Inhibition of Lipid Oxidation with Phosphates in Muscle Foods

by
Marsha L. Sickler

Submitted to the faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Food Science and Technology

Approved by:

Dr. James R. Claus, Chairman

Dr. William N. Eigel

Dr. Norman G. Marriott

Dr. Hengjian Wang

January 10, 2000
Blacksburg, VA

Key Words: Beef, Turkey, Phosphates, Lipid Oxidation

Inhibition of Lipid Oxidation with Phosphates in Muscle Foods

by

Marsha L. Sickler

Chairman: James R. Claus

Department of Food Science and Technology

ABSTRACT

Lipid oxidation degrades the quality and decreases the shelf-stability of muscle foods. The depletion of phosphates prior to cooking may be a major factor in this undesirable reaction. Thus, the effects on lipid oxidation with the use of an encapsulate to protect the phosphates during raw storage was investigated.

Unencapsulated and encapsulated sodium tripolyphosphate (STP) and sodium acid pyrophosphate (SAPP), at a level of 0.5%, were compared to control samples in cooked, ground beef patties at 0 and 6 days. The unencapsulated and encapsulated treated samples were different ($P<0.05$) from the controls with an 81.1% to 89.7% improvement in the reduction of lipid oxidation. However, encapsulated phosphates did not decrease the level of oxidation beyond the unencapsulated treatment. This observation was attributable to the lack of a storage time prior to evaluating rancidity. Therefore, with an increase of precooked storage time, the 0.10% active encapsulated STP was essentially as effective as 0.20% unencapsulated STP for both 3 and 11 days.

Unencapsulated STP (0.3% or 0.5%), encapsulated STP (0.3% or 0.5% active), a blend of unencapsulated (0.3%) and encapsulated (0.2% active) STP, and a control treatment was incorporated in ground turkey breast and stored at 3°C for 0, 5, and 10

days. The treated samples were cooked to two different endpoint temperatures (74°C and 79°C) and stored at 3°C (4 and 24 hr) before cooking. An improvement of 77% and 80% was found in the reduction of Thiobarbituric Acid Reactive Substances (TBARS) with the 0.3% and 0.5% encapsulated STP, respectively, in comparison to the unencapsulated STP. The best results were seen with a shorter storage time (4 hr) prior to cooking and a higher endpoint temperature (79°C). The unencapsulated and encapsulated STP were compared to commercial antioxidant blends, Lemo-fos and Freez-Gard FP 15, at a level of 0.5%, to determine differences in their capabilities of lipid oxidation reduction. The encapsulated phosphate was lower ($P<0.05$) in TBARS (3.5 mg/kg) in comparison to the treatments which ranged from 15.6 to 20.4 mg/kg. However, the CIE a* values were higher in the encapsulated samples due to the decrease in lipid oxidation.

The effect of liquid nitrogen on TBARS values was investigated to identify a means of analyzing a large quantity of samples. The use of cryogenic freezing was not significantly different in TBARS in comparison with a fresh, unfrozen control. Raw and cooked ground turkey samples were submerged into liquid nitrogen and stored intact or immediately reduced in particle size to compare particle reduction effects on TBARS. The different particle reduction methods were not significantly different, although, the immediately reduced sample was more efficient in TBARS determination. The samples stored in an ultralow freezer (-80°C) for 14 and 33 days were not different ($P>0.05$).

Overall, when encapsulated STP is used with sufficient pre-cook storage time, lipid oxidation can be more effectively reduced than with the use of unencapsulated phosphates. The use of cryogenic freezing and ultralow temperature storage can also aid

in the determination of lipid oxidation in large sample quantities due to the stability of TBARS values.

Acknowledgements

It is of great honor to acknowledge those who made this accomplishment possible. Starting the program as a provisional student, I feel a great sense of pride to say that I have completed the Food Science and Technology Master's program.

I would like to thank my committee for their effortless patience throughout my research. A special thanks goes to Dr. Wang who dedicated his time to solve problems and assist in the development in chemical analysis, statistics, keeping me positive, and curing my insomnia. My appreciation for the Food Science and Technology department brings me to a loss of words. The strength, happiness, and knowledge I received from all of them were inconceivable. The one word that comes to mind when I think of their involvement in my program is family.

To my friends and family, I would like to thank them for their endless support. Their confidence in me was unimaginable. As for my fiancé, Andrew William Pryor Jr., all I can say is that this thesis is dedicated to you. Without his constant love and support throughout my program, I would be at a great loss. I would also like to acknowledge my pets. Their unconditional love was pertinent to my sanity and completion to my thesis.

However, this accomplishment would not be possible without the constant encouragement and support of Dr. James R. Claus. His understanding of life's consequences was inconceivable for the average graduate professor. My gratitude for Dr. Claus is unfathomable… Thank you :)

Table of Contents

Chapter 1

Introduction

The investigations of lipid oxidation and its mechanisms began in the 1940's (Farmer et al., 1942; Bolland and Gee, 1946). Lipid oxidation can affect the appearance, flavor, and odor of foods. The cessation of oxidation is of paramount importance to the meat industry. The decrease in oxidation can be advantageous to the industry by an increase in shelf life and the production of more acceptable and flavorful product. The level of susceptibility to lipid oxidation varies from species to species. Meats, such as beef and lamb, which contain more saturated fat are much more resistant to lipid oxidation deterioration than are those containing more unsaturated fats, such as pork and turkey (Price et al., 1987). Therefore, a technique to decrease the level of oxidation susceptibility is highly desired by the industry, especially in poultry.

The use of phosphates to retard lipid oxidation and increase shelf stability has been extensively studied. Approximately 80% of added polyphosphate is lost by the time meat is cooked (Decker and Mei, 1996). Therefore, given that the legal limit of incorporation is 0.5%, only 0.1% polyphosphate (meat weight basis) remains after cooking. Li et al. (1993) demonstrated that 100% of sodium tripolyphosphate (STP) was lost in one day after incorporation in uncooked ground turkey. Akamittath et al. (1990) found that the protective effect of phosphates diminished over time due to prooxidative effects from salt, activated metmyoglobin, and the presence of free metal ions. However, Decker and Mei (1996) reported that the addition of STP to meat after cooking resulted in significant improvement in controlling oxidation during storage. Despite this

improvement, production inefficiencies and food safety issues from the addition of STP post-cooking would make this approach impractical to the industry.

Encapsulation of selected phosphates known for their strong antioxidant property can potentially be beneficial to the meat industry. The use of encapsulated phosphates during raw meat storage and the initial stages of cooking will prevent subsequent oxidation during post-cook storage times. Phosphatases play a key role in affecting the functionality of the phosphates due to the hydrolysis of polyphosphates before thermal processing (Sutton, 1973). Protection of the phosphates by encapsulation can provide time for some of the heat sensitive phosphatases to be inactivated prior to phosphate release. Therefore, the addition of encapsulated phosphates increases the availability of the phosphate post-cook, thus, improving the control of lipid oxidation during storage.

Encapsulation technology has been used to control the release of numerous food grade ingredients and act as a moisture barrier. The barrier against moisture transport is increased with a higher melting point of the lipid encapsulate (Kamper and Fennema, 1984). Ingredients that have been encapsulated include acids, sweeteners, flavorings, vitamins, and salt (Pszczola, 1998). For example, encapsulated ascorbic acid (citric acid) is used as a dough conditioner and to prevent premature coagulation of meat proteins in acidified processed meats. Some other examples of encapsulated ingredients are sodium acid pyrophosphate (SAPP) used to facilitate bread leavening, sugar used for chewing gum to extend the perception of sweetness, and salt due to its prooxidative abilities. Unfortunately, phosphates have not been encapsulated for the purpose of optimizing the ability to control oxidation. Encapsulated phosphates, if effective, could have numerous applications in red meat and poultry products. Catfish and dairy products, including fluid milk, may also benefit.

At times, it is not feasible to measure oxidation using Thiobarbituric acid reactive substances (TBARS) analysis on a large number of samples in one day. Therefore, the use of cryogenics to preserve samples may be extremely advantageous due to an increase in efficiency. The use of cryogenic freezing yields small ice crystals, which are highly desired with freezing due a minimum dislocation of water, reduction in textural change, decrease in fluid loss during freezing and thawing, and less chemical degradation (Sebranek, 1982). However, the application of liquid nitrogen and its direct effect on lipid oxidation has yet to be determined. Therefore, an applicable procedure would be a great asset to the research on lipid oxidation in muscle foods.

The objective of this research was to determine the effects of encapsulated phosphates on lipid oxidation. Cryogenic freezing was also evaluated as a method of increasing the efficacy of determining TBARS. The research consisted of four experiments. The experiment in Chapter 3 was designed to assess the impact of encapsulated phosphates in ground beef patties without any significant uncooked raw meat storage. The focus of this preliminary project was to determine if there is a benefit to incorporating encapsulated SAPP and STP to ground beef patties, and the effects the encapsulated phosphates have on lipid oxidation. Chapter 4 focused on the effects of various levels of encapsulated and unencapsulated phosphate on the development of lipid oxidation in ground turkey breast during storage. The significance of two different endpoint temperatures (74°C and 79°C) and storage time before cooking (4 and 24 hr) was determined. In Chapter 5, a comparison study was developed to determine the effects of encapsulated STP, unencapsulated STP, Lemo-fos, and Freez-Gard FP-15 on lipid oxidation in ground turkey breast. The study in Chapter 6 examined the use of cryogenic freezing and particle reduction with cooked and uncooked ground turkey breast

and the effects on lipid oxidation. These chapters are formatted in preparation for publication.

