has been effective because of their low cost, high stability, and effectiveness. However, their use in food has been decreasing because of possible mutagenicity and consumers' rejection of synthetic food additives (Wong et al., 1995). Natural antioxidants are commonly derived as plant polyphenolic compounds that may occur in all parts of the plant. Plant phenolics are multifunctional and can act as reducing agents, metal chelators, and singlet oxygen quenchers. Examples of common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, coumarins, tocopherols and polyfunctional organic acids (Shahidi & Wanasundara, 1992).

2.4.2 Tocopherols (vitamin E)

Vitamin E is a generic description for all tocopherol and tocotrienol derivatives. Tocopherols have a phytyl chain, while tocotrienols have a similar chain but contain three double bonds at positions 3′, 7′ and 11′. Both tocopherols and tocotrienols have four isomers, designated as α-, β-, γ- and δ-, which differ by the number and position of methyl groups on the chroman ring (Yoshida et al., 2003). In respect to antioxidant activity, α-tocopherol displays the highest biological activity and accounts for approximately 90% of the vitamin E activity found in tissues (Cohn, 1997). Furthermore, the activities of α-, β-, γ- and δ- tocopherols have been studied in SDS micelles and PC liposomes, reports indicate that the antioxidant activities increase in the order of $\delta < \gamma < \beta < \alpha$ (Pryor et al., 1988; Fukuzawa et al., 1982). Various structures of tocotrienol isomers and tocopherol are shown in Figure 3.

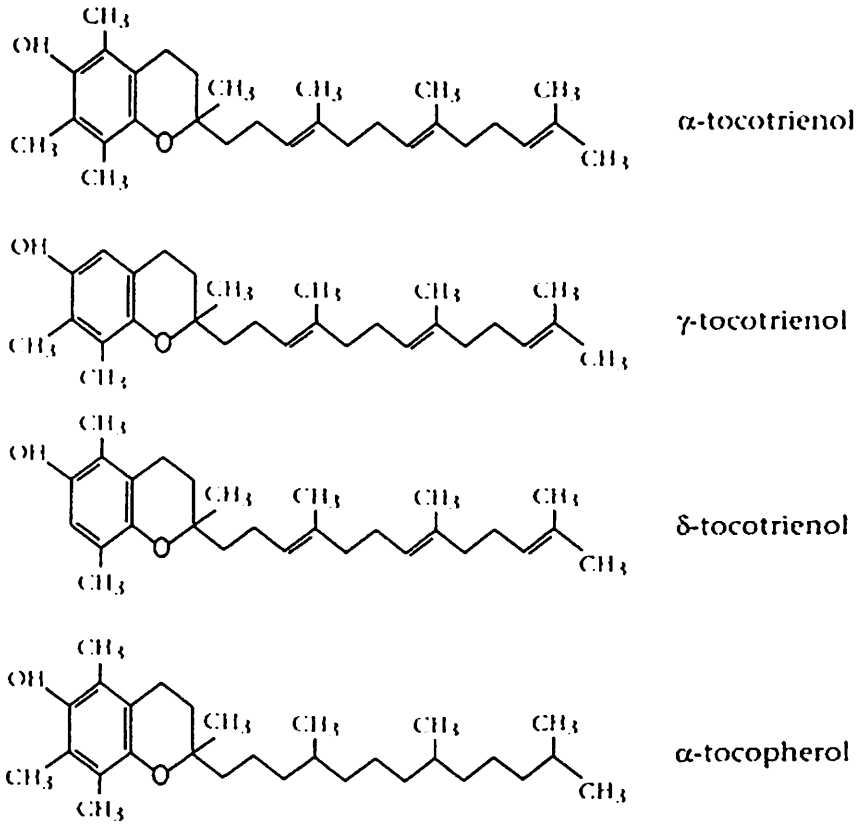


Figure 3. Structures of α-, γ- and δ- tocotrienol and α- tocopherol. http://www.carotech.net/toco.html.

Tocopherols are only present in trace quantities in the animal kingdom; however, the plant kingdom offers a range of natural phenolic compounds, among which α-tocopherol is best known as one of the most efficient naturally occurring liposoluble antioxidants (McCarthy et al., 2001). Tocopherols are commercially extracted from deodorizer sludge obtained from the deodorization of vegetable oils (Shahidi & Wanasundara, 1992).

Muscle-based foods that contain high concentrations of tocopherol demonstrate greater lipid and oxymyoglobin stability (Faustman et al., 1999). The increased stability the tocopherols impart is due to their ability to rapidly scavenge lipid peroxyl radicals,

thus creating a cellular defense mechanism. The reactions involved in the scavenging function of vitamin E are illustrated in figure 4.

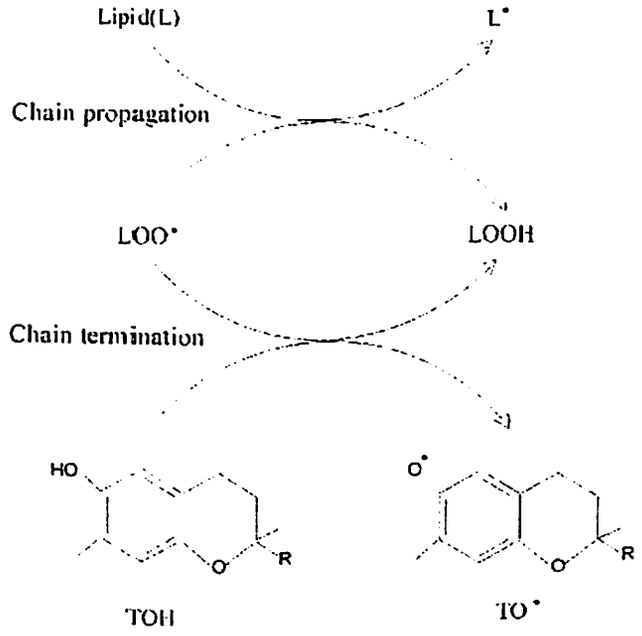


Figure 4. Scavenging reactions of α-tocopherol (Wang & Quinn, 2000).

Briefly, oxidation of lipids proceeds by a free radical mediated process, where a lipid peroxyl radical serves as a chain carrier. Chain propagation occurs by abstraction of a hydrogen atom from the lipid to produce a lipid hydroperoxide. The lipid hydroperoxide further reacts with oxygen and continues the chain reaction by producing additional peroxyl radicals. The principle role of α-tocopherol is to scavenge the lipid peroxyl radical before it is able to attack the target lipid substrate producing α-tocopheroxyl radicals (Wang & Quinn, 2000). Tocopheroxyl radicals and lipid hydroperoxides are formed when α-tocopherol reacts with a peroxyl radical by means of hydrogen transfer or sequential electron then proton transfer. Tocopheroxyl radicals are highly stable and

relatively unreactive due to delocalization of the unpaired electron of the fully substituted chromanol ring system (Kamal-Eldin & Appelqvist, 1996). In vitro measurements of the relative rate of chain propagation to chain inhibition by α-tocopherol have indicated that α-tocopherol scavenges the peroxyl radicals considerably faster than the peroxyl radical reacts with lipid substrates. In addition to the rapid rate of chain inhibition, α-tocopherol reacts approximately 200 times faster with a peroxyl radical than does the commercial antioxidant BHT (Burton & Traber, 1990).

2.4.3 Tocopherols in muscle tissue

Vitamin E is an ubiquitous, minor component of biological membranes. The concentration of α-tocopherol in cell membranes is relatively low. Despite its relatively low concentration compared to other membrane lipids, it plays an important role in preserving membrane integrity. Because vitamin E is a fat-soluble vitamin, it tends to partition into tissue lipids, locate in hydrophobic domains like lipoproteins, or partition into the membrane bilayer. Distribution of vitamin E in muscles differs greatly (Podda et al., 1996). Adipose tissue, liver and muscle represent the major sites of vitamin E in the body, with ~90% of the vitamin being contained in the adipose tissue. Of all the subcellular membrane fractions, the greatest concentrations of α-tocopherol were found in the Golgi membranes and lysosomes (Wang & Quinn, 2000). It is noteworthy that only α-tocopherol has been measured in any significant amount in animal tissues and may account for as much as 80-100% of the total tocopherols and tocotrienols (Sigfusson, 2000). Asghar et al. (1991) illustrated that although δ- and γ-tocopherols were present in feed components of plants or seed origin, only α-tocopherol could be detected in the adipose and muscle tissues, as well as subcellular fractions of pork muscle. Tocopherol

binding protein (TBP) is believed to be involved in the transport and metabolism of α-tocopherol in tissues. The TBP specifically binds α-tocopherol, in preference to δ- and γ-tocopherol, and may exclusively transport α-tocopherol to intracellular sites (Wang & Quinn, 2000). The importance of vitamin E for protecting the integrity of lipid structures (especially membranes) in vivo is underscored by the finding that it is the only major lipid-soluble, chain-breaking antioxidant that has been found in plasma, red cells, and tissues (Burton & Traber, 1990).

The concentration of α-tocopherol in cell membranes is relatively low, so the question arises as to how such a small amount of α-tocopherol can protect membranes against sustained free radical attack. One of the important features of α-tocopherol is that redox cycles occur in membranes to regenerate α-tocopherol in vitro from its tocopheroxyl radical form (Niki, 1991). Regeneration is mediated by vitamin A, vitamin C and coenzyme Q.

Codispersions of α-tocopherol with phospholipids in aqueous media have often been chosen as models to examine the effect of vitamin E on the lipid matrix of cell membranes. Previous studies have indicated that the formation of complexes of α-tocopherol with certain membrane components tends to stabilize the bilayer structure. While the chroman group of vitamin E is responsible for the antioxidant activity of the molecule, the phytyl group largely determines the kinetics of transport to, and retention within, membranes (Burton & Traber, 1990). Electron Spin Resonance (ESR) probe experiments have shown that the phytol chain of α-tocopherol anchors the molecule firmly within the phospholipid bilayer (Wang & Quinn, 2000). Biophysical methods used to study the orientation of α-tocopherol in phospholipid bilayers, suggest that the

chromanol moiety of α-tocopherol is oriented towards the lipid-water interface of the phospholipid bilayer, but does not extend into the lipid-water interface (Bisby &Ahmed, 1989). Although the phytyl chain reduces the mobility within and between membranes, tocopherols are capable of suppressing the oxidation of membrane lipids induced by free radicals formed either in the aqueous phase or within the membrane bilayer (Sigfusson, 2000).

α-Tocopherol is generally regarded as an acceptable, "consumer friendly" supplement and when incorporated into animal diets is a highly effective lipid-soluble chain breaking antioxidant. Dietary supplementation with α-tocopheryl acetate stabilizes the polyunsaturated fatty acids and cholesterol in muscle against oxidative deterioration. This is primarily due to the assimilation of the vitamin into the subcellular membranes, where it maximizes the antioxidant capacity of the system and also increases physical stability (Buckley et al., 1995). It is generally accepted that the oxidative stability of muscle lipids is dependant on the α-tocopherol concentration present in the tissue, which in turn is dependant upon the concentration of α-tocopheryl acetate present in the feed (McCarthy et al., 2001). As mentioned previously, the distribution of vitamin E varies greatly. In porcine, tissue α-tocopherol concentrations responded to dietary intake in the order: liver > heart > lung > kidney > muscle, however the response of the different tissues varied depending upon their metabolic activities (Morrissey et al., 1996). Morrissey et al., also demonstrated the effects of feeding α-tocopheryl acetate to pigs. Their results indicated that saturation of tissues with α-tocopherol is difficult to attain and the duration of supplementation may be an important determinant of membranal oxidative stability. Yang and others (2002a) investigated animal background feeding

(pasture versus grain) on WOF development in beef. Their results indicate that beef from cattle raised on good-quality pasture had an equivalent amount of α-tocopherol to that from grain-fed cattle supplemented with 2500 IU α-tocopheryl acetate per head per day for over four months. In addition, cooked meat from the pasture fed cattle appeared to be more resistant to the development of lipid oxidation and WOF (Yang et al., 2002b).

Direct addition of α-tocopherol to processed meat products has previously been reported to have no significant antioxidant effect (Kerry et al., 1998). Their findings also demonstrated that post-slaughter addition of α-tocopherol to pork offered no significant advantage in inhibiting lipid oxidation when compared to either dietary supplementation or control diets. It is believed dietary supplementation with vitamin E delays lipid oxidation more effectively than direct addition of α-tocopherol to ground beef because dietary vitamin E is incorporated into the polar membrane lipids, which are more prone to oxidation than neutral lipids (Ahn et al., 2002). This suggests that the α-tocopherol concentration in the unstable polar membrane lipids play an important role in the prevention of lipid oxidation in meat products. However, current methodologies for exogenous tocopherol supplementation are only capable of incorporating the antioxidant into the neutral lipid fraction and therefore don't maximize the oxidative stability.

2.4.4 Partitioning of antioxidants in foods

Oxidation is considered to be a surface phenomenon, occurring at a lipid-water interface. The determination of antioxidant partitioning between different phases is an important parameter by which to select antioxidants to favor their distribution toward the microenvironment that is most susceptible to oxidation (Huang et al., 1997). Thus, directing the added antioxidant to or near the origin of the oxidation initiation and/or

propagation is of particular importance. The exact location of the antioxidant, its concentration, the ease by which it donates hydrogen to lipid radicals, and the stability of the resulting antioxidant-radical determine the antioxidant efficiency (Sigfusson, 2000). Also, the location of the antioxidant influences its accessibility and diffusion rate to oxidation initiators or chain-carrying species in multiphase systems (Koga, 1994).

The majority of partitioning studies have previously been carried out on model systems such as simple butteroil-water systems, lipid micelles, or liposomes. Processed muscle foods represent a more complex emulsion than simple models because they contain multiple phases and components. However, most food emulsions can conveniently be considered to consist of three regions that have different physiochemical properties: the interior of the droplets, the continuous phase, and the interface (Figure 5).