References

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513-1517.

Bolland, J.L., and Gee, G. 1946. Kinetic studies in the chemistry of rubber and related materials. Trans. Faraday Soc. 42:236-252.

Decker, E.A. and Mei, L. 1996. Oxidative Processes in Meat. Antioxidant mechanisms and applications in muscle foods. Proceedings from the 49th Reciprocal Meat Conference, Provo, UT. Published by the Am. Meat Sci. Assoc. Chicago, IL. 49:64-72.

Farmer, E.H., Bloomfield, G.F., Sundralingam, A., and Sutton, D.A. 1942. The course and mechanism of autoxidation reactions in olefinic and polyolefinic substances, including rubber. Trans. Faraday Soc. 38:348-356.

Li, W., Bowers, J.A., Craig, J.A., and Perng, S.K. 1993. Sodium tripolyphosphate stability and effect in ground turkey meat. J. Food Sci. 58:501-504, 521.

Dugan, L.R., Jr. 1987. Meat Fats. Ch. 15 (part 1) in The Science of Meat and Meat Products, 3rd ed., J.F. Price and B.S. Schweigert. Food & Nutrition Press, Inc., Westport, CT. p. 507-530.

Kamper, S.L. and Fennema, O. 1984. Water vapor permeability of edible bilayer films. J. Food Sci. 49:1478-1481,1485.

Pszczola, D.E. 1998. Encapsulated ingredients: providing the right fit. Food Technol. 52(12):70-77.

Sebranek, J.G. 1982. Use of cryogenics for muscle foods. Food Technol. 36(4):120-127.

Sutton, A.H. 1973. The hydrolysis of sodium tripolyphosphate in cod and beef muscle. J. Food Technol. 8:185-195.

Chapter 2

Literature Review

2.1 Lipid Oxidation

2.1.1 Oxidation Reaction

In foods, both enzymatic and nonenzymatic mechanisms can cause the oxidation of lipids. Some examples of these mechanisms are light, temperature, metals, metalloproteins, microorganisms, and enzymes. The main reaction involved in the oxidation of lipids is autoxidation. Autoxidation is a chemical reaction that usually takes place at ambient temperatures between atmospheric oxygen and organic compounds (Chan, 1987). The low level of pH can also accelerate the protonation of bound oxygen and favor the release of superoxide anions (Livingston and Brown, 1981). The organic compounds include the unsaturated fatty acids, particularly polyunsaturated fatty acids (Allen and Foegeding, 1981).

The overall reaction of autoxidation consists of polyunsaturated fatty acids, which have three or more double bonds, and their interaction with oxygen. This reaction causes a breakdown of the polyunsaturated fatty acids which can produce free radicals and initiate the reaction of oxidation. Free radicals are atoms or compounds that are highly reactive and have at least one pair of unshared electrons (Claus and Marriott, 1995). According to Chan (1987), the process of autoxidation is autocatalytic. Therefore, the rate of oxidation is slow at the beginning but the rate increases progressively over time. The beginning of the autoxidation reaction consists of an induction period. This period is often too slow to be measured and signifies the involvement of radical-chain

mechanisms. The radical-chain reaction of autoxidation can be divided into three separate processes: initiation, propagation, and termination (Melton, 1983). The reaction is shown in Figure 1.

Figure 1. Radical-chain reaction of autoxidation.

Initiation: $RH+O_2 \rightarrow R\cdot + \cdot OH$

Propagation: $R\cdot + O_2 \rightarrow ROO\cdot$

$ROO\cdot + RH \rightarrow ROOH + R\cdot$

Termination: $R\cdot + R\cdot \rightarrow RR$

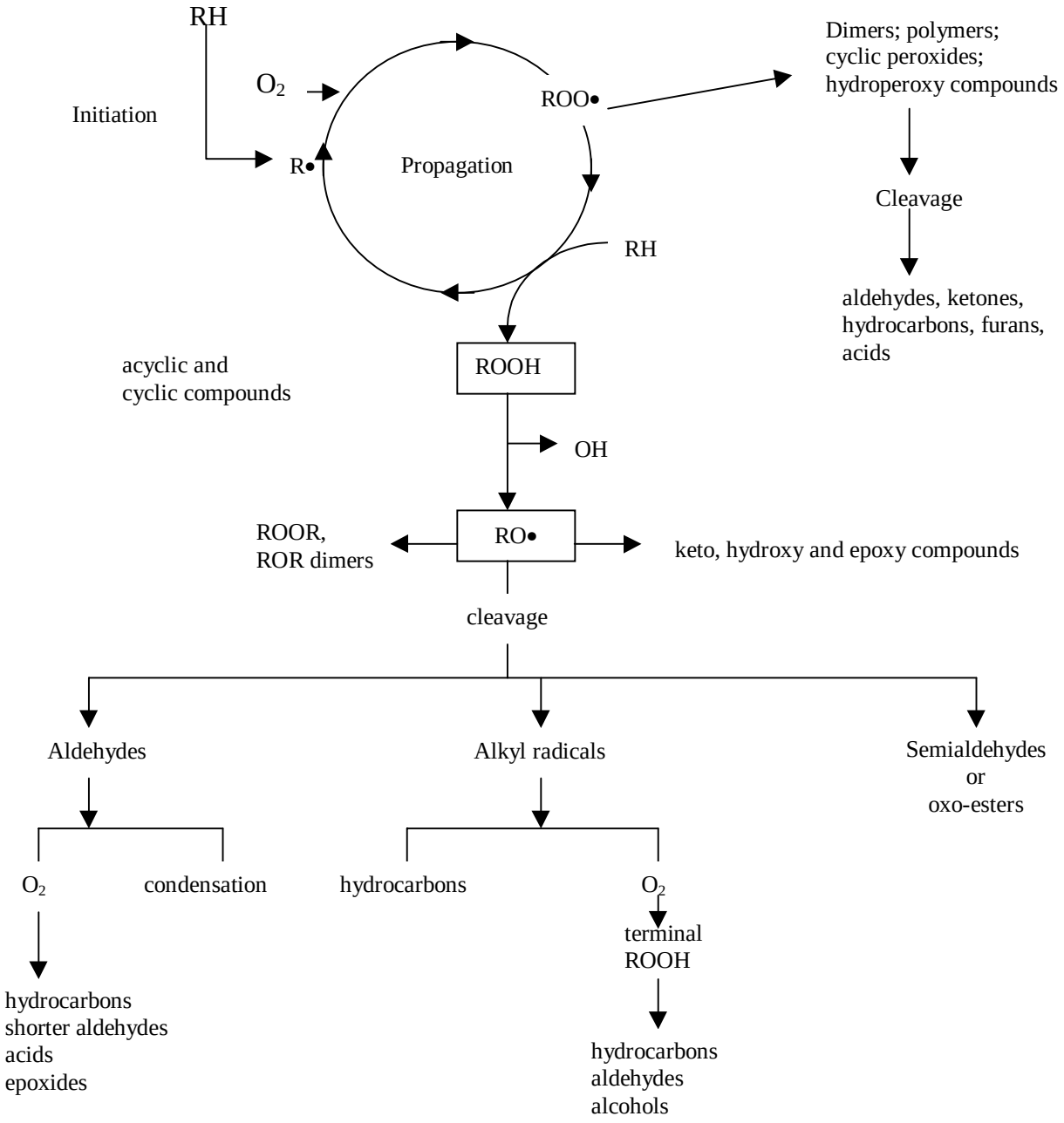
$R\cdot + ROO\cdot \rightarrow ROOR$

$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$

The initiation process is a thermodynamically difficult reaction due to an activation energy of 35 kcal (Chan, 1987). Therefore, catalysts, such as fatty acids (RH) and hydroperoxides (ROOH), must be present to begin the initiation reaction. These catalysts have a double bond in the singlet state. Single-state oxygen is highly electrophilic due to an opposite spin which gives a rapid reaction rate. The increase in reaction rate produces hydroperoxides which cleave and begin the free-radical chain reaction (Lillard, 1987). Metal catalysis and exposure to light can also replace the initiation process. The propagation step, also known as the rate determining step, entails the addition of oxygen which then forms a peroxy radical ($RO_2\cdot$). The $RO_2\cdot$ can react with other molecules, particularly RH, and produce ROOH and new free radicals ($R\cdot$). The $R\cdot$ is recycled, producing more ROOH. Once the cycle is initiated, it becomes

continuous. This cycle will continue until food additives such as antioxidants are implemented. The termination step is the main reaction under normal conditions. At this point, the lipid is completely oxidized. The overall reaction of lipid autoxidation is shown in Figure 2.

Figure 2. Overall autoxidation reaction (Nawar, 1996).



2.1.2 Factors Affecting Oxidation

Due to the complexity of food composition, a complete understanding of all the factors involved with lipid oxidation has not been thoroughly established in foods. Many factors can be involved simultaneously. The oxidation of lipids can begin through biochemical changes from the conversion of muscle to meat (Morrissey et al., 1994). Pre-slaughter events such as stress, electrical stimulation, or carcass temperature can also affect the rate of lipid oxidation (Hornstien and Wasserman, 1987). Likewise, heat, light, oxidizing enzymes, and metals are all factors linked to post-slaughter oxidizing reactions.

Species

The most significant variable between species in determining palatability and storage characteristics is the lipid content of muscle (Allen and Foegeding, 1981). Studies have demonstrated the lipid content of red fibers to be appreciably higher than that of white fibers. Wilson et al. (1976) stated that breast muscle in turkey contains about one-half as much total lipid as thigh muscle. Similarly, abdominal fat and skin in chicken contains several times the total lipid level of the thigh. More specifically, the study demonstrated the percentage of lipid made up by phospholipid in the turkey to be 35.4% for red muscle and 64.4% for white muscle (Wilson et al., 1976). Phospholipids contribute to the structural and functional component of cells and membranes (Dugan, 1987). The phospholipid content, as the percentage of total lipid for several different species, are approximately as follows: turkey (white 71%, dark 26%), chicken (white 48%, dark 21%), fish (white 24%, dark 7%), pork (*Longissmus dorsi* 21%, Psoas major 37%), beef (*Longissimus dorsi* 22% for 360 days old, *Longissimus dorsi* 5% for 480 days old), and lamb (*Longissimus dorsi* 10%, *Semitendinosus* 17%) (Allen and Foegeding,

1981). Phospholipids are highly unsaturated fats, therefore, susceptible to oxidation. The most susceptible phospholipid to oxidation is phosphatidyl ethanolamine (PE) (Igene and Pearson, 1979). Drippings, which were collected throughout meat cookery, contained a large amount of triglycerides with PE being virtually absent indicating that it was bound to the membrane. Since the concentration of phospholipids in meat is low, the effects on flavor are partly due to the high content of unsaturated fatty acids (Dugan, 1987).

Fatty Acids

Fatty acids with one or more hydrogen atoms are susceptible to oxidation. The composition of these fatty acids can affect the rate of oxidation. There are two types of fatty acids, saturated and unsaturated. Saturated fatty acids consist of only single bonds unlike unsaturated fatty acids that have at least one double or triple bond. The autoxidation of saturated fatty acids is extremely slow at room temperature. At the time oxidative rancidity of polyunsaturated fatty acids (PUFA) becomes detected, saturated fatty acids can be unaffected (Nawar, 1996). Moerck and Ball (1974) demonstrated that muscle food fatty acids, during refrigerated storage, must have three or more double bonds to be highly susceptible to oxidation. Moerck and Ball (1974) stated that with each additional double bond in a fatty acid the oxidation increases by a factor of two. For example, the relative rates of oxidation for arachidonic (20:4), linolenic (18:3), linoleic (18:2), and oleic (18:1) are 40:20:10:1, respectively (Nawar, 1996). The *cis* isomers are more subjective to oxidation than *trans* isomers (Nawar, 1996). Also, the nonconjugated double bonds are less reactive than the conjugated bonds (Nawar, 1996). Since high concentrations of fatty acids are present, the rate of oxidation will be accelerated.

Processing

Processing methods can also create effects that can change the composition of muscle. Ground turkey breast, stored for 7 days at 3°C, increased considerably in TBA values (Dawson and Schierholz, 1976). The increase in lipid oxidation is related to the length of storage and the incorporation of oxygen due to grinding. In cases of low oxygen concentration, such as packaging, the rate of oxidation is proportional to the level of oxygen concentration. Restructured meats are partially or completely disassembled products which are reformed in the same or different form. This restructuring can cause color instability and lipid oxidation, partially due to exposure to oxygen (Akamittath et al., 1990).

Temperature

Yamauchi (1972) found that cooking meat at a temperature of 70°C for 1 hr caused rancidity. However, heating to 80°C and above resulted in a decline in TBA values, and to 120°C resulted in no oxidation in beef, pork, and mutton after 4 days of storage at 4°C. The decline in TBA values with an increase in endpoint temperature is due to the oxidative degradation of myoglobin, which interacts with peroxides from polyunsaturated fatty acids present to produce substances with antioxidant activity (Faustman, 1994). These substances are maltol and reductic acid which are produced from Maillard browning. These peroxides can also break down and react with proteins, vitamins, and pigments (Gray, 1978; Karel, 1973). Maillard browning has many beneficial qualities such as producing a meaty flavor and decreasing the rate of oxidation. Further studies demonstrated that sensory and TBA values coincided with the use of retorting to decrease lipid oxidation (Einerson and Reineccius, 1977).

Moisture Content

The loss of moisture in can be detrimental to the sensory characteristics of a meat product. However, the reduction in moisture content can also be beneficial by retarding microbial spoilage (Lundquist, 1987). Furthermore, the range of moisture that occurs in food can alter the level of oxidation.

As the level of moisture increases, the sample composition moves from low, medium moisture, and high moisture (Fennema, 1996). The low level is the least mobile and the most strongly sorbed, and associates by water-ion or water-dipole interactions on accessible polar sites. The medium moisture level associates with water molecules and solute molecules by hydrogen bonding. The high moisture level is also known as the "bulk-phase water." This particular level of moisture has a decrease in viscosity, increase in molecular mobility, and has the ability to freeze (Fennema, 1996).

Karel and Yong (1981) found that the level of moisture has an affect on the rate of oxidation. They stated that the low level of moisture added to a dry sample binds to the hydroperoxides. The binding then causes an interference with the decomposition caused by the hydroperoxides and delays the onset of oxidation. Likewise, the water also hydrates metal ions and reduces their effectiveness. Water sorbed beyond the low level contributes to the increase in the rate of oxidation. The level of oxygen solubility is increased and the macromolecules have the ability to swell which exposes more catalytic sites. Although at even higher levels of moisture content, Karel and Yong (1981) found that the addition of water could decelerate the rate of oxidation. Karel and Yong (1981) indicated that the potency of the catalysts was reduced by dilution. The level of salt and freeze concentration can also cause an increase in the rate of oxidation by the decrease in water-holding capacity (Labuza, 1971).

pH

The binding properties of meat can be directly or indirectly related to the muscle pH (Dutson, 1983). The pH has a significant effect on the water-holding capacity of the muscle, likewise, the water-holding capacity of meat has an impact on the protein binding of meat (Honikel et al., 1981). For example, the use of polyphosphates to increase the binding of meat is due to the improvement of water-holding capacity by the increase in pH (Hamm, 1970). An elevation in pH causes an increase in the amount of salt-soluble protein that can be extracted which improves binding (Schmidt, 1987). The maximum gel strength of salt-soluble myosin and actomyosin is at a pH of 6.0 and weakens as the pH lowers (Yasui et al., 1980).

The increase in protein solubility at higher levels of pH is due to the isoelectric point. The isoelectric point of a protein occurs when the pH of the protein has a net charge of zero (Claus et al., 1994). Minimum solubility occurs at the isoelectric point. Wismer-Pedersen (1987) stated that water added to meat at a pH of 4.5 to 7.0 would show a pH of 5.0 to 5.1 after centrifugation. The resulting pH is a weighted average of the two major myofibrillar proteins, actin (4.7) and myosin (5.4). The charges, on the side groups of the amino acids, below and above the isoelectric point carry a positive or negative charge, respectively (Damodaran, 1996). These charges cause electrostatic repulsion and hydration of charged residues which cause an increase in the solubility of the protein (Damodaran, 1996).

Studies using prerigor, ground pork at a higher pH were less susceptible to lipid oxidation than postrigor pork at a lower pH (Judge and Aberle, 1980; Tay et al., 1983). Yasosky (1984) found that a pH value of 6.10 or higher was needed to attain maximum inhibition of oxidation. At a low pH, the heme protein is exposed to the external

environment due to the opening of the globin tertiary structure (Yin and Faustman, 1993), thus, creating a decrease in the stability of heme protein which enhances oxidation. Also, the low level of pH accelerates the protonation of bound oxygen and favors the release of superoxide anions (Livingston and Brown, 1981).

Metals

The scission of hydroperoxides and the further oxidation to secondary products are favored by pro-oxidant metals (Dugan, 1987). Pro-oxidant metals, also known as transition metals, such as cobalt, copper, iron, manganese, and nickel can also accelerate autoxidation by becoming an initiator for the reaction. The prooxidative activity of some metals are as follows: $Fe^{2+}>Cu^{2+}>Co^{2+}>Mg^{2+}$ (Tichivagana and Morrissey, 1985). An excessive amount of copper and iron are contained in phospholipids and bound to acidic groups (Pokorny, 1987).

Transition metals can initiate the oxidation of lipids by producing free radicals from single electron transfer or abstraction of hydrogen (Nawar, 1996). This produces superoxide radicals from reactions with triplet oxygen which lead to the formation of hydroperoxides. The metal oxidation state is also raised from interactions with oxygen or peroxides (Kanner et al., 1987). The concentration level of the highly active metals can be so low that it may only require one part in 10^6-10^8 to affect autoxidation (Pokorny, 1987). These metals can be from the soil, plants, animal, or from metallic equipment used for processing.

Antioxidants

Antioxidants can also play a role in the rate of oxidation. Many natural and synthetic antioxidants can inhibit the propagation step of autoxidation. The antioxidants form inert products by reacting with the free radicals in one of the termination steps

(Dugan, 1987). A few examples of synthetic antioxidants used in food are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), tert-butyl hydroquinone (TBHQ), ethoxyquin, ascorbic acid, and erythorbic acid (Nawar, 1996). Some natural antioxidants are vitamin E, selenium, β-carotene, and rosemary. Some chelators that are used include sodium tripolyphosphate and EDTA (Nawar, 1996).

The alpha-tocopherol (vitamin E) content of muscle foods during storage may also influence the rate of lipid oxidation and subsequent changes in fatty acid composition (Yamauchi et al., 1980). Dietary vitamin E is absorbed and stored in the liver and adipose tissue, which is contradictory to most synthetic antioxidants (Buckley and Connolly, 1980). Animals cannot produce vitamin E. A study by Buckley and Connolly (1980) revealed that porcine muscle containing the highest level of alpha-tocopherol per gram of fatty acids, with three or more double bonds, had the lowest TBA numbers after cooking and 3 days of storage.