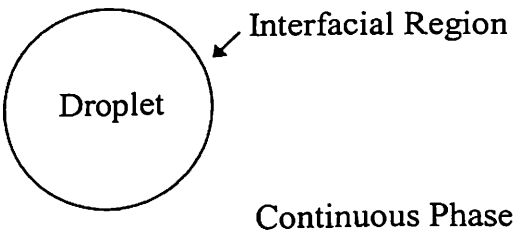


Figure 5. Ingredients in an emulsion partition themselves between the oil, water, and interfacial regions.

Molecules in an emulsion distribute themselves among these three regions according to their concentration and polarity. Nonpolar molecules tend to be located primarily in the oil phase, polar molecules in the aqueous phase, and amphiphilic molecules at the interface (McClements, 1998). The proportions of antioxidants residing in different phases therefore depend on the relative polarity of the antioxidants and the lipid substrates, pH, and the composition of the phases (Huang et al., 1997). Antioxidants

added to meat emulsions partition themselves similarly into the different phases; the aqueous phase; lipid phase (droplets containing mixtures of lipid soluble components, ie. triacylglycerols, diacylglycerols, free fatty acids, sterols, and membrane bilayers); the lipid-water interfacial region (mixtures of surface active components such as proteins and phospholipids). Lipid soluble antioxidants could also associate with myofibrillar proteins due to hydrophobic interactions or possibly hydrogen bonding (Seufert et al., 1970).

In a review by Porter et al. (1989) they postulated the general rule that in food systems of low surface-to-volume ratio (e.g., bulk vegetable oils) polar antioxidants with high hydrophilic-lipophilic balance, such as propyl gallate, TBHQ, and Trolox C, are more effective than nonpolar lipophilic antioxidants, such as BHA, BHT, and tocopherols. In contrast, foods of high surface-to-volume ratios (e.g., emulsified oils), lipophilic antioxidants of low hydrophilic-lipophilic balance are strongly favored. Porter et al. (1989) also used a polyamide fluorescence method to follow oxidation of lecithin liposome model catalyzed by hematin to evaluate a series of antioxidants. The fluorescence method allowed them to confirm the so-called "polar paradox" that polar antioxidants are more effective in nonpolar lipids, whereas nonpolar antioxidants are more active in polar lipid emulsions (Frankel, et al., 1994). Differences in the effectiveness of the antioxidants can be attributed to their affinities for the air-oil or water-oil interfaces in the two systems. Polar antioxidants are more effective in bulk oils because they form a protective membrane at the air-oil interface, which presumably reduces the accessibility of the lipid substrate to oxygen. In contrast, predominantly nonpolar antioxidants are more effective in emulsions because they form a protective membrane around the droplets (Coupland & McClements, 1996).

Partitioning studies of antioxidants in complex food systems (different lipid phases) have received little attention. Sigfusson and Hultin (2002a; 2002b) were among the first researchers to study partitioning of antioxidants in multifaceted food systems. Sigfusson and Hultin (2002a) investigated the uptake of δ-tocopherol by chicken muscle membranes in the presence or absence of added TAG. For this experiment, the uptake of tocopherol by membrane suspensions was determined by adding increasing concentrations of δ-tocopherol in an ethanol or oil carrier to the membranes. Results indicate that in aqueous suspensions of membranes only, the tocopherol uptake increased linearly with tocopherol concentrations, at about 50% of the added δ-tocopherol. When the TAG was added to isolated membranes treated with δ-tocopherol, little exchange of tocopherol between the different lipid fractions was observed. This indicates low migration between the lipid fractions when the tocopherol initially resides in one fraction. The carrier effect was observed when TAG and membranes free of δ-tocopherol were mixed and subsequently treated with δ-tocopherol dissolved in ethanol or oil. Adding the δ-tocopherol in ethanol favored incorporation of the antioxidant into membranes; little antioxidant was incorporated into the membranes when it was added in oil. These results indicate that the polarity of the tocopherol carrier influences partitioning of the antioxidant between the different lipid fractions and membrane stability can be further achieved with exogenous addition of δ-tocopherol in an ethanol carrier.

Sigfusson and Hultin (2002b) also determined the distribution of exogenous δ-tocopherol between the neutral and polar lipids in minced chicken muscles. Approximately 300 ppm (on total lipid basis) of ethanolic δ-tocopherol was added to minced chicken muscle. Addition of exogenous δ-tocopherol was preceded by

gravimetric separation of muscle membrane lipids and TAG. Membrane yields represented up to 35% of the total phospholipids and TAG yields represented up to 80% of the triacylglycerols of the muscle. Sample preparations derived from chickens containing low total lipid contents (3-5%) contained approximately equal concentrations of δ-tocopherol in both the neutral lipid and membrane fractions. When exogenous TAG was added to the chicken muscle (increasing total lipid content), the δ-tocopherol concentration in the membrane fraction increased relative to that in the triacylglycerols. These findings further support that antioxidants do not readily pass between the two lipid fractions once they are taken up.

2.5 Cooking Effects

2.5.1 General

Meat structure can be considered in its simplest form as a collection of parallel fibers, a myofibrillar structure, bound together by a connective tissue network. When meat and meat products undergo heating, a series of morphological changes occur as follows: up to 50°C slight effect, at 50°C compressing of myfibrillar proteins, at 60°C coagulation of thin and thick filaments, further myofibrillar shrinkage, granulation of sarcolema, at 70°C myofibrillar fragmentation at the z-disk, completed shrinkage of endomysium, at 80°C more disintegration of thin filaments, gelatinazation of collagen fibers in the perimysium, and at 90°C the structure becomes amorphous but the principal binding features of the sarcomeres can be identified (Palka & Daun, 1999). Heat-affected changes in meat components have been directly related to meat texture. During the cooking of meat there is first an increase in toughness between 40 and 50°C owing to the

beginning of denaturation of myofibrillar proteins, a further increase between 60 and 70°C because of shrinkage of intramuscular collagen at 65°C, and a third increase in the range 70-90°C when shrinkage and dehydration of the actomyosin occurs. It is generally agreed that heat-induced changes in connective tissue have a tenderizing effect while hardening of the myofibrillar proteins during cooking has a toughening one (Bailey & Light, 1989). Furthermore, total cooking losses depend on the temperature and rate of heating, and the cooking method applied (Palka & Daun, 1999).

Cooking of meat and meat products increases the rate and extent of oxidation. McCarthy et al. (2001) reported cooking of pork patties significantly increased TBARS values with a four-fold increase in oxidation levels being recorded in raw patties upon cooking. Kanner (1994) showed that high temperatures decreased the activation energy for oxidation, breaking down pre-formed hydroperoxides that propagate lipid peroxidation and the development of off-flavors. Cooking method and final internal temperature have an important effect on the formation and stability of the volatile compounds in meats. The rate of lipid autoxidation is also enhanced in meats cooked to higher internal temperatures. The enhanced myoglobin degradation and subsequent release of free iron and the disruption of the muscle membrane to expose the lipids to oxygen and catalysts augment the rate of lipid oxidation and deterioration of meat flavor (Drumm & Spanier, 1991).

2.5.2 Flavor formation

Meat flavor is thermally derived, since uncooked meat has little or no aroma and only a blood-like taste. During cooking, a complex series of thermally induced reactions occur between non-volatile components of lean and fatty tissue resulting in a large

number of reaction products. Over 1000 volatile compounds have been identified in meat (Mottram, 1998). The primary reactions during cooking, which result in aroma volatiles, are the Maillard reaction between amino acids and reducing sugars, and the degradation of lipids. Several hundred volatile compounds derived from lipid degradation have been found in cooked meat, including hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters. These compounds result from the oxidation of fatty acid components of lipids. During refrigerated storage, such reactions may lead to the development of off-flavors, but in freshly cooked meat, the reactions occur quickly and provide a different aroma profile that contributes to desirable flavors (Mottram, 1998). The phospholipids in meat are essential structural components of all cells, however they contain greater proportions of unsaturated fatty acids than the triglycerides. This makes them more susceptible to oxidation during heating and WOF flavor development during subsequent storage.

2.5.3 Ohmic heating

Most heating methods currently used by the meat industry are indirect, i.e. rely on conductive, convective and/or radiative mechanisms of heat transfer from the heating medium (air, water, oil etc.) to the meat product. Depending on the product geometry, it may take considerable time to conduct sufficient heat into the product core to reach a safe end-point temperature. This may cause some parts of the product to be overcooked and adversely affect quality and stability. Ohmic heating is a direct heating method capable of rapidly cooking foods by passing electrical current through the product. The use of ohmic energy to heat food products is not new. Food applications of ohmic heating can be found as far back as 1917. Ohmic heating has shown significant promise in a number

of food processes, including sterilization and pasteurization (Sastry & Li, 1996). The basic principle in ohmic heating is simple: an electrical current is applied to a food particle; electrical current passes through the liquid medium; electrical resistance by the surrounding particles builds (creating energy) internally. Ohmic heating operates very differently, depending on the concentration of particles in a solid-liquid mixture. For example, low-electrical-conductivity particles in a high-electrical-conductivity fluid could heat faster or slower than the fluid, depending on their concentration. High-solids volume fractions tend to favor faster heating of the solid phase and lower-solids fractions result in faster heating of the fluid phase (Sastry & Palaniappan, 1992). Unlike conventional heating, which relies on heat transfer from the carrier medium to the particles, ohmic heating causes large food particles to heat at rates comparable to the surrounding liquid (Ruan et al., 1999). Kim et al. (1996) demonstrated, using an industrial ohmic heating system and chemical and microbiological measurements, that the center of particulate foods received higher lethal treatment than the surface under typical conditions. However, ohmically heated products are not available in the U.S. market. No information is available in literature on the effects of ohmic heating on the quality and stability of meat products. Currently, research is unavailable on how cooking affects the retention of exogenous antioxidants in different lipid fractions. Furthermore, cooked membranes have not been isolated for partitioning studies. The purpose of this study was to utilize an ethanol carrier to add vitamin E to meats to evaluate the oxidative stability of cooked beef patties. Specifically, the objectives were to 1) evaluate the effect of ohmic heating on quality of beef patties compared to cooking with an impingement oven, 2) evaluate the effectiveness of vitamin E in retarding oxidative deterioration in

cooked beef patties, and 3) determine partitioning of vitamin E between membrane lipids and triacylglycerols as affected by cooking.

CHAPTER 3

PARTITIONING OF EXOGENOUS δ-TOCOPHEROL BETWEEN THE TRIACYLGLYCEROL AND MEMBRANE LIPID FRACTIONS OF GROUND BEEF MUSCLE AS AFFECTED BY COOKING

T.M. Wills, C.M. DeWitt, H. Sigfusson

ABSTRACT

The partitioning of exogenous ethanolic δ-tocopherol between the neutral triacylglycerols and membranes of ground beef muscle was investigated. In addition, the effect of cooking method on subsequent partitioning of δ-tocopherol was determined. The lipid fractions were separated using differential ultracentrifugation techniques. Based on absolute tocopherol amounts in raw meat, approximately 52% of the added tocopherol partitioned into membrane lipids, and 48% into triacylglycerols. This corresponds to approximately 10-fold higher tocopherol concentrations in the membrane lipids. Cooking of beef patties resulted in a 25% loss in δ-tocopherol, part of which can be attributed to mass loss (40%). The tocopherol lost during cooking is primarily membrane-associated; approximately 50% of the initial amount in membranes. The amount of tocopherol in triacylglycerols increased approximately 30% after cooking.

INTRODUCTION

Processed meat items are commonly manufactured from muscle tissue high in total fat to aid in the overall texture or to act as a stabilizer in emulsion type products. Lipids of processed meat products are much more susceptible to oxidation than lipids of whole muscle. This low oxidative stability is largely due to incorporation of oxygen and release of heme catalysts and/or degradative enzymes due to cellular disruption during the grinding, mixing, or mincing process (Dawson & Gartner, 1983). Furthermore, the membrane phospholipids are more susceptible to oxidative changes than the neutral triacylglycerols (Dawson et al., 1990). As a consequence, antioxidants are added to muscle-based foods to improve the lipid and oxymyoglobin stability (Faustman et al., 1999).

Oxidation is considered to be a surface phenomenon, occurring at a lipid-water interface (Boyd et al., 1990). Determining the partitioning characteristics of antioxidants between different phases is an important parameter used to select antioxidants that favor distribution toward the microenvironment that is most susceptible to oxidation (Huang et al., 1997). Thus, directing the added antioxidant to or near the origin of the oxidation initiation and/or propagation is of particular importance. The partitioning of antioxidants has generally been studied in various model or simple food systems (Cornell et al., 1970; Frankel et al., 1994; Huang et al., 1997). Results from these studies indicate partitioning is influenced by both the lipid and antioxidant surface (Porter et al., 1989).

Direct addition of α-tocopherol to processed meat products has previously been reported to have no significant antioxidant effect (Mitsumoto et al., 1993; Buckley et al., 1995; Kerry et al., 1998; Higgins et al., 1998). However, recent studies with chicken

muscle have shown that tocopherol partitioning between membrane lipids and triacylglycerols is significantly affected by the carrier used to deliver the antioxidant into the system (Sigfusson and Hultin 2002a,b). Using ethanol, significant amounts of tocopherol were incorporated into membrane lipids, whereas virtually no incorporation was observed when corn oil was used as a carrier. However, no information is available in the literature on partitioning of exogenous antioxidants between different lipids of beef muscle. Furthermore, membranes have not been isolated in any appreciable quantity from cooked meat. The objective of this study was to determine the partitioning of exogenous ethanolic δ-tocopherol between the lipids of ground beef, and the effect of cooking and subsequent storage on oxidative stability.

MATERIALS AND METHODS

Materials. Beef trim was obtained from a local meat processor. The #4 Mahogany casings used to make sample patties were obtained from Devro Teepak (Lisle, IL). HEPES buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid buffer hemisodium salt), Subtilisin A. type VIII protease (EC 3.4.21.62), and δ-tocopherol (90% pure) were obtained from Sigma Chemical Co. (St. Louis, MO). High purity δ-tocopherol (97%) for standard curve preparation was purchased from Supelco, Inc. (Bellefonte, PA). Whatman polytetrafluoroethyl acetate (PTFE) membrane filters (0.45μm pore size) were obtained from VWR. Ethanol (190 proof) was obtained from Pharmco Products Inc. (Brookfield, CT). All reagents were of ACS grade and all solvents of HPLC grade unless otherwise noted.

Sample Preparation. Experiments were performed at refrigerated temperatures (0°C to 4°C) in the Oklahoma State University Food and Agriculture Products Center pilot plant. Frozen beef trim (vacuum-packaged; approximately 10 to 20% total lipids) was thawed and coarse ground through a 9.3 mm grinder plate followed by finely grinding through a 3.2 mm plate. Approximately 45 kg of meat was acquired and triplicate random 25g samples were collected to accurately determine total lipids according to a modified method of Lee et al. (1996).

Tocopherol Addition. Ethanol was used as the antioxidant carrier to deliver δ-tocopherol into the beef lipids. A stock solution of δ-tocopherol was made fresh by dissolving 100μL of δ-tocopherol standard in 9.9mL ethanol. Approximately 300ppm ethanolic δ-tocopherol on a total lipid basis (μL tocopherol/g fat based on total lipid content of meat) was added by hand spraying the stock solution onto ground beef while paddle mixing for 60 s. Approximately 65-85 mL of the ethanolic tocopherol stock solution was added to the meat. Ethanol and water-treated batches served as negative and positive controls, respectively. The controls received an equal volume of dH_2O or ethanol.

Sample Patties. After adding tocopherol and mixing, treatments were vacuum stuffed with a Vemag model 500 (Vemag, Canton, MA) into #4 mahogany casings. Stuffed casings were crust frozen for 3 h in a blast freezer and a band saw was used to cut sample patties 15mm thick. Patties from the three treatments consisting of control, ethanol, and vitamin E were cooked with a Lincoln model 1022 impingement oven (Lincoln, Fort Wayne, IN) to core temperatures of 85°C. All patties were vacuumed-packaged using a double-chambered UltraVac Model 2100-D packaging machine and

stored for 22 d at 2°C. The antioxidant partitioning between the major lipids (triacylglycerols, membrane lipids) was determined and the oxidative stability (TBARS) of beef patties monitored every 7 d over a period of 22 d. A composite from four patties per day for each treatment was used for measurements.

Isolation of Polar Membrane Lipids. The method of Sigfusson and Hultin (2002) was used to isolate muscle membrane lipids from cooked beef. Briefly, an aqueous Protease Type VIII Bacterial enzyme-sodium ascorbate solution was added to cooked ground beef to give a final concentration of 0.05 and 0.2% (w/w) of enzyme and ascorbate respectively. The enzyme was added to aid in releasing membranes from the muscle and the ascorbate to provide protection to the added tocopherol during the centrifugation procedure. This was followed by chopping in a Cuisinart Mini-Prep Plus food processor for 30 s and the meat was stored at 5°C for 1 h to allow the enzyme time to adequately break down the muscle components. Four volumes of cold 0.1 M HEPES buffer (pH 7.5) containing 0.2% (w/v) sodium ascorbate were added to the enzyme-treated meat, followed by homogenization for 60 s with a PowerGen 700 Fisher Scientific homogenizer set on speed four. The pH of the homogenate was adjusted to 7.5 and then centrifuged at 10,000 g for 20 min at 4°C in a Sorvall RC-5C Plus centrifuge. The supernatant obtained was collected and re-centrifuged at 130,000 g for 30 min at 4°C in a Beckman XL-70 Ultracentrifuge. The sediment was gently suspended in cold 0.1 M HEPES buffer (pH 7.5) with 0.2% (w/v) ascorbate, transferred to a Potter-Elvehjem tube and homogenized mechanically with a pestle using a Con-Torque Eberbach tissue grinder. The membrane homogenate was suspended in 0.6 M KCl in the HEPES buffer previously described. This suspension was then centrifuged at 130,000 g for 30 min at

4°C, and the resulting sediment was gently suspended in the HEPES buffer and finally homogenized with the tissue grinder. The KCl (salt wash) step was utilized to further remove any membrane bound proteins. Control and samples were kept on ice throughout the experiment. The protein content, total lipid, phospholipid and tocopherol content of the resulting control and sample membrane preparations were determined as described below.

Isolation of Triacylglycerols. The method of Liang (1998) as modified by Sigfusson & Hultin (2002) was used to isolate triacylglycerols from ground muscle. Duplicate 40g meat samples were centrifuged for 40 min at 130,000 g at 37°C in a Beckman XL-70 Ultracentrifuge. After centrifugation, the top oil layer was collected, transferred to 10 mL Kimble graduated centrifuge tubes and re-centrifuged at 270 g for 5 min in a Fisher Scientific Centrific Centrifuge Model 225 for clarification. Cooked beef samples required the addition of 10 mL dH_2O to allow the oil to migrate to the top of the ultracentrifuge tubes. The oil collected was weighed and yields determined. Duplicate 100 μL aliquots of the clear oil layer obtained were dissolved in 4 mL of chloroform and stored at -80°C for subsequent analysis of phospholipids and tocopherols.

Chemical Analysis. Moisture of ground beef was determined using the AOAC method 950.46. Protein contents of ground beef/patties (2g duplicate samples) were determined by means of a Foss-Tecator, 2300 Kjeltec protein analyzer. Protein content of isolated membranes was determined by the Biuret reaction (Gornall et al., 1949) using Bovine serum albumin (BSA) as a standard.

Total lipids (TL) of ground beef/patties and isolated membrane preparations were determined gravimetrically according to a modified method of Lee et al. (1996). In a 250

mL Eberbach model 8580 blender, 5 g ground muscle was homogenized for 60 s at moderate speed (setting 50) with 50 mL of cold 2:1 chloroform-methanol using a Waring Commercial Blender base model 33BL79 (Waring Products Division, Dynamics Corporation of America, New Hartford, CT). A variable Autotransformer type 3PN1010B (Staco Energy Products Co., Dayton, OH) was used to control speed. Homogenate was filtered through Whatman #4 filter paper into a 125 mL separatory funnel, pressing the homogenate cake gently with a spatula to squeeze out any remaining solvent. Then, 20 mL of cold 0.5% (w/v) sodium chloride solution were added to the extract to break up the emulsion and facilitate phase separation, the funnel tilted four times, and immediately transferred to a cold room (0-4°C) where phase separation was achieved in 60 min. The lower chloroform phase was collected and duplicate 5 mL aliquots were transferred to pre-weighed 20 mL beakers and evaporated on a Corning model PC-320 Stirrer/Hotplate at a low setting (low-2). Following evaporation, beakers were weighed back and the TL content calculated.

Total lipid content of membrane fractions was determined by pipetting 5 mL of the membrane suspension to a 60 mL separatory funnel and extracted with 30 mL of cold 1:1 chloroform-methanol solvent. The funnel was inverted 5 times to facilitate mixing. This was followed by adding 12 mL of cold 0.5% sodium chloride solution and inverting funnel four times before transferring to cold room for phase separation to occur. As mentioned previously, the lower chloroform phase was collected and aliquots taken for total lipid determinations. Aliquots of the chloroform layer were also stored at -80°C for subsequent analysis of phospholipids and tocopherols.

Phospholipid contents of ground beef/patties, isolated membrane preparations and triacylglycerols were determined as lipid phosphorus on aliquots of the organic chloroform phase obtained after total lipid extraction using a modified version of Anderson & Davis (1982). Lipid extracts (100μL) were deposited as close to bottom of a 12 x 150mm test tubes as possible. Chloroform was evaporated in a vacuum oven at 60°C for 30 min. Samples were cooled to room temperature and 300 μL concentrated sulfuric acid added and samples were vortexed. Digestion was facilitated by heating at 155°C for 10 min (Electrothermal Digi-Block, Barnstead/Thermolyne, Dubuque, IA) with screw caps removed. Tubes were cooled and 150 μL of 6% hydrogen peroxide added, vortexed, and heated again at 155°C for 90 min. Samples were finally cooled and 6 mL dH_2O added. Phosphorus content was determined by Inductively Coupled Plasma Spectrophotometry-ICP (Spectroflame- Spectro Analytical Instruments Company, Fitchburg, MA) with inorganic phosphorus (High Purity Standards- Charleston, SC) used as a standard. A minimum of four points were used for the calibration curve.

Tocopherol contents of organic chloroform extracts and oil triacylglycerols were determined by separation and quantification of tocopherols using HPLC with fluorescence detection as described by Katsandidis & Addis (1999). The HPLC consisted of a Waters 616 pump and a Waters 474 Fluorescence Detector (Waters, Milford, MA). Fifteen microliters were injected into a silica column equipped with a guard module (Zorbax RX-SIL, 5-μm particle size 4.6 x 250 mm, guard column was of same particle size, 4.6 x 15 mm). Mobile phase consisted of 99:1 HPLC grade hexane-isopropanol run at a flow rate of 1.3 mL/min. A programmed excitation of 325 nm was used for detection of tocopherols. All samples were filtered through 0.45 μL pore size

PTFE disk filters prior to injection to remove particulate debris. Samples were stored in amber HPLC vials at -80°C until injection into the HPLC. Values are reported as the average of measurements on at least duplicate samples.

The progress of lipid oxidation during refrigerated storage of cooked beef patties was monitored by determining secondary lipid oxidation products (thiobarbituric reactive substances, TBARS). TBARS were determined spectrophotometrically according to the method of Lemon (1975) using malonaldehyde bis (diethyl acetate) as a standard. Beef samples (2 g) were initially extracted with a 6 mL solution containing 7.5% trichloroacetic acid (TCA) in water, 0.1% propyl gallate dissolved in ethanol, and 0.1% ethylenediaminetetraacetic acid in water. Samples were homogenized with 6 mL extracting solution for 30 s using a PowerGen 700 Fisher Scientific homogenizer set on speed four. Homogenate was carefully poured into funnels containting Whatman #1 filter paper and filtered. Filtrate (2 mL) was placed in a 12 x 125 mm screw cap test tube and 2 mL of 0.2 M thiobarbituric acid added, followed by heating in a boiling water bath for 40 min with caps tightly screwed on. Caps were unscrewed and sample tubes cooled under running tap water for 5 min. Absorbance was measured (against a blank of 2 mL TCA + 2 mL TBA) at 532 nm.

Statistical analysis. The Statistical Analysis System (SAS), version 8 (SAS, Cary, N.C., U.S.A.) was used for statistical analyses. The experiment was set up as a complete randomized block design. An analysis of variance (ANOVA) was used to evaluate sample treatment and storage time as main effects. Interactions between treatment and storage time were included in the model. Mean separation was

accomplished using Tukey's to determine significant differences among the treatments at $p<0.05$.

RESULTS

Sample Composition. Proximate composition for the raw and cooked samples is shown in Table 1 on a wet basis. Reference values from the USDA Nutrient Database (Agriculture Research Service 2001) were similar to the ground beef used for this study. In the raw sample, moisture content ranged from 61-67% (w/w), the protein content from 17-21%, and the total lipid content from 13-20%. The phospholipid content ranged from 0.6-1.5% (w/w); the remainder of the lipid content (≈90%) is found as triacylglycerols. Cooked beef samples moisture content ranged from 50-55% (w/w), protein content from 28-33%, and phospholipid content from 0.8-1.0% (w/w). Chemical composition of the cooked, vacuum packaged beef patties did not change during refrigerated storage (Appendix A).

Isolation of Triacylglycerols. The triacylglycerols were isolated from the ground beef by utilizing ultracentrifugation techniques at elevated temperatures. Centrifuging the meat at elevated temperatures liquefies the lipid fraction thus separating it from the water phase. The neutral lipids migrate to the top of the centrifuge tube, forming an oil layer. Yields of triacylglycerols are expressed in Appendix B. The isolated triacylglycerols in the raw meat represented 30-50% of the total triacylglycerols of the beef muscle. The yields of triacylglycerols from cooked beef patties represented 7-41% of the total triacylglycerols in the cooked beef. Phosphorus was not detected in the isolated triacylglycerol lipid fractions (Appendix C) indicating the fraction was of high purity with little or no phospholipid contamination present.

Isolation of Membrane Lipids. When performing partitioning studies in complex systems such as muscle lipids, it is necessary to obtain lipid fractions with little contamination. Obtaining a membrane lipid fraction of low neutral lipid contamination is essential for assessing the antioxidant distribution between the different lipid fractions. Tocopherol could associate with membrane bound proteins, but neutral lipid contamination poses a much larger problem as tocopherol has much more affinity for this fraction. Membrane contamination by neutral lipids and proteins (Table 2) was estimated by determining chemical compositions of the membrane fraction and calculating the ratio of phospholipid to total lipid content (PL_{sed}/TL_{sed}), and the phospholipid to protein content ($PL_{sed}/Protein_{sed}$). High ratios indicate low neutral lipid or protein contamination. Membrane isolation may appear to be a relatively straightforward laboratory operation, but, in practice, this is rarely the case. Since neutral lipids and membrane-associated proteins are integral components of membranes, evaluation of membrane contamination is difficult at best.