Another supplement that has been used is selenium. Vitamin E and selenium interact to prevent muscular dystrophy, exudative diathesis, and other forms of muscular degeneration (Larbier and Leclercq, 1994). The relationship of vitamin E and selenium is essential for the protection of the cells from oxidation. It was discovered (Rotruck et al, 1973) that selenium is an essential component of the enzyme glutathione peroxidase (GSH-Px). These antioxidant enzymes, found inside the cell, can reduce the amount of superoxide anions, lipid peroxides, and hydrogen peroxides. For example, GSH-Px reduces hydrogen peroxide to water within the cell. These enzymes can also interact with transition metals which can reduce the rate of oxidation (Halliwell and Gutteridge, 1990). DeVore et al. (1983) discovered that selenium-supplemented (0.25 ppm) uncooked red chicken muscles showed a fourfold increase in the activity of GSH-Px after storage for 4

days at 4°C. However, Lee et al. (1996) found that GSH-Px at higher concentrations, such as beef, would be more beneficial in the deterring the development of warmed-over flavor (WOF).

2.1.3 Heme-lipid Interaction

Interaction of unsaturated fatty acids and iron endogenous to muscle was found to accelerate the development of warmed-over flavor. Another reason for the high oxidation rate may be due to the significant amounts of free catalytic iron (Kanner et al., 1988). These free catalytic iron concentrations are heme cataylsts such as hemoglobin, myoglobin, and cytochromes. The removal of heme pigments due to the addition of 156 mg/kg of nitrite inhibited the oxidation in cooked meat (Igene et al., 1979). This technique demonstrated that heme pigments are involved in the initiation of lipid oxidation in meat.

Heme and non-heme iron can affect WOF due to catalytic reactions. Researchers have found that the main cause for rapid oxidation in meat was due to non-heme iron rather than heme iron (Sato and Hegarty, 1971; Love and Pearson, 1974). The oxidation of heme and non-heme iron can decrease the quality in meat. Fresh meat in the oxidized ferric state, also known at metmyoglobin, is brown in color. The hydroperoxides then react with metmyoglobin to produce ferryl-myoglobin (Fe^{4+}), which then produces more free radicals (Kanner and Harel, 1985). Not only is the brown color of the meat undesirable, but the oxidized state gives a higher catalytic reaction for further oxidation.

The cooking process breaks down the heme producing free iron. The free iron catalyzes autoxidation by accelerating the propagation step of the free-radical chain mechanism (Igene et al., 1979b). It was also observed that slow heating released more

nonheme iron (Chen et al., 1984). The increase of the nonheme iron from slow cooking is caused by the release of heme iron from the polyphyrin rings. The use of rapid heating could have caused the myoglobin molecules to coagulate in such a way that the heme iron was unable to release from the polyphyrin rings. The highest amount of released nonheme iron is found at a temperature of approximately 63 to 70°C (Pearson et al., 1983). Chen et al. (1984) reported an increase in the release of iron of 5.3% for fast heated extract and 71.8% for slow heated extract in comparison to the unheated extract. The final temperature for both the fast and slow extracts was set at 70°C. The fast extract was cooked for 50 sec and the slow extract was cooked for 8 min and 50 sec. Schricker and Miller (1983) also found that microwave heating resulted in a lower amount of nonheme iron than braising and roasting.

2.1.4 Warmed-over flavor

Warmed-Over Flavor (WOF) is defined as the rapid development of oxidized flavor in refrigerated uncured, cooked meats, in which a "rancid" or "stale" flavor becomes apparent within 48 hr when stored at 4°C (Tims and Watts, 1958). WOF differs from the usual rancidity found in raw meats, fatty tissues, or rendered fat. Raw products can have a delayed onset of oxidative rancidity detection until a storage time for weeks or months.

WOF has also been detected in cooked meat that has been ground and exposed to air (Sato and Hegarty, 1971), mechanically separated, and restructured meats (Love, 1987). For example, an increase in TBA values in beef, pork, lamb, and turkey meat were found immediately after grinding and throughout storage at 5°C (Keskinel et al., 1964). It is known that additional storage time causes an increase in oxidation. Many

factors affecting the level of WOF are very similar to lipid oxidation factors such as temperature, storage time, species, and iron content. The same pathways are also involved (St.Angelo and Bailey, 1987). One pathway consists of the reaction, by Strecker degradation, of carbonyl compounds with the amino groups of cysteine and ammonia. A second pathway consists of the reaction of an amino group in phosphatidyl ethanolamine with sugar-derived carbonyl compounds. The interaction of free radicals from oxidized lipids in the Maillard reaction can be a third pathway.

Turkey is the most susceptible to WOF, followed by chicken, pork, beef, and mutton, respectively (Wilson et al., 1976). Wilson et al. (1976) found a higher correlation of phospholipids and TBA values than between total lipid values and TBA values. The decomposition of the hydroperoxides creates the distinctive odors and flavor characteristics associated with WOF. Even after cooking, an off odor present before cooking will still remain (Mottram, 1987). These preexisting off-odors may be masked, by higher intensity odors, in the cooked product but not removed.

2.2 Phosphates

The composition and level of phosphate play a major role in the decrease of lipid oxidation (Miller et al., 1986). Phosphates have the ability to chelate free metals (Tims and Watts, 1958), increase pH (Miller et al., 1986), reduce phosphatase activity (McComb et al., 1979), and increase water-holding capacity (Claus et al., 1994). Salt facilitates the disassociation of actomyosin, solubilizes myosin, and improves water-holding capacity (Claus et al., 1994). These reactions are beneficial to product quality through the increase in moisture content, reduction of lipid oxidation, and improved color

(Townsend and Olson, 1987). However, a regulation by the USDA only allows 0.5% phosphate to be added in processed meats.

Two groups of phosphates used commercially are acidic and alkaline phosphates (Townsend and Olson, 1987). Examples of acidic phosphates used are sodium hexametaphosphate, sodium acid pyrophosphate, and monosodium phosphate. Sodium tripolyphosphate, sodium pyrophosphate, and disodium phosphate are alkaline phosphates. Alkaline phosphates are primarily used to increase the moisture content by raising the pH. Sodium tripolyphosphate (STP) is the most common of the alkaline phosphates (Lindsay, 1997). Lindsay (1997) also found that STP could be combined with sodium hexametaphosphate for an increase in calcium tolerance due to the occurrence of precipitate with ortho- and pyro-phosphates.

Phosphates can differ in structure and composition (McComb et al., 1979). These differences are classified as ortho-, pyro-, and meta-phosphates. Orthophosphoric acid has phosphorus in the center of a tetrahedron. The phosphorus is bound to four oxygen molecules, with one bond being a double bond. Two orthophosphoric acids can be bound, with the elimination of water, to create pyrophosphoric acid. Two or more phosphorus compounds are also known as polyphosphates (Sofos, 1986). These polyphosphates can be manufactured by high temperature heating (Sofos, 1986). Metaphosphates are composed of chains or rings of three or more phosphorus atoms. Phosphates can also react with other compounds and enzymes.

The best sequestering agents are long-chain polyphosphates (Sofos, 1986). When phosphates sequester metal ions, the metals are still present in the food but are incapable of participating with any reactions (Dziezak, 1990). The polyphosphates, such as sodium hexametaphosphate, best sequester calcium and magnesium (Ellinger, 1972). Although,

sodium tripolyphosphate can be used to sequester many ions, this phosphate can effectively sequester heavy metals, such as copper and iron. However, the sequestering efficiency of short-chain polyphsophates can decrease as pH increases (Ellinger, 1972).

2.3 Phosphatases

Phosphatases consist of two types of enzymes, acidic and alkaline, which are dependent on the optimum pH for rate of activity (McComb et al., 1979). The activity of these enzymes is also affected by such conditions as phosphate concentration, enzyme concentration, temperature of incubation, and ionic strength (McComb et al., 1979). Alkaline and acidic phosphatases are "nonspecific" meaning that their primary specificity is for the monophosphate and their secondary specificity is for the alcohol moiety of the substrate (McComb et al., 1979). The function of the phosphatase enzyme is to hydrolyze phosphate esters which can be located in all tissues of an organism. The resulting effect from these phosphatases can cause changes in the food stability (Takata et al., 1993). Therefore, the inhibition of these phosphatases is a concern for food processing and preservation.

Many compounds of phosphorus are substrates for these phosphatases which cleave the O-P linkage (Sofos, 1986). However, phosphatases have a high affinity for inorganic phosphates, orthophosphates, which bind competitively with phosphate esters and reduce the activity of the phosphatase (McComb et al., 1979). Barman and Gutfreund (1966) found that the covalent bond between the enzyme and phosphate is formed at the active site. This was determined by the loss of enzymatic activity which paralleled with the loss in the ability to bind phosphate during denaturation experiments with bovine milk alkaline phosphatase. However, it has been shown that the amount of

binding capability may vary from phosphatase to another. One to two moles of phosphorus can already be contained per mole of enzyme which can limit the amount of binding sites (Bloch and Schlesinger, 1973). Chlebowski and Coleman (1976) discovered that several hours were required in order for the complete exchange of phosphatases with exogenous phosphate. The reason for this delay is due to the difficulty encountered in displacing the phosphate presently bound at the active site.

Acid phosphatases have an optimum activity at a pH of 4.5-5.5 and at a temperature of 35°C. Two factors of concern with this enzyme are a higher thermal stability than most enzymes and a higher activity at a pH of 5, which is an average pH of most foods. Andrews (1974) reported that acid phosphatase survived normal pasteurization and that heating at a pH of 6-7 for nearly 5 sec at 100°C or 20 sec at 90°C was needed in order to inactivate 90% of the enzyme. Although, the residual phosphatase activity does not completely depend on the core temperature itself, but depends on the integral heat treatment of the core (Koermendy et al., 1991). The composition, such as NaCl, polyphosphate and protein content, and pH, may also influence the inactivation of the enzyme (Koermendy et al., 1991).

Acid phosphatase is a lysosomal enzyme (Whitaker, 1972). However, the lysosome cannot be intact for the activity of the phosphatase to be detected (Kas et al., 1983). Furthermore, muscles contain a low amount of lysosomes, which are found in the muscle fibers of the sarcoplasm. In a study conducted by Kas et al. (1983), the maximum activity of acid phosphatase was obtained between the second and fourth day postmortem in raw beef sirloin stored at 4°C when the majority of lysosomes had leaky membranes. At 10 days postmortem, a decrease in activity was noticed (Kas et al., 1983). The change in activity was similar in beef and pork (Kas et al., 1983). The activity can be confined in

the space among the muscle fibers 2 hr postmortem, although it can be observed inside the muscle fibers with time (Kas et al., 1983). In 1965, Lind discovered that there was no significant difference between acid phosphatase activity of pork samples stored for one month at 4°C and without storage. The samples were immediately minced, minced and stored 5 days at 4°C, or canned and heat processed for analysis. Townsend (1989) observed a greater loss in residual phosphatase activity during the first 15 months of storage in comparison to the remaining 21 months of storage of heated cured/canned picnics stored at -34°C. The study also showed a greater loss of activity in samples that were heat processed to 61°C and 62.7°C, which contained the highest residual phosphatase activity, in comparison to temperatures of 65.5°C, 68.3°C and 70.9°C.

In a study conducted by Davis and Townsend (1994), the acid phosphatase activity was measured in broiler breast, turkey breast, and dark turkey meat. Results indicated that freezing prior to cooking did not lower the acid phosphatase activity. However, the increase in endpoint temperature did lower the acid phosphatase activity. Also, the dark turkey muscle was lower in acid phosphatase activity in comparison with the turkey and broiler breast. However, the broiler breast decreased in activity at a greater rate than the turkey breast. Mg^{2+} can activate some acid phosphatases (McComb et al., 1979). Inhibition of acid phosphatase can be accomplished by orthophosphate but not by metal chelating agents (Sofos, 1986).

Alkaline phosphatase is a non-heme metalloprotein usually containing Zn^{2+} with high concentrations in the lactating mammary gland and the kidney (McComb et al., 1979). The activity of alkaline phosphatase is enhanced as the pH of the medium is increased to 9-10. The greatest impact from this enzyme is in the dairy industry. It is used as an indicator for the effectiveness of HTST pasteurization. The activity can be

lost during the heat treatment but partially recovered during prolonged storage at lower temperatures (Whitaker, 1972). For example, alkaline phosphatase activity in milk dramatically decreases at about 72°C, whereas acid phosphatases have to be heated to above 90°C (Andrews, 1974). In contrast, the acid phosphatase activity in beef decreases between 63° and 71°C, however alkaline phosphatase activity does not change within this range (Personal communication, Smith 1996, Michigan State University). The reactivity of alkaline phosphatase, which can be detected after 6 hr of pasteurization, is influenced by the presence of Mg^{2+} and Zn^{2+} (Fox, 1991).

2.4 Encapsulation

The use of encapsulation with moisture-sensitive ingredients has proven to be successful in the food industry. Food grade acids used in the industry are commonly coated with hydrogenated vegetable oils. These acids can accelerate food component deterioration, such as starch hydrolysis, color degradation, and flavor loss, before heating (Gunstone and Padley, 1997). The coating can aid in the reduction of the acid's hygroscopicity, reduce dusting, and improve the flow by the minimization of clumping (Werner, 1980).

Encapsulated ingredients are used in all aspects of the food industry. For instance, Cordray and Huffman (1985) studied uses for encapsulation with glucono-δ-lactone, and lactic acid in restructured pork. Also, encapsulated ascorbic acid has many applications, particularly as a dough conditioner in order to maintain a barrier from water and oxygen (Pszczola, 1998). Sweeteners are also a popular ingredient for encapsulation to extend the perception of sweetness (Pszczola, 1998). Flavorings and vitamins are encapsulated in fat for protection. For example, nucleoside-5'-phosphate is used for a

flavoring in several foods and beverages (Gunstone and Padley, 1997). This flavoring is susceptible to the decomposition by phosphatases. The use of a high melt fat to encapsulate the flavoring in instant soups can deter decomposition until the phosphatases have become inactivated or until the product is heated and consumed (Fujita et al., 1973).

2.5 References

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513-1517.

Allen, C.E., and Foegeding, E.A. 1981. Some lipid characteristics and interactions in muscle food. A review. Food Technol. 35: 253-257.

Andrews, A.T. 1974. Bovine milk acid phosphatase. J. Dairy Res. 41:229-237.

Barman, T.E. and Gutfreund, H. 1966. The catalytic-centre activity and kinetic Properties of bovine milk alkaline phosphatase. Biochem. J. 101:460-466.

Bloch, W. and Schlesinger, M.J. 1973. The phosphate content of *Escherichia coli* alkaline phosphatase and its effect on stopped flow kinetic studies. J. Biol. Chem. 248:5794-5805.

Buckley, J. and Conolly, J.F. 1980. Influence of alpha-tocopherol (vitamin E) on storage stability of raw pork and bacon. J. Food Proc. 43: 265-267.

Chan, H.W.S. 1987. The mechanism of autoxidation. Ch.1 in Autoxidation of Unsaturated Lipids, Chapter 1. Academic Press, N.Y. 1-17.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H., and Ku, P.K. 1984. Some factors influencing the nonheme content of meat and its implications in oxidation. J. Food Sci. 49:581-584.

Chlebowski, J.F. and Coleman, J.E. 1976. Metallophosphoryl and apophosphoryl alkaline phosphatases. J. Biol. Chem. 251:1202-1206.

Claus, J.R., Colby, J.W., and Flick, G.J. 1994. Processed meats/poultry/seafood. Ch.5 in Muscle Foods: Meat, Poultry, and Seafood Technology, D.M. Kinsman, A.W. Kotula, and B.C. Breidenstein (Eds.). Chapman & Hall, New York. p.106-162.

Claus, J.R. and Marriott, N.G. 1995. Influence of vitamin E on improving meat colour and lipid stability. Meat Focus Inter. 4(Part 3):109-112.

Cordray J.C. and Huffman, D.L. 1985. Restructured pork from hot processed sow meat: effect of encapsulated food acids. J. of Food Prot. 48:965-968.

Damodaran, S. 1996. Amino acids, peptides, and proteins. Ch. 6 in Food Chemistry, 3rd ed., O.R. Fennema (ed). Marcel Dekker, Inc., N.Y. 17-94.

Davis, C.E. and Townsend, W.E. 1994. Rapid fluorometric analysis of acid phosphatase activity in cooked poultry meat. J. Food Prot. 57(12): 1094-1097.

Dawson, L.E. and Schierholz, K. 1976. Influence of grinding, cooking, and refrigerated storage on lipid stability in turkey. Poult. Sci. 55: 618-622.

Dawson, L.E., Stevenson, K.E., and Gertonson, E. 1975. Flavor, bacterial, and TBA changes in ground turkey patties treated with antioxidants. Poult. Sci. 54:1134-1139.

DeVore, V.R., Jensen, L.S., and Greene, B.E. 1983. Thiobarbituric acid values and