Neutral lipid contamination in the raw membrane fraction determined by the PL_{sed}/TL_{sed} ratio was relatively low (0.879 ± 0.112) as suggested by the high ratio. Low neutral lipid contamination in the membranes increases confidence in the partitioning results. The low $PL_{sed}/Protein_{sed}$ ratio (0.215 ± 0.059) indicates the presence of proteins in the raw membrane fraction. The amount of phospholipid in the membrane preparation that was obtained from 100g of original muscle was 151 ± 2mg. Membrane yields were estimated by determining the absolute amount of lipid phosphorus obtained from the membrane fraction and comparing it to the lipid phosphorus content of the original ground muscle (PL_{sed}/PL_{muscle}). Yields of membranal phospholipids (Appendix D)

obtained in the raw beef were 15% of the total muscle phospholipids. The purity and yield of the membrane fraction recovered from the cooked muscle was much lower than the raw muscle as indicated by the low neutral lipid (0.588 ± 0.160) and protein (0.073 ± 0.028) ratios. Cooking also resulted in a reduction in membrane yields to 1-2% of the total muscle phospholipids.

Partitioning of δ-Tocopherol in Ground Beef. The partitioning of the added δ-tocopherol between the membrane and triacylglycerol lipid fractions was estimated by comparison of the tocopherol concentrations determined in each lipid fraction after separation. Approximately 350 ppm δ-tocopherol on a muscle total lipid basis was added to the ground muscle. Lipid extracts from the whole muscle were utilized to determine the amount of tocopherol incorporated into the ground beef. As some tocopherol (5-10%) is destroyed upon addition and mixing, partitioning calculations are based on tocopherols recovered from the ground beef immediately after its addition. The high recovery is in part due to uniform distribution of the tocopherol, indicating spraying the antioxidant evenly dispersed it throughout the product, and a minute amount was lost or destroyed during sample preparation.

Prior to cooking, the absolute amounts (μg TOH / g Lipid) of tocopherol recovered from the lipid fractions were determined. Membrane lipids displayed significantly ($p<0.05$) higher tocopherol concentrations than the neutral lipids. Tocopherol amounts recovered from the membrane fraction ranged from 2,600–3,500 ppm compared to 85–230 ppm in the neutral lipid fraction. This corresponds to a 10-fold increase in tocopherol concentration in the membranes than in the triacylglycerols. On an absolute tocopherol weight basis for 100g raw beef the exogenous δ-tocopherol

recovered had partitioned almost equally between the two lipid fractions. Approximately 52% (2,828 μg TOH) of the added δ-tocopherol was recovered from 0.8g of phospholipid. The remaining tocopherol, 48% (2,700 μg TOH) was recovered from 16.2g of neutral lipids (Figure 6). These results further support the significant concentration effect the ethanol carrier contributes to antioxidant delivery to the membranes.

Effects of Cooking on the Partitioning of δ-Tocopherol in Ground Beef. Partitioning of the added δ-tocopherol between the membrane and triacylglycerol lipid fractions of cooked beef was estimated by comparison of the tocopherol concentrations determined in each lipid fraction after separation and further compared to the raw meat. Comparing results from the two treatments proved to be difficult due to cooking losses. Additionally, the membrane fraction was not as pure as expected and low membrane yields were encountered.

Impingement cooking of patties resulted in approximately 25% overall loss of added δ-tocopherol, some of which may be accounted for by a mass loss upon cooking of approximately 40% (Table 3). The membrane tocopherol concentration decreased by approximately 50%, whereas the tocopherol concentration in the triacylglycerols increased by approximately 30% (Figure 6). This may suggest a considerable redistribution of the added δ-tocopherol upon cooking.

To determine the effects of refrigerated storage on partitioning of δ-tocopherol between the different lipid fractions, samples were subsequently analyzed for tocopherol in the various lipid fractions over a 21 d period (Figure 7). The tocopherol concentration in the membrane fraction was significantly ($p<0.05$) higher than both the neutral lipids

and whole muscle, indicating a concentration effect. As expected, no significant differences were found between the whole muscle lipid extract and the neutral lipids. However, during this time the membrane tocopherol concentrations decreased ($p<0.05$) linearly, from approximately 3,300ppm to 1,500ppm. This underscores the importance of providing antioxidant protection to the less stable membrane lipids.

Oxidative Stability of Cooked Ground Beef During Refrigerated Storage. Secondary oxidation products or thiobarbituric reactive substances (TBARS) of beef patties were monitored during refrigerated storage (Figure 8). Trends of TBARS values are characteristic of a normal oxidation curve, increasing during the first days of storage then decreasing as the secondary products react with other meat components. Statistical analysis of TBARS revealed a significant replication effect. TBARS results from the second replicate were withdrawn and not used in the statistical calculations. High concentrations of α-tocopherol were discovered in this replicate when δ-tocopherol was being quantified by HPLC. Meat obtained from the abattoir more than likely came from an animal that was supplemented with α-tocopherol. Meat containing endogenous α-tocopherol wouldn't form lipid hydroperoxides at the same rate as other replicates. Results from replicate one and three indicated the tocopherol treated samples displayed significantly ($p<0.05$) lower TBARS values than the control samples. No significant differences were present between the control and ethanol treated samples, indicating the ethanol carrier had no significant effect on the oxidative stability of the cooked beef patties. Day 2 also displayed significantly ($p<0.05$) lower TBARS values than all other days; however, no other days were significantly different from each other.

DISCUSSION

To confidently estimate the partitioning of the exogenous δ-tocopherol between the different lipid fractions, the separated lipids should be of high yield and purity. The isolated triacylglycerols were found to be free of phospholipid contamination, indicating the tocopherol recovered from this fraction is purely associated with the neutral lipids. Applying a centrifuge force of 130,000 g for 30 min at 37°C, 30-50% of the total triacylglycerols in the raw beef were recovered compared to 12-41% in the cooked beef. Yields were based on gravimetric comparison of the amount of oil (triacylglycerols) obtained after centrifugation to the amount initially present in the beef muscle. The yields of triacylglycerols are comparable to what Sigfusson and Hultin (2002) recovered from chicken muscle (11-30% for lean muscle; up to 80% when exogenous triacylglycerols were added to native chicken muscle). Neutral lipid yields from the cooked beef patties were slightly lower than their raw counterpart. Differences between the two are more than likely due to moisture loss during cooking. Raw samples would have an adequate amount of evenly distributed, native water to assist in the removal of fat. On the other hand, neutral lipids in the cooked product that would be the easiest to remove are lost during cooking. The cooked beef did not have sufficient moisture to remove the remaining oil from its partially bound state. Increasing the amount of exogenous water added to the centrifugation procedure could help increase triacylglycerol yields.

The membrane fractions isolated in these studies are derived from a variety of membrane sources, containing different lipid and protein compositions. It is noteworthy however, all biological membranes, no matter how carefully purified, are found to

contain proteins and lipids. The percentage and exact nature of the adhering proteins vary considerably with membrane type (Stryer, 1988). The chemical properties of the isolated membranes were further compared to determine the purity of the samples. The chemical characteristics of the raw membrane fractions indicated minor contamination with triacylglycerols and moderate protein contamination, as indicated by the PL/TL and PL/Protein ratios. The PL/TL ratio of the raw membrane fraction is in agreement with the results of Sigfusson and Hultin (2002) for the salt-washed membrane fraction of broiler meat (0.8 – 0.95) and slightly higher than the range of 0.70 – 0.75 for broiler microsomes reported by Asghar et al. (1989). The PL/Protein ratio of the raw membrane fraction was also slightly lower than 0.30 – 0.40 reported by Sigfusson and Hultin (2002) for chicken meat. The differences in protein contamination are more than likely due to species effects; however, without characterizing the membrane-bound proteins through SDS-Page analysis, the variation cannot be completely explained. The PL/TL and PL/Protein ratios in cooked beef membranes display elevated levels of contamination. Evaluating these results are much more difficult due to mass loss and membrane breakdown resulting from cooking. Low membrane yields in the cooked beef can partially explain contamination, but once again without characterizing the membrane suspension through SDS-Page analysis or microscopy studies, the resulting cooked membrane sediment cannot be confidently differentiated from the raw membrane sediment. Enzyme concentrations, mincing times, and centrifugation procedures could further be investigated to optimize recovery conditions and yields.

Recovery of total δ-tocopherol following addition was approximately 90-95%. Elevated recoveries signify tocopherol did not undergo oxidative degradation to other

oxidation products, which would have escaped detection. Comparison of the tocopherol concentrations (ppm) determined in each lipid fraction after separation suggested an approximately even distribution of tocopherol between the lipids in raw muscle. Furthermore, the ethanol carrier promoted a significant tocopherol concentration in the minute membrane lipid fraction as opposed to the bulk of the neutral lipids. The concentration effect the ethanol imparts is due to the hydrophobic nature of the carrier and the polar head-group of the tocopherol molecule. This combination of antioxidant and carrier are very compatible with the hydrophilic membrane surface. Sigfusson and Hultin (2002) hypothesized the ethanol carrier could transfer the tocopherol more rapidly into the membrane lipids than across the more hydrophobic surface of the triacylglycerol oil droplets.

The effects of cooking on the partitioning of δ-tocopherol between the membrane and triacylglycerol lipid fractions of beef were determined after separation of lipids and further compared to the raw meat. Although cooking resulted in a 40% reduction in mass, the majority (75%) of δ-tocopherol still remained in the cooked product. Kerry et al. (1998) exogenously added α-tocopherol to pork loins, followed by cooking. Results from their experiment revealed the cooking process decreased the vitamin E content in loins by 10% as a result of leaching. Cooking resulted in a 50% reduction in membrane tocopherol levels, but a 30% increase in δ-tocopherol occurred in neutral lipids. One would assume some tocopherol associated with the oil would be lost in the drippings; however, the disruption of membrane lipids, onset by cooking, could allow a quantity of membrane bound tocopherol to re-associate with the neutral lipid fraction. It is believed the tocopherol associated with the ruptured membranes would aggregate in the aqueous

phase where they could then re-associate with oil droplets. If the drippings had been collected during the cooking process, analysis could be performed to gain a better understanding of the amount tocopherol lost in each fraction during cooking through subsequent phosphorus, total lipid, and tocopherol determinations. Igene and others (1981) investigated the effects of cooking on beef phospholipids and their fatty acid compositions. These investigators analyzed the phospholipids recovered from the drippings of cooked beef. Their results indicate drippings were free of phosphatidyl ethanolamine, suggesting that it is more tightly membrane bound than phosphatidyl choline or other phospholipid components. Drippings also contained low levels of PUFA's, indicating the unsaturated phospholipid components are firmly held by the membrane. These results further support the fact that the membrane bound phospholipids (mainly unsaturated) existing after cooking play a significant role in membrane oxidation. Thus increasing the tocopherol concentration in the cooked membrane fraction could slow or inhibit the oxidation process.

Tocopherol was effectively utilized in the cooked beef by inhibiting oxidation. Figure 7 illustrates the reduction of membrane tocopherol levels as storage progresses. Since the neutral lipid fraction of muscle systems makes a minimal contribution to the oxidation of meat, (Dawson et al., 1990) it's reasonable the majority of tocopherol oxidized was located in the membrane fraction. Vitamin E treated samples significantly ($p<0.05$) reduced oxidative deterioration. Direct addition of α-tocopherol to processed meat products has previously been reported to have no significant antioxidant effect (Kerry et al., 1998; Higgins et al., 1998; Mitsumoto et al., 1993). Kerry et al. (1998) demonstrated that exogenous addition of α-tocopherol to pork offered no

significant advantage in inhibiting lipid oxidation when compared to either dietary supplementation or control diets. Furthermore, Higgins et al. (1998) determined the oxidative stability of turkey breast patties supplemented with exogenous α-tocopherol was less stable to oxidative attack than the endogenous meat, although greater concentrations of tocopherol were in the exogenous meat. The majority of researchers investigating dietary supplementation vs. postmortem addition have utilized oil as the antioxidant carrier. This clearly explains why exogenous samples containing greater amounts of tocopherol were not as effective inhibiting oxidation, as the majority of the antioxidant would locate in the neutral lipids where oxidation is of less concern. Dietary supplementation with α-tocopheryl acetate stabilizes the polyunsaturated fatty acids and cholesterol in muscle against oxidative deterioration. The protective effect is primarily due to the assimilation of the vitamin into the subcellular membranes, where it maximizes the antioxidant capacity of the system (McCarthy et al., 2001). Although cooking of meat results in a significant reduction in membrane tocopherol levels, ethanol as an antioxidant carrier may raise the membrane tocopherol levels sufficiently to retard oxidative deterioration during refrigerated storage of cooked meats. Increasing the concentration of vitamin E in the membrane lipids may effectively extend the product shelf life and reduce the formation of off-flavors and odors associated with pre-cooked, refrigerated meat products. The results from this study confirm the importance of choosing an appropriate antioxidant carrier for delivering the antioxidant to the site where oxidation is initiated and/or propagated.

Future work could be conducted on sensory analysis with a consumer panel. A sensory panel would be useful to determine if the exogenous vitamin E could inhibit

warmed over flavor. Furthermore, sensory analysis is needed to ensure the ethanol carrier does not impart an alcohol taste. To determine the presence of warmed over flavors, panelists could be trained to detect its' presence in three training sessions. Session one would be used to train panelists to detect the presence of hexanal. This could be accomplished by preparing oil solutions containing 0.05 to 10 ppm of hexanal standards in test tubes. Panelists would then be asked to rank the tubes in order of increasing intensity based on their smell. Session two could provide cooked beef patties to panelists stored 0, 1, 3, 5, and 7 days in zip-lock bags. Panelists would then be asked to smell and taste the beef samples and rank them according to the increasing intensity of WOF based on the presence of hexanal. Training session three would educate panelists how to score WOF of cooked beef samples. Panelists would be provided with five samples aged 0, 1, 3, 5, and 7 days. Panelists would then be asked to use day 0 and 7 as reference samples. Panelists would be asked to taste the lower and upper reference samples and score the three remaining samples (days 1, 3 and 5) on a line scale that indicated the intensity of WOF as compared to the references (day 0 no WOF, day 7 extensive WOF). Sensory analysis for the project would then follow by providing vacuum packaged sample patties from the different treatments stored 1, 8, 14, and 21 days. Panelists would be given day 0 and 7 reference samples stored in zip-lock bags and would be asked once again to rank the treatment patties on a line scale, comparing them to the low and high controls.