glutathione peroxidase activity in meat from chickens fed a selenium-supplemented diet. J. Food Sci. 48:300-302.
Dugan, L.R., Jr. 1987. Fats. Ch. 3 (part 2) in The Science of Meat and Meat Products, 3rd ed., J.F. Price and B.S. Schweigert. Food & Nutrition Press, Inc., Westport, CT. p. 103-114.
Dutson, T.R. 1983. The measurement of pH in muscle and its importance to meat quality. Proc. Recip. Meat Conf. Meetings, Chicago, IL. 36:92-97.
Dziezak, J.D. 1990. Phosphate improve many foods. Food Technol. 44(4):80-92.
Einerson, M.A. and Reineccius, G.A. 1977. Inhibition of warmed-over flavor in retorted turkey by antioxidants formed during processing. J. Food Proc. and Preserv. 1:279-291.
Ellinger, R.H. 1972. Some general chemical characteristics of phosphates. Ch.3 in "Phosphates as Food Ingredients" CRC Press, Boca Raton, FL.
Faustman, C. 1994. Postmortem changes in muscle foods. Ch.3 in Muscle Foods: Meat, Poultry, and Seafood Technology, D.M. Kinsman, A.W. Kotula, and B.C. Breidenstein (Eds.). Chapman & Hall, NY.
Fennema, O.R. 1996. Water and Ice. Ch. 2 in Food Chemistry, 3rd ed., O.R. Fennema (ed). Marcel Dekker, Inc., N.Y. 17-94.
Fujita, E., Ishii, K., Nakatani, H., Nakai, Y., and Katagini, K. 1973. U.S. Patent 3,389,000, cited in Fat Encapsulation, Edible Coatings, and Soluble Packaging (R. Daniel). Noyes Data Corporation, Park Ridge, NJ. 306-308.
Gray, J.I. 1978. Measurement of lipid oxidation: A review. J. Am. Oil Chem. Soc. 55:539-546.
Gunstone, F.D. and Padley, F.B. 1997. Lipid technologies and applications. Marcel Dekker, Inc., NY. 470-471.
Halliwell, B. and Gutteridge, J.M. 1990. Role of free radicals and catalytic metal ions in human disease: and overview. Meth. Enzymol. 186:1-85.
Hamm, R. 1970. Interactions between phosphates and meat proteins. Symposium: Phosphates in Food Processing. J.M. deMann and P. Meinychyn (Eds.). AVI Publishers, Wesport, CT.
Honikel, K.O., Fisher, C., Hamid, A., and Hamm, R. 1981. Influence of postmortem changes in bovine muscle on the water-holding capacity of beef. J. Food Sci. 46:1-6.
Hornstein, I. And Wasserman, A. 1987. Chemistry of meat flavor. Ch. 8, part 2 in The Sci. of Meat and Meat Prod., 3rd ed., J.F. Price and B.S. Schweigert (Ed.), p. 329-348. Food & Nutrition Press, Inc., Westport, CT.
Igene, J.O., and Pearson, A.M. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. J. Food Sci. 44:1285-1290.
Igene, J.O., King, J.A., Pearson, A.M., and Gray, J.I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27:838-842.
Judge, M.D. and Aberle, E.D. 1980. Effect of prerigor processing on the oxidative rancidity of ground light and dark porcine muscles. J. Food Sci. 45:1736-1741.
Kanner, J., and Harel, S. 1985. Lipid peroxidation and oxidation of several compounds by H_2O_2 –activated metmyoglobin. Lipid. 20:625-628.
Kanner, J., German, J.B., and Kinsella, J.E. 1987. Initiation of lipid peroxidation in biological systems. CRC Crit. Rev. Food Sci. Nutri. 25:317-346.

Kanner, J., Hazan, B., and Doll, L. 1988. Catalytic free iron ions in muscle food. J. Agric Food Chem. 36:412-415.
Karel, M. 1973. Symposium: Protein interactions in biosystems. Protein lipid interactions. J. Food Sci. 38:756-763.
Karel, M. and Yong, S. 1981. Autoxidation-initiated reactions in food. Water Activity: Influences on Food Quality, L.B. Rockland and G.F. Stewart (Eds.). Academic Press, New York. P.511-529.
Kas, J., Vana, V., Kopecny, J., and Rauch, P. 1983. Stability of lyosomes in beef and pork muscle post mortem. Zeitschrift fuer Lebensmittel Untersuchung und Forschung 177(6): 461-467.
Keskinel, A., Ayres, J.C., and Synder, H.E. 1964. Determination of oxidative changes in raw meats by the 2-thiobarbituric acid reactive substances method. Food Tech. 18:101-104.
Koermendy, L., Zsarnoczay, G., and Mihalyi, V. 1992. A new, modified acid phosphatase assay for determining the extent of heat treatment in canned hams. Food Chem. 44:367-375.
Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. CRC Crit. Rev. Food Tech. 2:355-405.
Larbier, M. and Leclercq, B. 1994. Nutrition and feeding of poultry, Wiseman, J. (ed). Nottingham University Press, France.
Lee, S.K., Mei, L., and Decker, E.A. 1996. Lipid oxidation in cooked turkey as affected by added antioxidant enzymes. J. Food Sci. 61:726-728.
Lillard, D.A. 1987. Oxidative deterioration in meat, poultry, and fish. Warmed-Over Flavor of Meat, St. Angelo, A.J. and Bailey, M.E. (ed.). Academic Press Inc., NY. 41-67.
Lind, J. 1965. Report of research on determination of the activity of acid phosphatase in canned hams. Danish Meat Products Laboratory. The Royal Veterinary and Agricultural College Denmark. Sept. 23, 1965.
Lindsay, R.C. 1996. Food Additives. Ch. 12 in Food Chemistry, 3rd ed., O.R. Fennema (ed). Marcel Dekker, Inc., N.Y. 17-94.
Livingston, D.J. and Brown, W.D. 1981. The chemistry of myoglobin and its reaction. Food Technol. 35:244-249.
Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and nonheme iron as prooxidants in cooked meat. J. Agric. Food Chem. 22: 1032-1034.
Lundquist, B.R. 1987. Protective packaging of meat and meat products. Ch.14 in Sci. of Meat and Meat Prod. 3rd ed., J.F. Price and B.S. Schweigert (Ed.), p. 487-506. Food & Nutrition Press, Inc., Westport, CT.
McComb, R.B., Bowers, G.N., Jr., and Posen, S. 1979. Alkaline phosphatase. Plenum Press, NY.
Melton, S.L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technol. 37(7):105-111.
Miller, M.F., David, G.W., Seidemann, S.C., Ramsey, C.B., and Rolan, T.L. 1986. Effects of various phosphates on the palatability, appearance, and storage traits of flaked and formed restructured beef steaks. J. Food Sci. 51:1435-1438.
Moerck, K.E., and Ball, H.R. 1974. Lipid oxidation in mechanically deboned chicken meat. J. Food Sci. 39:876-879.
Morrissey, P.A., Buckley, D.J., Sheehy, P.J.A., and Monahan, F.J. 1994. Vitamin E and meat quality. Proc. Nut. Soc. Sept. 8-10. Belfast, Ireland. 53:289-295.

Mottram, D.S. 1987. Lipid oxidation and flavour in meat and meat products. Food Sci. Technol. Today. 1:159-163.
Nawar, W.N. 1996. Lipids. Ch. 5 in Food Chemistry, 3rd ed., O.R. Fennema (ed). Marcel Dekker, Inc., NY. 225-319.
Pearson, A.M. and Gray, J.I. 1983. Mechanism responsible for warmed-over flavor in cooked meat. In "Maillard reaction in foods and nutrition," ed. G.R.Waller and M.S. Feather, Sympos. Series. 215, p. 287. Am. Chem. Soc., Washington DC.
Pokorny, J. 1987. Autoxidation of Unsaturated Lipids, Chapter 5. Academic Press, NY. 141-207.
Pszczola, D.E. 1998. Encapsulated ingredients: providing the right fit. Food Technol. 52(12): 70-77.
Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hokestra, W.G. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Sci. 179:588-590.
Sato, K. and Hegarty, G.R. 1971. Warmed over flavor in cooked meats. J. Food Sci. 38:398-403.
Schmidt, G.R. 1987. Functional behavior of meat components in processing. Ch.14 in The Science of Meat and Meat Products 3rd ed., J.F. Price and B.S. Schweigert (Ed.), p. 487-506. Food & Nutrition Press, Inc., Westport, CT.
Schricker, B.R., and Miller, D.D. 1983. Effects of cooking and chemical treatment on heme and nonheme iron in meat J. Food Sci. 48:1340.
Smith, D.M. 1996. Personal communication. Michigan State University, E. Lansing.
Sofos, J.N. 1986. Use of phosphates in low-sodium meat products. Food Technol. 40(9):52-69.
St. Angelo, A.J. and Bailey, M.E. (Ed.). 1987. Warmed-over flavor of meat. Academic Press Inc., NY.
Takata, R., Yoshimoto, M., Miyamoto, T., Sakamoto, J., Soeda, T., and Hatano, S. 1993. Inhibitory monoclonal antibody against Japanese radish acid phosphatase. Biosci. Biotech. Biochem. 57(11): 1924-1928.
Tay, H.C., Aberle, E.D., and Judge, M.D. 1983. Iron catalyzed oxidative rancidity in prerigor ground pork. J. Food Sci. 48:1328-1331.
Tichivangana, J.Z., and Morrissey, P.A. 1985. Metmyoglobin and inorganic metals as pro-oxidants in raw and cooked muscle systems. Meat Sci. 15:107-116.
Tims, M.J., and Watts, B.M. 1958. Protection of cooked meats with phosphate. Food Technol. 12:240-243.
Townsend, W.E. 1989. Stability of residual acid phosphatse activity in cured/canned picnic samples stored at -34°C for 15 and 36 months. J. Food Sci. 54(3):52-53.
Townsend, W.E. and Olson, D.G. 1987. Cured meats and cured meat products processing. Ch. 3 (part 2) in The Sci. of Meat and Meat Prod., 3rd ed., J.F. Price and B.S. Schweigert. P.103-114. Food & Nutrition Press, Inc., Westport, CT.
Werner, L.E. 1980. Encapsulated food acids. Cereal Foods World. 25:102-108.
Whitaker, J.R. 1972. Principles of enzymology for the food sciences. Marcel Dekker, Inc., New York.
Wilson, B.R., Pearson, A.M., and Shorland, F.B. 1976. Effect of total lipids and phospholipids on warmed-over flavor in red and white muscles from several species as measured by thiobarbituric acid analysis. J. Agric. Food Chem. 24:7-11.
Wismer-Pedersen, J. 1987. Water. Ch. 3 (part 5) in The Science of Meat and Meat

Products, 3rd ed., J.F. Price and B.S. Schweigert. Food & Nutrition Press, Inc., Westport, CT. p. 103-114.
Yamauchi, K. 1972. Effect of heat treatment on the development of oxidative rancidity in meat and its isolated tissue fractions. Bull. Fac. Agric., Miyazaki Univ. 19: 147-154.
Yamauchi, K., Nagai, Y., and Ohashi, T. 1980. Quantitative relationship between alpha tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in porcine skeletal muscle. Agric. Biol. Chem. 44:1061-1067.
Yasosky, J.J., Aberle, E.D., Peng, I.C., Mills, E.W., and Judge, M.D. 1984. Effects of pH and time of grinding on lipid oxidation of fresh ground pork. J. Food Sci. 49:1510-1512.
Yasui, T., Ishioroshi, M., and Samejima, K. 1980. Heat-induced gelation of myosin in the presence of actin. J. Food Biochem. 4:61-78.
Yin, M.C. and Faustman, C. 1993. Influence of temperature, pH, and phospholipid composition upon the stability of myoglobin and phospholipid: liposome model. J. Agric. Food Chem. 41:853-857.

Chapter 3

Antioxidative Effects of Encapsulated Sodium Acid Pyrophosphate (SAPP) and Encapsulated Sodium Tripolyphosphate (STP) in Ground Beef Patties Cooked Immediately after Antioxidant Incorporation

3.1 Abstract

Cooked, ground beef patties with 1% NaCl were incorporated with 0.5% unencapsulated sodium tripolyphosphate (STP), 0.5% encapsulated sodium tripolyphosphate (STP), 0.5% unencapsulated sodium acid pyrophosphate (SAPP), and 0.5% encapsulated sodium acid polyphosphate (SAPP) were stored for 0 and 6 days at 3°C. The pH was higher and lower than the control for the STP and SAPP samples, respectively. Overall, the samples with unencapsulated STP had the least amount of cooking loss and lowest TBARS values. TBARS for the all phosphate treatments were lower ($P<0.05$) than for the control samples. Therefore, the use of phosphates as an antioxidant can aid in the reduction of oxidation. Although, a longer period of time before thermal processing may be necessary for the encapsulated phosphate to have significant benefits.

3.2 Introduction

A means of reducing oxidation in meat products has been investigated for years. Attributes, such as flavor, odor, and appearance, are important traits to the success of a processed food. Several types of ingredients have been used in order to aid the cessation of oxidation. Examples of ingredients with anti-oxidative properties include nitrites, phosphates, ascorbic acid, BHA, BHT, propylgallate, vitamin E, and chelators (Claus et al., 1994; Dugan, 1987).

Phosphates are commonly used in the meat, poultry, and seafood industry (Dziezak, 1990). Not only do phosphates aid in the extension of shelf stability but they also improve water-holding capacity (Claus et al., 1994). It was also stated that products containing phosphates retain more natural juices and added water during heat processing and subsequent reheating. Furthermore, the combination of phosphates and salt create a synergistic effect on extracting myofibrillar proteins which aid in the retention of water (Claus et al., 1994). Some examples of incorporated phosphates are sodium tripolyphosphate, sodium hexametaphosphate, sodium pyrophosphate, disodium phosphate, sodium acid pyrophosphate, and monosodium phosphate (Townsend and Olson, 1987). Incorporated either as a single phosphate or in blends, sodium tripolyphosphate (STP) is the most predominate in the industry, accounting for 80% of the phosphates added (Barbut, 1988). Due to a restricted level of 0.5% added phosphate in a product by the USDA, the amount available can be minimal due to the breakdown by phosphatases prior to heating.

In general, the primary function of a phosphatase is to hydrolyze phosphate esters, which can be located in all tissues of an organism (McComb, 1979). Phosphatases play a key role in affecting the functionality of the phosphates due to hydrolysis of polyphosphates before thermal processing (Sutton, 1973). However, not all phosphatases are inactivated by normal meat cookery temperatures (McComb, 1979).

Encapsulation technology has been used to control the release of numerous food grade ingredients. However, phosphates have not been encapsulated for the purpose of optimizing the ability to control oxidation. Encapsulated phosphates, if effective, could have numerous applications in red meat and poultry products. Seafood and dairy products including fluid milk may also benefit. The hypothesis is that by

encapsulating the phosphates, the encapsulate will protect the phosphate from degradation by the phosphatases active in uncooked meat. The objectives of this research were to evaluate the effects of incorporated unencapsulated and encapsulated, SAPP and STP, in ground beef on pH, cooking loss, and TBARS at different storage times.

3.3 Materials and Methods

3.3.1 Formulation and Processing

Beef *semimembranous* muscles were obtained from the Virginia Polytechnic Institute and State University Meat Laboratory. The *semimembranous* muscle (SM) was removed 24 hr postmortem from three different ungraded steer carcasses stored at 4°C. The SM was trimmed of external fat and sliced into strips 2.54 cm thick. Each SM was used for one replication. The strips were coarse ground (Model 4532, Hobart Manufacturing Co., Troy, OH) through a 12.7-mm plate and mixed in a bowl mixer using a dough hook attachment (Model A-200, Hobart Manufacturing Co., Troy, OH) for one minute.

The ground meat was then separated for five treatments. All treatments consisted of 400 g of ground beef and 1% NaCl (based on meat weight) with the addition of one of the following: 1) no added phosphate (control), 2) unencapsulated STP, 3) encapsulated STP (STP-165-50), 4) unencapsulated SAPP, or 5) encapsulated SAPP (CAP-SHURE® SAPP-140-50) all incorporated to provide 0.5% active STP or SAPP on a meat weight basis. Active refers to the amount of pure phosphate added to the meat. The phosphates were provided by Rhodia Co. (Cranbury, NJ). The STP was encapsulated by Balchem Inc. (Slate Hill, NY). Phosphates were encapsulated with hydrogenated vegetable oil

designed to release the phosphate once the temperature reached either 74°C (STP) or 60°C (SAPP). The encapsulated ingredients were composed of 51.1% phosphate and 48.9% encapsulating oil. The treated meat samples were mixed for 2 min at speed 1 and ground through a 4.8-mm plate (model K45SS Kitchen Aid Classic Mixer™, Kitchen Aid Inc., St. Joseph, MI).

The treated samples were formed into 85-g patties with a stainless steel hand hamburger patty press (4 1/2 inch diameter, Twoyco, Taiwan, R.O.C). Two patties per treatment per replication were formed for 0 and 5 days of storage after slaughter. The patties were randomly placed on aluminum broiler pans and heated at 163°C in an electric oven (Model Mark V, Blodgett Inc., Burlington, VT) to an internal temperature of 76°C. The internal temperature was measured by thermocouples placed into the center of the patty (AMSA, 1995). Thermocouples were placed randomly and attached to a datalogger (Model 5100, Electronic Controls Design, Milwaukie, OR). Day 0 patties were analyzed immediately after cooking for pH, cooking loss, and TBARS. The patties were stored in the dark in unsealed bags (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at a temperature of 3°C. Before analysis, the patties were ground through a 4.8-mm plate (Model K45SS Kitchen Aid Classic Mixer™, Kitchen Aid Inc., St. Joseph, MI).

3.3.2 pH

The pH of the cooked patties was measured (Model 340, Corning Inc., Corning, NY) for each treatment. A 5-g sample was placed into a beaker and homogenized at a speed of 40% (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) for 1 minute with 50-mL of distilled water. The pH of the samples was measured on Day 0 and Day 5 of storage.

3.3.3 Cooking Loss

The cooking loss was determined by the equation: Cooking loss = (weight of raw patty minus the weight of cooked patty)/ (weight of raw patty) multiplied by 100. Cooked patty weights were determined after the patties were blotted for excess exudate and cooled to approximately 25°C.

3.3.4 Thiobarbituric Acid Reactive Substances

TBARS determination (modification of Spanier and Traylor, 1991) on a 5-g sample in triplicates was homogenized in a 250-mL beaker with 40 mL of distilled water, 0.1 mL of 10% sodium dodecyl sulfate (SDS), and 10 mL of solution III (0.05-g propylgallate and 0.10-g ethylenediaminetetraacetic acid, EDTA, dissolved in 500-mL distilled water). The Macro Ultrafine generator (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) was used for homogenization at a speed setting of 40% for one minute. The homogenate volume was increased to 100 mL with distilled water and rehomogenized. All homogenates were maintained in an ice slush. A mixture of 0.8 mL of homogenate with 3.2 mL of solution I (0.375% TBA, 0.506% SDS, and 11.7% of 80% acetic acid for a final volume of 100 mL, diluted with distilled water) with a final pH of 3.4 was placed into a centrifuge tube. Spanier and Traylor (1991) used 0.4 mL of homogenate and 1.6 mL of solution I. These volumes were doubled for analysis to scale up to the available equipment. The centrifuge tube was placed into a 95°C water bath (10-L, Fisher Scientific, Pittsburgh, PA) for 60 min. Samples were shaken every 15 min throughout heating. The tubes were then cooled in tap water to room temperature. After cooling, 1 mL (modified from 0.5 mL, Spanier and Traylor, 1991) of 4°C distilled water and 5 mL (modified from 2.5 mL,

Spanier and Traylor, 1991) of solution II (1:15 ratio of pyridine to n-butanol) were added to the centrifuge tube and vortexed at maximum speed for 10 sec. The samples were centrifuged (Model PR-2, International Portable Refrigerated Centrifuge, International Equipment Company, Boston, MA) at room temperature (25°C) at 2180 x g for 15 min. The organic layer, located at the top of the tube, was then pipetted into a cuvette. Absorbance of the sample was measured at 532 nm (Model 21D, Spectronic, Milton Roy Co., Rochester, NY).