Table 1. Composition of Raw and Cooked Ground Beef on a Wet Basis.

	RAW	COOKED
Moisture %	64.81 ± 2.35	53.44 ± 1.73
Protein %	19.19 ± 1.53	30.80 ± 1.90
Total Lipids (TL) %	17.85 ± 3.25	17.22 ± 4.33
Phospholipids (PL) %	1.06 ± 0.50	0.91 ± 0.06
TAG (=TL-PL) %	16.61 ± 3.27	17.62 ± 4.80

Data represents means ± standard deviation

Table 2. Comparison of Chemical Characteristics of Membrane Fractions Obtained from Raw and Cooked Beef Patties.

Membrane Fraction	PL_{sed}/TL_{sed}	$PL_{sed}/Protein_{sed}$	PL_{sed}/PL_{muscle}
Raw Beef	0.879 ± 0.112	0.215 ± 0.059	0.145 ± 0.019
Cooked Beef	0.588 ± 0.160	0.073 ± 0.028	0.016 ± 0.003

Numbers reported are the averages and standard deviations from duplicate measurements and expressed as the weight ratios (w/w). See text for definition of ratios. The abbreviations PL and TL represent phospholipid and total lipid contents, respectively. The subscripts refer to the membrane pellet (sediment) obtained after centrifugation, or to the muscle from which the membranes were obtained.

Table 3. Effects of Cooking on the Retention of δ-Tocopherol in Whole Muscle.

Whole Muscle	μg TOH/g Lipid	g Lipid	Total μg TOH
Raw	290 ± 40	17	4930 ± 680
Cooked	380 ± 75	10	3800 ± 750

Data represent means ± standard deviations of μg TOH/g Lipid derived from whole muscle. Lipid content is based on the average of at least quadruplicate determinations. Total μg TOH is product of μg TOH (x) g Lipid.

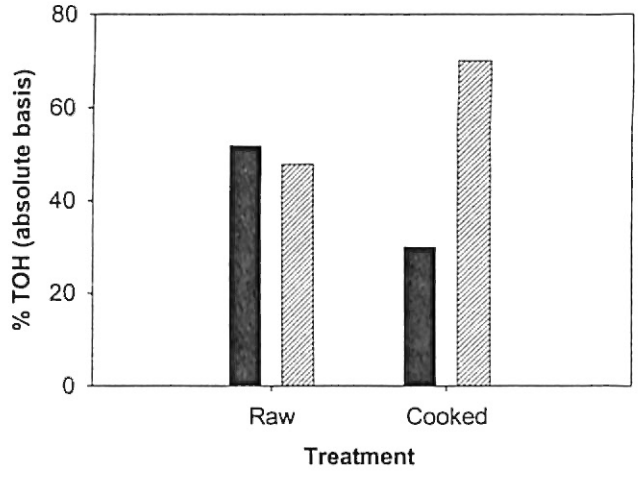


Figure 6. Comparison of the partitioning of δ-tocopherol between the membrane and triacylglycerol fractions as affected by cooking. Results are expressed as the % tocopherol recovered, residing in the different lipid fractions. (■) Membranes; (▨) Triacylglycerols.

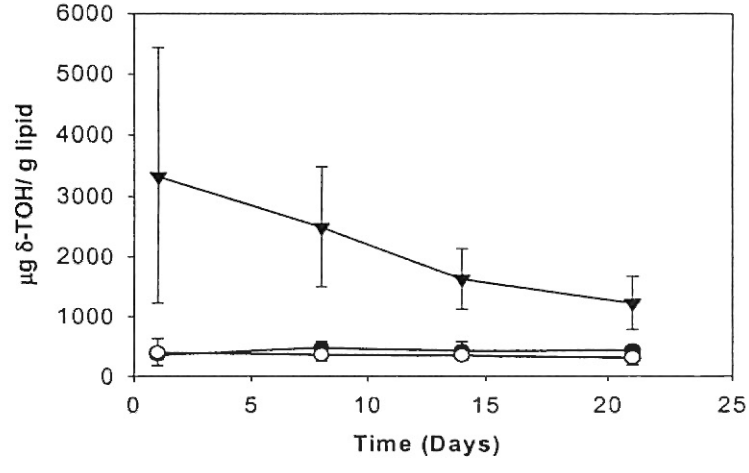


Figure 7. Changes in δ-tocopherol concentrations (ppm) of cooked patties, triacylglycerols and membrane lipids upon refrigerated storage. Results are means and standard deviations of at least duplicate measurements from three replicate experiments. (●) Cooked patties; (○) Triacylglycerols; (▼) Membrane Lipids.

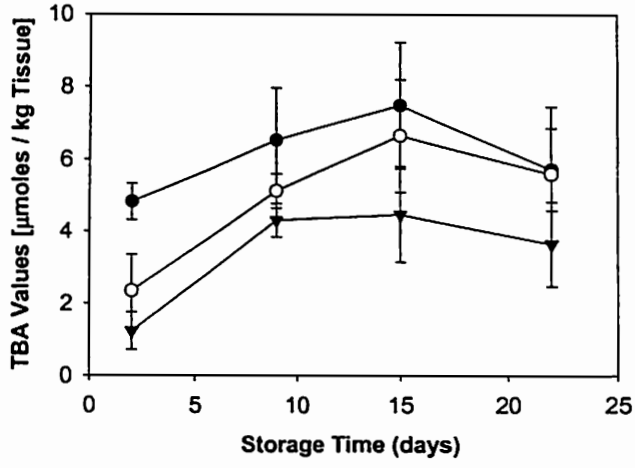


Figure 8. TBARS values of control, ethanol, and vitamin E treated samples of vacuum packaged/refrigerated (2°C) patties. Values are means and standard errors, expressed on a ground muscle basis. (●) Control; (○) Ethanol; (▼) Vitamin E.

CHAPTER 4

EFFECT OF ETHANOLIC TOCOPHEROL ADDITION AND COOKING METHOD ON OXIDATIVE STABILITY AND QULATIY OF BEEF PATTIES DURING REFRIGERATED STORAGE

T.M. Wills, C.M. DeWitt, H. Sigfusson

ABSTRACT

Exogenous ethanolic δ-tocopherol was spray applied to beef patties. The influence of tocopherol in an ethanolic carrier and cooking method on progress of oxidation in beef patties during refrigerated storage was monitored and quality characteristics determined. The addition of ethanolic δ-tocopherol delayed oxidative deterioration of cooked beef patties in both cooking methods. Ohmically cooked samples developed significantly higher ($p<0.05$) TBARS than impingement-cooked samples. Formation of TBARS significantly ($p<0.05$) progressed with storage time in both cooking treatments. Cooking method significantly ($p<0.05$) influenced color and textural attributes of beef patties. Samples cooked by ohmic heater displayed significantly ($p<0.05$) larger L* and b* color values. Additionally, ohmic samples were significantly ($p<0.05$) harder, chewier and gummier than samples cooked with impingement oven.

INTRODUCTION

Most heating methods currently used by the meat industry are indirect, i.e. they rely on conductive, convective and/or radiative mechanisms of heat transfer from the heating medium (air, water, oil etc.) to the meat product. Depending on the product geometry, it may take considerable time to conduct sufficient heat into the product core to reach a safe end-point temperature. This may cause some parts of the product to be overcooked and adversely affect quality and stability. Ohmic processing, sometimes described as resistive heating, consists of passing current directly through a conductive food, which in turn generates heat. Because heating accompanies the current, heat distribution throughout the product is far more rapid and even, which can result in better flavor retention and particulate integrity compared to conventional methods (Skudder, 1993). Ohmic heating has shown significant promise in a number of food processes, including sterilization and pasteurization (Sastry & Li, 1996). Unlike conventional heating, which relies on heat transfer from the carrier medium to the particles, ohmic heating causes large food particles to heat at rates comparable to the surrounding liquid (Ruan et al., 1999). Kim et al. (1996) demonstrated, using an industrial ohmic heating system and chemical and microbiological measurements, that the center of particulate foods received higher lethal treatment than the surface under typical conditions. However, ohmically heated meat products are not available in the U.S. market due to concerns about whether the particles are adequately heated to reach sterility (Piette et al., 2004).

Sigfusson and Hultin (2002) recently investigated the partitioning of δ-tocopherol between the neutral and polar membrane lipids of chicken muscle. Their results indicated

that antioxidant carriers play a large role in the efficiency of exogenous antioxidants. Using ethanol as an antioxidant carrier, they were able to significantly concentrate the tocopherol in the membrane lipids. When corn oil was used as the carrier, virtually no membrane incorporation was observed. Although these researchers found large concentrations of tocopherol in the membrane lipids, the oxidative stability of the product was not determined.

Food preparation and safety are major issues fast food chains are facing. Fast food hamburger patties are often cooked while frozen on industrial or clamshell grills. Due to the high latent heat of melting ice, the center temperature of the patties may not rise to the necessary level for killing all pathogenic microorganisms (Ozkan et al., 2004). For this reason, ohmic heating of beef patties may be a suitable alternative to effectively reach adequate end point temperatures throughout the product; however, no information is available in literature on the effects of ohmic heating on the quality and stability of meat products. The objective of this study was to compare the composition, quality characteristics and oxidative stability of ohmically heated beef patties to those cooked with an impingement oven.

MATERIALS AND METHODS

Sample Preparation. Experiments were performed at refrigerated temperatures (0°C to 4°C) in the Oklahoma State University Food and Agriculture Products Center pilot plant. Frozen beef trim (vacuum-packaged; approximately 10 to 20% total lipids) was thawed at 4°C and coarse ground (Biro, model 542-48-52, Marblehead, OH) through a 9.3 mm grinder plate followed by finely grinding through a 3.2 mm plate.

Approximately 45 kg of meat was acquired and triplicate random 25 g samples were collected to accurately determine total lipids according to a modified method of Lee et al. (1996).

Antioxidant Treatment. Ethanol was used as the antioxidant carrier to deliver δ-tocopherol into the beef lipids. A stock solution of δ-tocopherol (90% purity, Sigma Chemical Co., St. Louis, MO) was made fresh by dissolving 100 μL of δ-tocopherol standard in 9.9 mL ethanol. Approximately 300 ppm ethanolic δ-tocopherol on total lipid basis (μL tocopherol/g fat based on total lipid content of meat) was added by spraying (hand held, 500 mL spray bottle) the stock onto ground beef while paddle mixing for 60 s. Ethanol and water-treated batches served as negative and positive controls, respectively. The controls received an equal volume of dH_2O or ethanol.

Sample Patties. After adding tocopherol and mixing, treatments were vacuum stuffed with a Vemag model 500 (Vemag, Canton, MA) into #4 mahogany casings (Devro Teepak, Lisle, IL). Stuffed casings were crust frozen for 3 h in a blast freezer and a band saw was used to cut sample patties 15 mm thick. Patties from the three treatments consisting of control, ethanol, and vitamin E were cooked with a Lincoln model 1022 impingement oven (Lincoln, Fort Wayne, IN) and an InstantBurger™ -ohmic heater (Smokaroma Inc., Boley, OK) to core temperatures of 85°C. All patties were vacuum-packaged using a double-chambered UltraVac Model 2100-D packaging machine (Koch, Kansas City, MO) and stored for 22 d at 2°C. Primary oxidation products or lipid hydroperoxides (LOOH) and secondary oxidation products, thiobarbituric reactive substances (TBARS) of beef patties was monitored every 7 days over a period of 22 days. A composite from four patties per day for each treatment was used for measurements.

Chemical Analysis. Moisture of ground beef was determined using the AOAC method 950.46. Protein contents of raw ground beef and cooked patties (2 g duplicate samples) were determined by means of a Foss-Tecator, 2300 Kjeltec protein analyzer. Total lipids (TL) of raw ground beef and cooked patties were determined gravimetrically according to the method of Lee et al., (1996). Modifications were previously described in Chapter 3. Lipids were extracted using a 2:1 mixture of chloroform-methanol solvent, followed by separation of the organic (chloroform) and aqueous phases and evaporation of the organic phase to determine total lipid contents. Aliquots of the chloroform layer were also stored at -80°C for subsequent analysis of phospholipids. The progress of lipid oxidation during refrigerated storage of cooked beef patties was monitored by determining primary (LOOH) and secondary lipid oxidation products (TBARS). LOOH were determined from lipid extracts spectrophotometrically using a modified method of Shantha & Decker (1994) with cumin hydroperoxide as a standard. Briefly, a Fe(II)Cl_2 solution was prepared by dissolving 0.5 g ferrous sulfate in 50mL dH_2O and 0.4 g barium chloride dihydrate in 50 mL dH_2O. The solutions were mixed together with constant stirring and 2 mL of 10M HCl added. The barium sulfate precipitate was filtered off and the remaining solution stored in a brown bottle in the dark. An ammonium thiocyanate solution was prepared by dissolving 30g ammonium thiocyanate in 100 mL dH_2O. Lipid extracts (300 μL) were placed in a 12 x 150 mm test tube and brought to a 10 mL volume with 2:1 chloroform methanol. Fifty micro-liters of ammonium thiocyanate was added to tubes and vortexed for 2-4 s, followed by adding 50 μL Fe(II) solution and vortexing again. Tubes were incubated at room temperature for 5 min in the dark and absorbance measured (against a blank of 10 mL 2:1 chloroform-methanol) at 500 nm. TBARS were

determined spectrophotometrically according to the method of Lemon (1975) using malonaldehyde bis (diethyl acetate) as a standard. Phospholipid contents of ground beef/patties were determined as lipid phosphorus on aliquots of the organic chloroform phase obtained after total lipid extraction using the modified version previously described in chapter 3 of Anderson & Davis (1982). Inorganic phosphorus was used as a standard.

Color Determination. Cooked beef patties were allowed 2-3 h to come to room temperature prior to determination of color values on day 0. Hunter L* a* b* values from the cooked beef patties were recorded using a HunterLab spectrocolorimeter MiniScan XE Plus (HunterLab, Reston, VA). Measurements were taken under cool white fluorescent lighting with a 10° viewing angle. Duplicate readings were taken from separate areas on 5 patties for each of the treatments.

Texture Analysis. Texture profile analysis (TPA) was performed using a Stable Micro Systems' TA-XT2i texture analyzer (Texture Technologies Inc., Scarsdale, NY). Before beginning TPA tests, patties were allowed to cool to room temperature to avoid temperature changes during testing. For each treatment, two 2 cm sample cores were tested from 5 patties. A macro was programmed into the software (Texture Expert Exceed, v. 2.56, Texture Technologies Inc., Scarsdale, NY) to calculate TPA parameters. The samples were compressed twice, with a 5 s delay between the two descents, to 25% of their original height using a 4 cm acrylic cylinder. The probe descended into the beef sample to a distance of 7.0 mm at a rate of 1.0 mm per s and TPA parameters were determined; hardness, chewiness, springiness, cohesiveness, and gumminess (parameter calculations are described in Appendix F).

Statistical analysis. The Statistical Analysis System (SAS), version 8 (SAS, Cary, N.C., U.S.A.) was used for statistical analyses. An analysis of variance (ANOVA) was used to evaluate treatment and storage time as main effects. Interactions between treatment and storage time were included in the model. Mean separation was accomplished using Tukey's to determine significant differences among the treatments at $p<0.05$.

RESULTS

Sample Composition. Proximate composition for the raw and cooked impingement oven and ohmically heated samples are shown in Table 4 on a wet basis. Reference values from the USDA Nutrient Database (Agriculture Research Service 2001) were similar to the ground beef used for this study. In the raw sample, moisture content ranged from 61-67% (w/w), the protein content from 17-21%, and the total lipid content from 13-20%. The phospholipid content ranged from 0.6-1.5% (w/w); the remainder of the lipid content (≈90%) is found as triacylglycerols. Results of proximate analysis indicated that cooking method affected sample composition. Ohmically heated samples contained ($p<0.05$) higher moisture and ($p<0.05$) lower protein contents than patties cooked with an impingement oven. Additionally, ohmically heated samples tended to display lower fat contents. Antioxidant treatments had no effect on sample composition; furthermore, composition of the cooked, vacuum packaged beef patties did not change during refrigerated storage (Appendix A).

Oxidative Stability of Cooked Ground Beef During Refrigerated Storage. The influence of sample treatment and cooking method on lipid oxidation was evaluated

by monitoring primary (LOOH) and secondary (TBARS) oxidation products of beef patties during refrigerated storage. Figures 9 and 10 illustrate the data obtained with these determinations. Peroxide values did not significantly change during patty storage. Furthermore, no significant differences were observed between cooking methods. Peroxide values ranged from 0.1 to 0.4 meq/Kg fat, which are lower than the findings of Shantha & Decker, (1994) when they conducted a comparative study on methods used to obtain peroxide values in cooked ground beef. Their shelf study indicated peroxide values ranging from 0.21 to 4.68 meq/Kg lipid. Rodriguez-Estrada et al. (1997) investigated the effects of different cooking methods on lipid oxidation in ground beef. Peroxide values observed from the different cooking methods (roasting, microwave, barbeque and pan frying) were not significantly different and values were reported from 0.7 to 2.4 meq/Kg lipid. The low peroxide value in this study more than likely represents lipids in advanced stages of oxidation. Due to the vulnerability of peroxides breaking down into secondary products, the complete oxidative history of the peroxides cannot be determined due to rapid decay prior to analysis. Peroxide values were not useful in assessing either treatment or cooking effects on the oxidative stability of the cooked beef patties.

Measurement of secondary products provided a much more clear indication of oxidative changes. Trends of TBARS values (Figure 10) are characteristic of a normal oxidation curve, increasing during the first days of storage then decreasing as the secondary products react with other meat components. The tocopherol treated samples displayed significantly ($p<0.05$) lower TBARS values than the control samples throughout storage, indicating that tocopherol retarded the development of lipid oxidation

in cooked ground beef. Although ethanol treated patties tended to have lower TBARS values than controls, no significant differences were present between the two samples. TBARS also significantly increased ($p<0.05$) after day 2; however, no other days were significantly different from each other. Cooking patties with the ohmic heater also resulted in significantly higher ($p<0.05$) TBARS values than patties cooked by the impingement oven. Heat may affect development of oxidative rancidity in several ways including cooking methods, cooking temperatures, cooking time and end-point temperature of the products (Su et al., 1991). Rapid cooking and the passage of electrons could explain why ohmically heated samples developed greater TBARS values. Increased reaction rates could result from such rapid cooking. Additionally, passage of current could stimulate oxidation through electron transfer (generating radicals) or excessive membrane destruction.

Dietary supplementation with vitamin E has been reported to delay lipid oxidation more effectively than exogenous addition to ground beef. This is because dietary vitamin E is incorporated into the polar membrane lipids, which are more prone to oxidation than neutral lipids. Furthermore, direct postmortem addition of tocopherol has previously been reported to have much less of an antioxidant effect because the antioxidant is primarily incorporated into the neutral lipid fractions (Mitsumoto et al., 1993; Buckley et al., 1995; Kerry et al., 1998; Higgins et al., 1998). Previous researchers have utilized oil as the medium to carry the fat-soluble vitamin E. However, recent studies with chicken muscle have shown that tocopherol partitioning between membrane lipids and triacylglycerols is significantly affected by the carrier used to deliver the antioxidant into the system (Sigfusson and Hultin 2002a,b). Using ethanol, significant amounts of

tocopherol were incorporated into membrane lipids, whereas virtually no incorporation was observed when corn oil was used as a carrier. The ethanol carrier used in this study allowed the tocopherol to concentrate in the membrane lipid fraction thus slowing oxidative attack.

Cooked Color. Effects of sample treatment and cooking method on cooked beef color were determined (Table 5 and Appendix E). Addition of tocopherol did not influence color. L* value results demonstrated ohmic heated sample patties were significantly ($p<0.05$) lighter in color than impingement patties. The a* value (redness) was not affected by cooking method. The b* values (yellow) were significantly ($p<0.05$) larger in ohmically heated patties than those cooked by impingement method. Differences in external meat color are more than likely due to moisture loss, Maillard Browning and grill marks.

Texture Profile Analysis. The results for the instrumental texture evaluation of beef patties are presented in Figures 11-15. Significant differences ($p<0.05$) were present between cooking methods; however, no significant differences were observed between sample treatments. Sample patties cooked by ohmic heating were significantly ($p<0.05$) harder, chewier, and gummier than samples cooked with the impingement oven. Springiness and cohesiveness were not significantly affected by cooking method. The main factors considered as affecting meat texture are: myofibrillar proteins, muscle cytoskeleton and intramuscular connective tissue (Jones et al., 1977; Silva et al., 1993). Heat-affected changes in meat components are largely responsible for textural attributes of cooked meat. During the cooking of meat there is first an increase in toughness between 40 and 50°C owing to the beginning of denaturation of myofibrillar proteins, a

further increase between 60 and 70°C because of shrinkage of intramuscular collagen at 65°C, and a third increase in the range 70-90°C when shrinkage and dehydration of the actomyosin occurs (Palka & Daun 1999). It is generally agreed that heat-induced changes in connective tissue have a tenderizing effect while hardening of the myofibrillar proteins during cooking has a toughening one (Laakkonen, 1973). Temperature variation can partially explain textural differences observed between the two cooking methods in this study. Samples cooked by the impingement oven would attain higher overall temperatures. Since internal temperature measurements were used to determine degree of doneness, the exterior portion of the patties would inherently reach higher temperatures than the center. Temperature variation in ohmically heated patties poses much less of a concern, due to uniform heating of particles; however, such rapid cooking and passage of current could promote myofibrillar protein hardening and sarcomere shortening. Furthermore, the tenderizing effect onset by gelatinization of collagen might not readily occur due to the rapid cooking.

CONCLUSION

Cooking beef patties with an ohmic heater resulted in significantly larger TBARS values compared to the impingent oven; however, the addition of 300ppm d-tocopherol using an ethanol carrier was effective in retarding lipid oxidation in beef patties cooked by both the impingement oven and ohmic heater. Tocopherol addition had no effect on color or texture attributes of sample patties. Although cooking method produced different texture values, further work is needed to determine the effects of particle size on ohmically heated samples texture results.

Table 4. Composition of Raw and Cooked Ground Beef on a Wet Basis.

	RAW	IMPINGEMENT	OHMIC
Moisture %	64.81 ± 2.35^{c}	53.44 ± 1.73^{a}	58.09 ± 1.08^{b}
Protein %	19.19 ± 1.53^{a}	30.80 ± 1.90^{c}	27.59 ± 2.30^{b}
Total Lipids (TL) %	17.85 ± 3.25^{a}	17.22 ± 4.33^{a}	15.54 ± 3.38^{a}
Phospholipids (PL) %	1.06 ± 0.50^{a}	0.91 ± 0.06^{a}	0.80 ± 0.10^{a}
TAG (=TL-PL) %	16.61 ± 3.27^{a}	17.62 ± 4.80^{a}	14.90 ± 4.10^{a}

Data represents means ± standard deviation.
[a,b,c]Means within same row without common superscript are different ($p<0.05$).

Table 5. Color Values of Ground Beef When Cooked by Different Methods and Treated With Ethanol and δ-Tocopherol.

TREATMENT^	L*[x]	a*[y]	b*[z]
CI	32.86 ± 3.37^{a}	5.72 ± 0.57^{a}	18.16 ± 3.52^{a}
EI	30.83 ± 2.96^{a}	5.80 ± 0.60^{a}	17.60 ± 3.79^{a}
VI	32.37 ± 3.78^{a}	5.78 ± 0.59^{a}	18.54 ± 3.24^{a}
CO	39.61 ± 3.30^{b}	5.90 ± 0.47^{a}	19.81 ± 1.35^{b}
EO	40.12 ± 3.07^{b}	5.87 ± 0.41^{a}	19.73 ± 2.04^{b}
VO	41.79 ± 3.18^{b}	5.77 ± 0.65^{a}	19.93 ± 1.68^{b}

^ (C) Control; (E) Ethanol; (V) Vitamin E + Ethanol; (I) Impingement; (O) Ohmic.
[x] L*: 0 = Black, 100 = White.
[y] a*: Negative Values = Green, Positive Values = Red.
[z] b*: Negative Values = Blue, Positive Values = Yellow.
Data represent means ± standard deviation.
[a,b]Means within same column without common superscript are different ($p<0.05$).

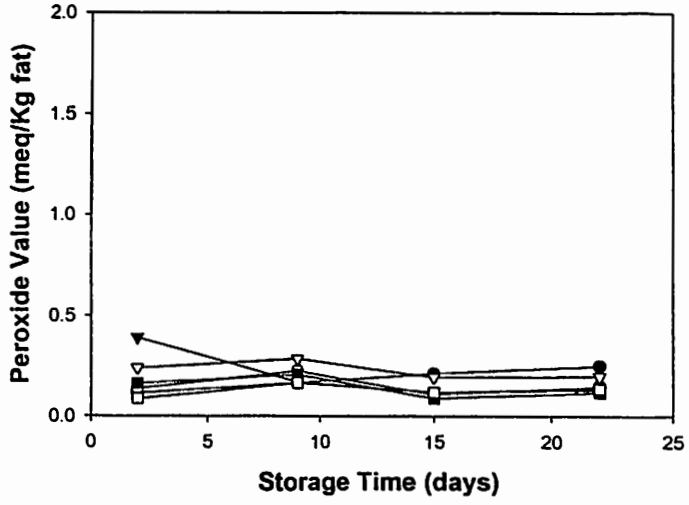


Figure 9. Lipid peroxide values (LOOH) of control, ethanol, and vitamin E treated samples cooked by both impingement and ohmic methods; (▼) Ethanol-Impingement; (▽) Ethanol-Ohmic; (■) Vit E-Impingement; (□) Vit E-Ohmic; (●) Control-Impingement; (○) Control-Ohmic. Samples were vacuum packaged and refrigerated at 2°C. Results are expressed on a fat basis of means of at least duplicate measurements from three replicate experiments.

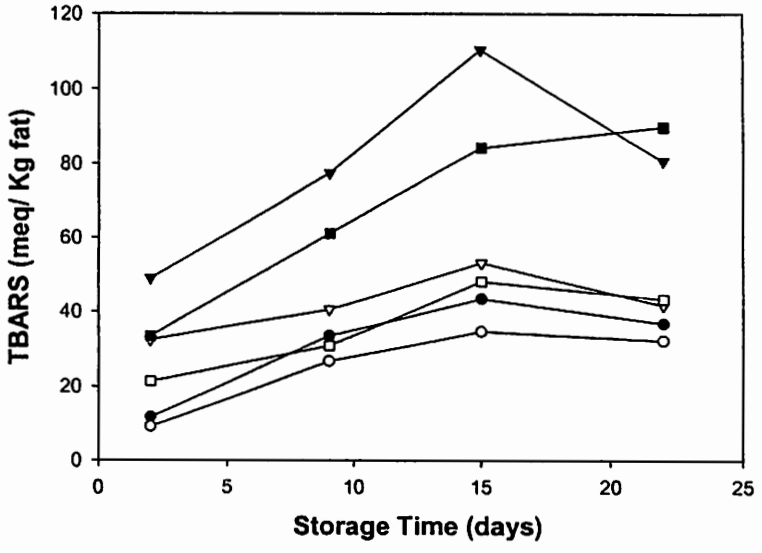


Figure 10. Values of thiobarbituric acid reactive substances (TBARS) of control, ethanol, and vitamin E treated samples cooked by both impingement and ohmic methods; (▼) Control-Ohmic; (▽) Control-Impingement; (■) Ethanol-Ohmic; (□) Ethanol Impingement; (●) Vit E-Ohmic; (○) Vit E-Impingement. Samples were vacuum packaged and refrigerated at 2°C. Results are expressed on a fat basis of means of at least duplicate measurements from three replicate experiments.

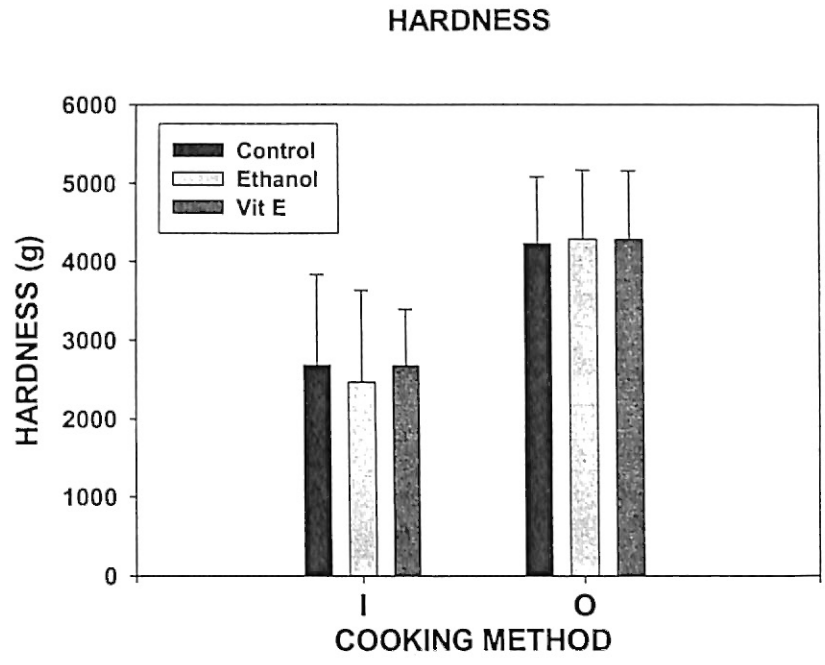


Figure 11. Hardness of Cooked Beef Patties. (I) Impingement; (O) Ohmic. Data Represent Means ± Standard Deviation.

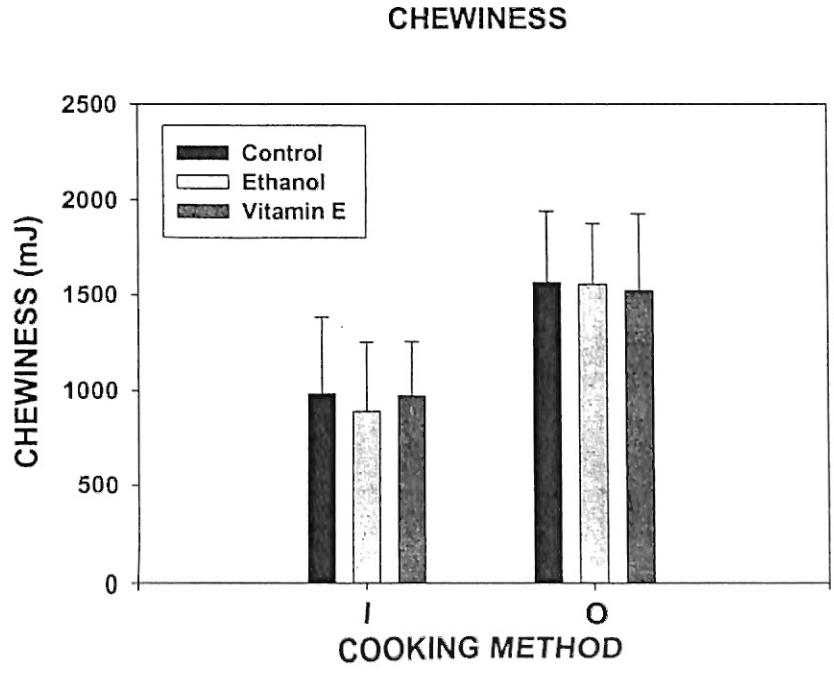


Figure 12. Chewiness of Cooked Beef Patties. (I) Impingement; (O) Ohmic. Data Represent Means ± Standard Deviation.

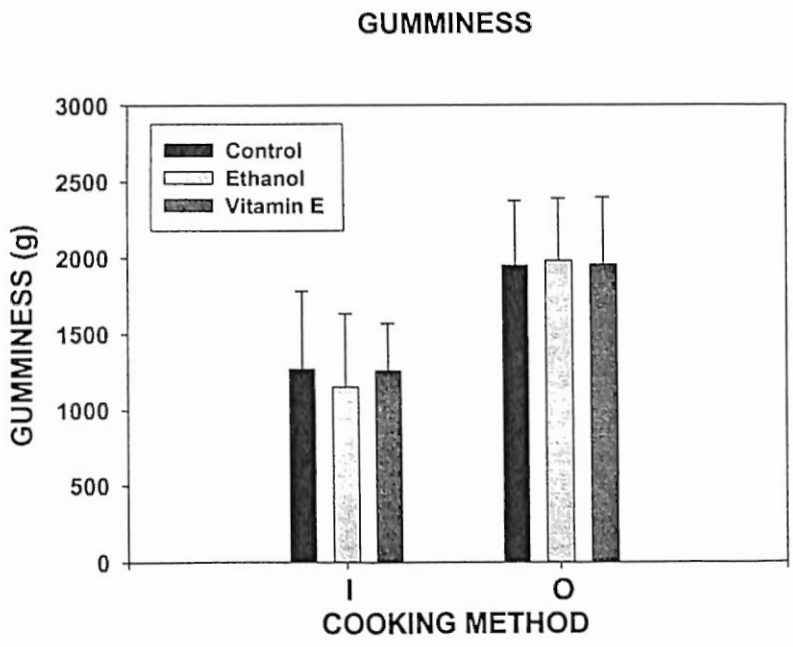


Figure 13. Gumminess of Cooked Beef Patties. (I) Impingement; (O) Ohmic. Data Represent Means ± Standard Deviation.

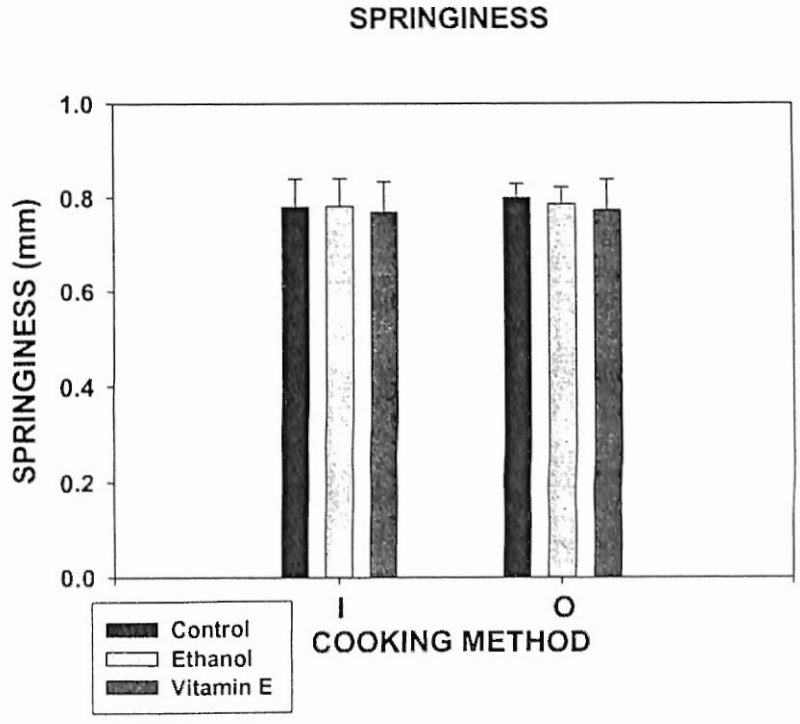


Figure 14. Springiness of Cooked Beef Patties. (I) Impingement; (O) Ohmic. Data Represent Means ± Standard Deviation.

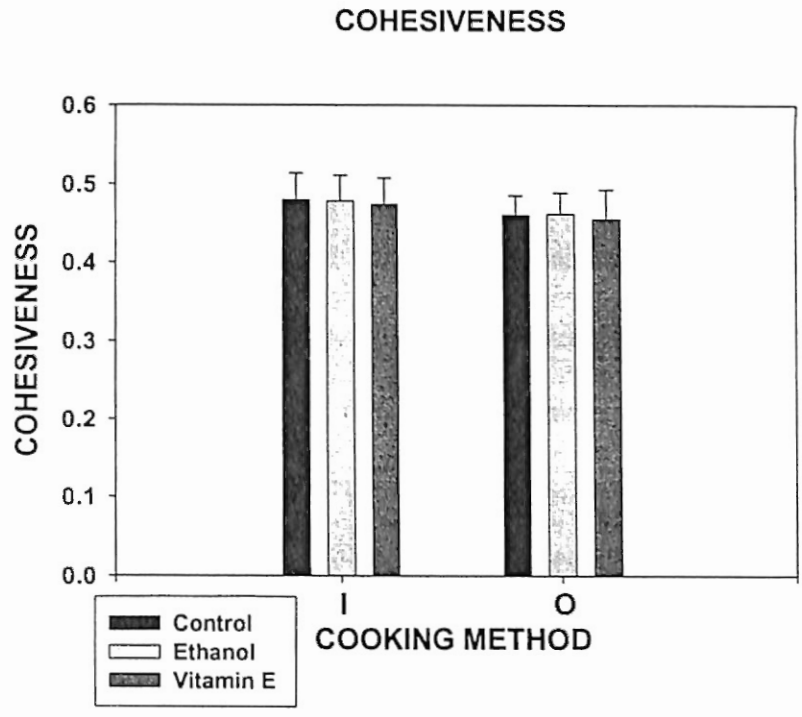


Figure 15. Cohesiveness of Cooked Beef Patties. (I) Impingement; (O) Ohmic. Data Represent Means ± Standard Deviation.

SUMMARY OF RESULTS AND CONCLUSION

The partitioning of exogenous antioxidants between neutral and membrane lipids is greatly influenced by the antioxidant carrier. Although neutral lipid yields were higher in raw muscle than cooked patties, the isolation procedures were adequate for beef muscle. The yields of raw polar membrane lipids obtained in this study were comparable to previous findings. Although low membrane recoveries were encountered in cooked samples, a high concentration of tocopherol was found to be membrane associated. By analyzing the chemical composition of membrane lipid fractions, it was determined contamination was present. Further isolation conditions should be investigated to optimize membrane recovery while minimizing contamination. Cooking resulted in a overall loss of membrane associated tococpherol, but an increase in tocopherol content of neutral lipids. The ethanol carrier transported and promoted concentration of vitamin E in membranes; however, vitamin E decreased linearly over storage time. The reduction in membrane tocopherol levels underscores the importance of incorporating the antioxidant into the membrane fraction. Vitamin E treated samples inhibited the formation of oxidation products, thus, increasing the concentration of vitamin E in the membrane lipids may effectively extend the product shelf life and reduce the formation of off-flavors and odors associated with pre-cooked, refrigerated meat products.

Cooking method had a pronounced effect on the chemical composition and quality of beef patties. Samples cooked with the ohmic heater expressed higher moisture and lower protein contents than samples cooked with the impingement oven. Chemical composition of patties did not change during refrigerated storage. Primary oxidation products (LOOH) were not a useful indicator of shelf stability due to their rapid

formation and decomposition. Secondary oxidation products (TBARS) indicated vitamin E hindered the oxidation reaction. Patties cooked with the ohmic heater developed larger TBARS values than patties cooked with the impingement oven. Furthermore, ohmic patties were more pale in color, harder, chewier, and gummier than patties cooked with the impingement oven.

Most meat processors have little, if any, knowledge of animal feed backgrounds. Many animals not suitable for retail display are sent to processing plants to have value added through additional processing. The meat from cattle which have not been supplemented with dietary vitamin E are much more susceptible to oxidation. Since animal backgrounds aren't always known, exogenously adding vitamin E to processed meat products is a suitable practice to slow oxidative deterioration; however, an appropriate antioxidant carrier must be selected to maximize antioxidant effectiveness.

APPENDIX A

PROXIMATE COMPOSITION OF COOKED BEEF PATTIES

Cooking Method	Sample	Day	Moisture	Fat	Protein	Proximate %
Impingement	Control	1	53.09 ± 2.23	17.34 ± 5.42	31.45 ± 1.85	101.88 ± 1.93
	Control	9	53.31 ± 0.05	17.54 ± 4.09	31.28 ± 2.03	102.12 ± 1.65
	Control	15	52.22 ± 1.80	17.65 ± 3.67	31.39 ± 0.97	101.25 ± 1.88
	Control	22	53.41 ± 1.00	17.24 ± 3.31	30.83 ± 1.57	101.48 ± 0.97
	Ethanol	1	52.87 ± 1.81	17.49 ± 4.89	31.17 ± 1.94	101.53 ± 1.31
	Ethanol	9	53.33 ± 1.45	17.73 ± 3.91	30.23 ± 1.87	101.29 ± 0.68
	Ethanol	15	52.61 ± 1.67	16.86 ± 4.35	30.75 ± 2.19	100.20 ± 0.69
	Ethanol	22	52.53 ± 2.09	17.83 ± 3.05	31.73 ± 1.25	102.09 ± 0.42
	Vit E	1	54.35 ± 1.35	16.84 ± 4.63	29.78 ± 2.22	100.97 ± 2.02
	Vit E	9	53.49 ± 0.57	17.69 ± 4.43	30.72 ± 2.39	101.89 ± 1.67
	Vit E	15	53.56 ± 0.83	17.41 ± 2.50	29.91 ± 0.15	100.89 ± 1.58
	Vit E	22	54.06 ± 1.82	17.05 ± 3.20	30.28 ± 1.44	101.39 ± 1.56
Ohmic	Control	1	58.07 ± 1.07	14.54 ± 3.46	28.08 ± 1.10	100.61 ± 1.77
	Control	9	58.66 ± 1.34	16.16 ± 3.33	28.03 ± 1.28	102.85 ± 1.04
	Control	15	58.40 ± 1.01	15.68 ± 4.39	27.25 ± 1.41	101.32 ± 2.28
	Control	22	57.91 ± 2.07	16.33 ± 4.27	28.05 ± 1.86	102.28 ± 1.01
	Ethanol	1	58.14 ± 1.12	15.74 ± 4.00	27.79 ± 2.62	101.66 ± 2.02
	Ethanol	9	58.63 ± 1.24	15.91 ± 3.76	28.23 ± 2.21	102.77 ± 0.65
	Ethanol	15	58.49 ± 1.13	15.68 ± 2.92	27.76 ± 2.55	101.94 ± 0.71
	Ethanol	22	58.73 ± 1.03	14.62 ± 3.45	27.38 ± 0.86	100.72 ± 1.62
	Vit E	1	58.15 ± 0.18	16.42 ± 4.00	26.82 ± 3.49	101.38 ± 1.39
	Vit E	9	58.22 ± 1.30	16.67 ± 6.20	26.83 ± 3.63	101.73 ± 2.21
	Vit E	15	57.98 ± 1.72	16.86 ± 5.63	26.32 ± 2.76	101.16 ± 1.70
	Vit E	22	58.50 ± 0.79	16.29 ± 4.41	27.04 ± 4.30	101.83 ± 0.46

Results are reported as means and standard deviations of three replicates with at least duplicate measurements within each replicate.

APPENDIX B

TRIACYLGLYCEROL YIELDS FROM CENTRIFUGED MUSCLE

Sample[a]	Day[b]	Sample wt.[c]	TL %[d]	PL %[e]	TAG %[f]	TAG (g)[g]	Oil Collected (g)[h]	NL Collected / NL Muscle %[i]	Std Dev[j]
RC	0	50.01	20.28	1.07	19.21	9.61	3.85	40.31	10.88
RV	0	50.12	18.12	0.59	17.53	8.78	3.40	38.91	1.87
CI	1	40.04	19.14	1.06	18.07	7.24	2.00	24.80	17.24
VI	1	40.13	16.21	0.81	15.40	6.18	2.00	29.38	17.38
CI	8	40.19	17.10	0.83	16.27	6.53	2.20	33.22	2.95
VI	8	40.17	17.04	0.85	16.19	6.50	2.15	33.18	0.56
CI	14	40.46	17.49	0.88	16.61	6.73	1.55	22.54	5.05
VI	14	40.21	16.47	0.94	15.53	6.25	1.40	23.47	6.03
CI	21	40.03	17.40	0.88	16.52	6.61	1.75	25.54	8.22
VI	21	40.13	17.87	0.90	16.96	6.81	1.65	23.78	4.52

[a]Sample Treatment. R=Raw, C=Control, V=Vitamin E, I=Impingement.
[b]Day partitioning study occurred.
[c]Sample weight of ground beef.
[d]Total lipid % derived from chemical analysis of lipid extract.
[e]Phospholipid % derived from ICP.
[f]Triacylglycerol % estimated by subtraction. TL% - PL% = TAG%.
[g]Grams of triacylglycerols. Calculated by multiplying sample wt. x TAG%.
[h]Oil collected following centrifugation. Based on visual measurement in graduated Kimble tubes.
[i]Neutral lipid collected / neutral lipid muscle. Recovery based on theoretical comparison.
[j]Standard deviation of recoveries based on duplicate measurements in duplicate experiments.

APPENDIX C

PHOSPHORUS CONTENT OF TRIACYLGLYCEROLS

Sample[a]	Day[b]	ICP Reading (ppm)[c]	μg/g PL[d]	% PL[e] [wet wt.]	% TAG[f]
RC	0	< 0.0200	32.7500	0.003	99.997
RV	0	< 0.0200	32.7500	0.003	99.997
CI	1	< 0.0200	32.7500	0.003	99.997
VI	1	< 0.0200	32.7500	0.003	99.997
CI	8	< 0.0200	32.7500	0.003	99.997
VI	8	< 0.0200	32.7500	0.003	99.997
CI	14	< 0.0200	32.7500	0.003	99.997
VI	14	< 0.0200	32.7500	0.003	99.997
CI	21	< 0.0200	32.7500	0.003	99.997
VI	21	< 0.0200	32.7500	0.003	99.997

Data represents means of duplicate measurements from experiments run in triplicate.

[a]Sample treatment. R=Raw, C=Control, V=Vitamin E, I=Impingement.

[b]Day partitioning study occurred.

[c]Phosphorus (ppm) detected by ICP.

[d]μg phosphorus / g phospholipid. Calculation is as follows:

(ICP Reading ppm) * (6.55/0.1) * 25

6.55= volume of sample (mL)

0.1= amount lipid extract from chloroform extraction (mL)

25.0= factor for converting lipid phosphorus to phospholipid

[e]Phospholipid %.

(μg/g PL / 1,000,000) * 100

[f]Triacylglycerol %.

100- % PL

APPENDIX D

MEMBRANE YIELDS OF RAW AND COOKED GROUND BEEF

Sample[a]	Day[b]	Sample wt.[c]	PL %[d]	Theoretical PL (g)[e]	PL mg/mL membranes[f]	mL of membrane suspension[g]	Phospholipid (g)[h]	Recovery %[i]	Std Dev[j]
RC	0	70.02	1.07	0.75	7.53	7.93	0.06	9.55	5.36
RV	0	70.03	0.59	0.42	7.60	8.20	0.06	15.08	2.32
CI	1	70.11	1.06	0.75	0.85	10.20	0.01	1.19	0.37
VI	1	70.10	0.81	0.57	0.93	10.40	0.01	1.73	0.36
CI	8	70.02	0.83	0.58	0.70	10.25	0.01	1.23	0.27
VI	8	70.01	0.85	0.60	0.69	10.70	0.01	1.23	0.10
CI	14	70.02	0.88	0.62	0.66	10.55	0.01	1.12	0.10
VI	14	70.12	0.94	0.66	0.67	11.35	0.01	1.15	0.02
CI	21	70.13	0.88	0.62	0.82	10.05	0.01	1.34	0.12
VI	21	70.06	0.90	0.63	0.70	10.65	0.01	1.18	0.17

[a]Sample Treatment. R=Raw, C=Control, V=Vitamin E, I=Impingement.
[b]Day partitioning study occurred.
[c]Sample weight of ground beef.
[d]Phospholipid % in beef patties determined by ICP.
[e]Theoretical (g) phospholipid. Calculated as (sample wt * PL %).
[f]Amount phospholipid (mg) determined in membrane suspension by ICP.
[g]Membrane suspension volume collected.
[h]Phospholipid (g) content of total suspension. Calculated as (PL mg/mL in membranes * mL membrane suspension) / 1000.
[i]Theorectical % membrane recovered from original sample.
[j]Standard deviation of % recovery from duplicate measurements in duplicate experiments.

APPENDIX E

COLOR VALUES (*L* a* b**) OF COOKED BEEF PATTIES

Sample[a]	L*[b]	a*[c]	b*[d]
CI_1	32.19 ± 3.31	5.12 ± 0.50	13.95 ± 2.59
CI_2	29.97 ± 0.66	6.05 ± 0.20	19.64 ± 0.74
CI_3	36.40 ± 1.29	5.96 ± 0.41	20.88 ± 1.40
EI_1	29.62 ± 3.43	5.08 ± 0.45	13.13 ± 2.95
EI_2	30.09 ± 2.05	6.14 ± 0.15	20.70 ± 0.65
EI_3	32.78 ± 2.71	6.18 ± 0.26	18.96 ± 1.36
VI_1	32.58 ± 2.18	5.09 ± 0.47	14.82 ± 2.95
VI_2	28.40 ± 2.61	6.18 ± 0.18	20.52 ± 1.15
VI_3	36.13 ± 1.02	6.08 ± 0.26	20.30 ± 0.79
CO_1	43.82 ± 1.77	5.41 ± 0.36	18.70 ± 0.83
CO_2	37.52 ± 1.04	6.23 ± 0.36	19.31 ± 0.70
CO_3	37.47 ± 0.84	6.05 ± 0.24	21.42 ± 0.26
EO_1	40.98 ± 1.59	5.48 ± 0.25	17.66 ± 1.55
EO_2	36.41 ± 1.23	6.27 ± 0.32	19.53 ± 0.41
EO_3	42.96 ± 0.83	5.85 ± 0.21	21.99 ± 0.39
VO_1	44.94 ± 1.06	5.07 ± 0.43	18.04 ± 1.15
VO_2	38.01 ± 1.03	5.97 ± 0.40	20.21 ± 0.43
VO_3	42.43 ± 1.54	6.28 ± 0.37	21.56 ± 0.73

[a]Number is trial run. C=Control, E=Ethanol, V=Vitamin E, I=Impingement, O=Ohmic.

[b]L*. 0 = Black, 100 = White.

[c]a*. Negative Values = Green, Positive Values = Red.

[d]b*. Negative Values = Blue, Positive Values = Yellow.

APPENDIX F

TEXTURE (TPA) RESULTS OF COOKED BEEF PATTIES

Sample[a]	Hardness[b]	Springiness[c]	Cohesiveness[d]	Gumminess[e]	Chewiness[f]
CI_1	1876 ± 396	0.816 ± 0.048	0.502 ± 0.032	936 ± 177	760 ± 127
CI_2	2912 ± 1369	0.776 ± 0.078	0.485 ± 0.036	1393 ± 620	1085 ± 492
CI_3	3253 ± 1053	0.759 ± 0.029	0.454 ± 0.012	1479 ± 487	1129 ± 404
CO_1	4438 ± 766	0.798 ± 0.029	0.484 ± 0.023	2159 ± 430	1730 ± 389
CO_2	3754 ± 525	0.801 ± 0.027	0.461 ± 0.015	1728 ± 248	1387 ± 223
CO_3	4525 ± 1038	0.812 ± 0.031	0.439 ± 0.013	1990 ± 485	1622 ± 425
EI_1	1709 ± 587	0.814 ± 0.028	0.504 ± 0.020	856 ± 280	696 ± 228
EI_2	2120 ± 904	0.776 ± 0.049	0.479 ± 0.032	1001 ± 404	781 ± 329
EI_3	3571 ± 1040	0.764 ± 0.078	0.453 ± 0.025	1599 ± 391	1218 ± 290
EO_1	4536 ± 967	0.790 ± 0.029	0.463 ± 0.026	2107 ± 498	1664 ± 399
EO_2	3919 ± 877	0.808 ± 0.026	0.483 ± 0.018	1891 ± 419	1525 ± 334
EO_3	4438 ± 720	0.775 ± 0.044	0.441 ± 0.018	1951 ± 293	1507 ± 205
VI_1	2377 ± 464	0.784 ± 0.047	0.492 ± 0.029	1164 ± 209	916 ± 193
VI_2	2960 ± 895	0.810 ± 0.035	0.487 ± 0.023	1435 ± 403	1166 ± 339
VI_3	2678 ± 665	0.721 ± 0.075	0.443 ± 0.027	1172 ± 232	852 ± 219
VO_1	4582 ± 935	0.822 ± 0.030	0.490 ± 0.017	2246 ± 473	1846 ± 396
VO_2	3946 ± 538	0.809 ± 0.023	0.463 ± 0.015	1830 ± 291	1483 ± 259
VO_3	4344 ± 1030	0.702 ± 0.049	0.415 ± 0.028	1800 ± 427	1264 ± 325

[a]Number is trial run. C=Control, E=Ethanol, V=Vitamin E, I=Impingement, O=Ohmic.
[b]Hardness (g) = Peak force of the 1st compression.
[c]Springiness (mm) = Length of 2nd compression / length of 1st compression.
[d]Cohesiveness = Area 2nd compression / area of 1st compression.
[e]Gumminess (g) = Hardness x Springiness.
[f]Chewiness (mJ) = Gumminess x Springiness.

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VITA

Todd Michael Wills

Candidate for the Degree

Master of Science

Thesis: Partitioning of Ethanolic Delta-Tocopherol and Cooking Effects on Oxidative Stability and Quality of Beef Patties During Refrigerated

Major Field: Food Science

Biographical:

Personal Data: Born in Liberal, Kansas, January 31, 1979, the son of James D. Wills and the late Judith Ann Lee.

Education: Graduated from Liberal High School, Liberal, Kansas, May 1997; Received Bachelor of Science Degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma, December 2001; Completed the Requirements for the Master of Science degree with a major in Food Science at Oklahoma State University in July, 2004.

Experience: Employed by Oklahoma State University department of Animal Science as an undergraduate lab assistant 2000-2001, Farmland National Beef as a quality control intern in 2000, Oklahoma State University Food & Ag Products Center as HACCP coordinator and a research assistant, 2001- present.

Professional Organizations: Institute of Food Technologists, American Oilseed Chemists Society.