A standard curve was obtained by pipetting 0.2 mL of 5-mM Tetramethoxypropane (TMP) into 100-mL dilution flask. A total of 1 mL of 10% SDS and 10 mL of solution III was added. The mixture was then diluted to 100 mL with distilled water to give a concentration of 10-uM TMP solution. Five standards of 0, 0.2, 0.4, 0.6, and 0.8 mL of 10-uM TMP solutions were made. After 0.8 mL of the standard and 3.2 mL of solution I were added to the centrifuge tubes, the standard solutions were then treated as the samples above.

3.3.5 Statistical analysis

Data for dependent variables were analyzed as a split-plot design. The ingredient treatment represented the main effects and storage time represented the split. The dependent variables included pH, cooking loss, and TBARS. The statistical analysis was achieved by using the General Linear Model procedure (SAS, 1997). When significant, the means were separated by the Least Significant Difference procedure of SAS (1997).

3.4 Results and Discussion

pH

Cooked, ground beef patties formulated with sodium tripolyphosphate had higher ($P<0.05$) pH values than those containing sodium acid pyrophosphate (Table 1). The control was lower in pH than the alkaline STP and higher in pH than the acidic SAPP. Barbut et al. (1988) found a similar relationship in pH between the control, STP, and SAPP. However, the encapsulation had no effect ($P<0.05$) on the final pH of the samples. The lack of significance between the unencapsulated and encapsulated phosphates could be a result of insufficient storage time.

Cooking Loss

The unencapsulated STP was lower ($P<0.05$) in cooking loss than the other treatments (Table 1). This observation is attributable to water having the ability to bond with selected side groups of amino acids within the protein. Therefore, an increase in the water-holding capacity can be achieved by a difference in pH from the isoelectric point of a protein, which has a neutral charge (Claus et al., 1994). Furthermore, Townsend and Olson (1987) stated that alkaline phosphates are more effective in the improvement of water-holding capacity by the increase in pH than acid phosphates due to a decrease in pH and an ultimate decrease in shrinkage. The isoelectric point of a protein is approximately at a pH of 5.0-5.1 (Wismer-Pedersen, 1987). The low pH of the unencapsulated and encapsulated SAPP caused an increase in cooking loss due to the ph being closer to the isoelectric point of the myofibrillar proteins. The increase in cooking loss from the encapsulated samples may be due to the delayed release of the phosphate which increases water-holding capacity. A delayed release would limit the ability of the phosphate to facilitate myofibrillar protein extractability during mixing.

TBARS

Regardless of the phosphate incorporated or degree of encapsulation, phosphates resulted in lower ($p<0.05$) TBARS values in comparison to the control samples on all days (Figure 1). Molins et al. (1985) found an increase in orthophosphate concentration due to heat-induced hydrolysis of STP and SAPP from cooking. The increase in orthophosphate can reduce the activity of phosphatase enzymes, hence, reducing oxidation (Sofos, 1986). Phosphates also reduce the phosphatase activity by sequestering metals needed to enhance enzymatic activity (McComb et al., 1979). At 0 days, the unencapsulated STP produced a 72% improvement in TBARS values compared with the control samples. Furthermore, the other treatments reduced TBARS from 26% to 40% in comparison to the control samples. The decrease in pH, caused by the acidic SAPP, can reduce the stability of heme iron (Yin and Faustman, 1993), which causes an increase in oxidation. Livingston and Brown (1981) also found that a low level of pH accelerates the protonation of bound oxygen and favors the release of superoxide anions. The significance between the unencapsulated and encapsulated STP at day 0 may be due to the lower level of phosphate activity prior to analysis. At 6 days, the samples containing phosphates were 81% to 90% more effective in the reduction of TBARS than the controls. The lack of difference between the phosphate treatments, at 6 days, may be due to the wide variation in TBARS associated with the control. Many researchers (Lee et al., 1996; Trout and Dale, 1990; Salih et al., 1989; Whang and Peng, 1987; Igene et al., 1985; Tims and Watts, 1958) have found similar results with an increase in TBARS of the controls, especially cooked, with an increase in storage time. Nevertheless, the general trend among the phosphate treatments at 6 days were similar to that observed at 0 days in comparison to the control. An insufficient time available for the phosphatases to

breakdown the unencapsulated phosphates could be the cause of the lack of difference ($P>0.05$) from the encapsulated phosphates.

3.5 Conclusions

The use of phosphates in ground beef patties has been demonstrated to be effective in the reduction of lipid oxidation. Although the phosphates differed in their ability to control lipid oxidation, STP was the most effective phosphate in fresh cooked ground beef. Encapsulated STP in comparison with unencapsulated STP did not provide any additional control of lipid oxidation. An extension of the time available for the phosphatases to break down the unencapsulated phosphates prior to cooking may be necessary to demonstrate the functional benefit of the encapsulated phosphates. Therefore, future research should focus on the effects of encapsulated phosphates in raw meat stored prior to cooking.

3.6 References

AMSA. 1995. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. Am. Meat Sci. Assoc. and Nat. Livesock and Meat Board, Chicago, IL.

Barbut, S., Maurer, A.J., and Lindsay, R.C. 1988. Effects of reduced sodium chloride and added phosphates on physical and sensory properties of turkey frankfurters. J. Food Sci. 53: 62-66.

Claus, J.R., Colby J., and Flick, G.J. 1994. Processed meats/ poultry/ seafood. Ch. 5 in *Muscle Foods: Meat, Poultry, and Seafood Technology*, D.M. Kinsman, A.W. Kotula, and B.C. Breidenstein (Ed.), p. 106-162. Chapman & Hall., New York.

Dugan, L.R., Jr. 1987. Meat Fats. Ch. 15 (part 1) in The Science of Meat and Meat Products, 3rd ed., J.F. Price and B.S. Schweigert (Eds.). Food & Nutrition Press, Inc., Westport, CT. p. 507-530.

Dziezak, J.D. 1990. Phosphates improve many foods. Food Technol. 44(4):80-92.

Igene, J.O., Yamauchi, K., Pearson, A.M., Gray, J.I., and Aust, S.D. 1985. Evaluation of 2-thiobarbituric acid reactive substances (TBRS) in relation to warmed-over flavor (WOF) development in cooked chicken. J. Agric. Food Chem. 33:364-367.

Lee, S.K., Mei, L., and Decker, E.A. 1996. Lipid oxidation in cooked turkey as affected

by added antioxidant enzymes. J. Food Sci. 61:726-728.
Livingston, D.J. and Brown, W.D. 1981. The chemistry of myoglobin and its reaction. Food Technol. 35:244-249.
McComb, R.B., Bowers, G.N., Jr., and Posen, S. 1979. Alkaline phosphatase. Plenum Press, New York.
Molins, R.A., Kraft, A.A., and Olson, D.G. 1985. Adaptation of a method for the determination of soluble orthophosphate in cooked and uncooked pork containing acid-labile poly- and pyrophosphates. J. Food Sci. 50:1482-1483.
Salih, A.M, Price, J.F., Smith, D.M., and Dawson, L.E. 1989. Lipid oxidation in turkey meat as influenced by salt, metal cations, and antioxidants. J. Food Qual. 12:71-83.
SAS Institute, Inc. 1997. SAS Users Guide to the Statistical Analysis System. SAS. Cary, NC.
Sofos, J.N. 1986. Use of phosphates in low-sodium meat products. Food Technol. 40(9):52-69.
Spanier, A.M., and Traylor, R.D. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. J. Muscle Foods 2:165-176.
Sutton, A.H. 1973. The hydrolysis of sodium tripolyphosphate in cod and beef muscle. J. Food Technol. 8:185-195.
Tims, M.J., and Watts, B.M. 1958. Protection of cooked meats with phosphate. Food Technol. 12:240-243.
Townsend, W.E. and Olson, D.G. 1987. Cured meats and cured meat products processing. Ch. 12 in The Science of Meat and Meat Products, 3rd ed., J.F. Price and B.S. Schweigert (Eds.). Food & Nutrition Press, Inc., Westport, CT. p. 431-456.
Trout, G.R. and Dale, S. 1990. Prevention of warmed-over flavor in cooked beef: effect of phosphate type, phosphate concentration, a lemon juice/phosphate blend, and beef extract. J. Agric. Food Chem. 38:665-669.
Whang, K. and Peng, I.C. 1987. Lipid oxidation in ground turkey skin and muscle during storage. Poul. Sci. 66:458-466.
Wismer-Pedersen, J. 1987. Water. Ch. 3 (part 5) in The Science of Meat and Meat Products, 3rd ed., J.F. Price and B.S. Schweigert. Food & Nutrition Press, Inc., Westport, CT. p. 103-114.
Yin, M.C. and Faustman, C. 1993. Influence of temperature, pH, and phospholipid composition upon the stability of myoglobin and phospholipid: liposome model. J. Agric. Food Chem. 41:853-857.

3.7 Table & Figure

Table 1-- pH and cooking loss means for ground beef patties cooked immediately after the addition of phosphates.

	pH		Cooking Loss
Treatment[1]	Day 0	Day 6	(%)
Control	5.53[b]	5.50[b]	31.9[b]
0.5% Unencapsulated STP	6.02[a]	5.98[a]	25.2[c]
0.5% Encapsulated STP	5.85[a]	5.89[a]	34.6[ab]
0.5% Unencapsulated SAPP	5.28[c]	5.31[c]	34.3[ab]
0.5% Encapsulated SAPP	5.28[c]	5.33[c]	35.4[a]
Std Errors	0.11	0.065	1.5

[a-c]Means bearing unlike superscripts within a column are different ($P<0.05$).

[1]Treatments- all treatments had 1% NaCl and cooked to internal of 76°C; control had no added phosphates; phosphate treatments had 0.5% phosphate added on a meat a weight basis.

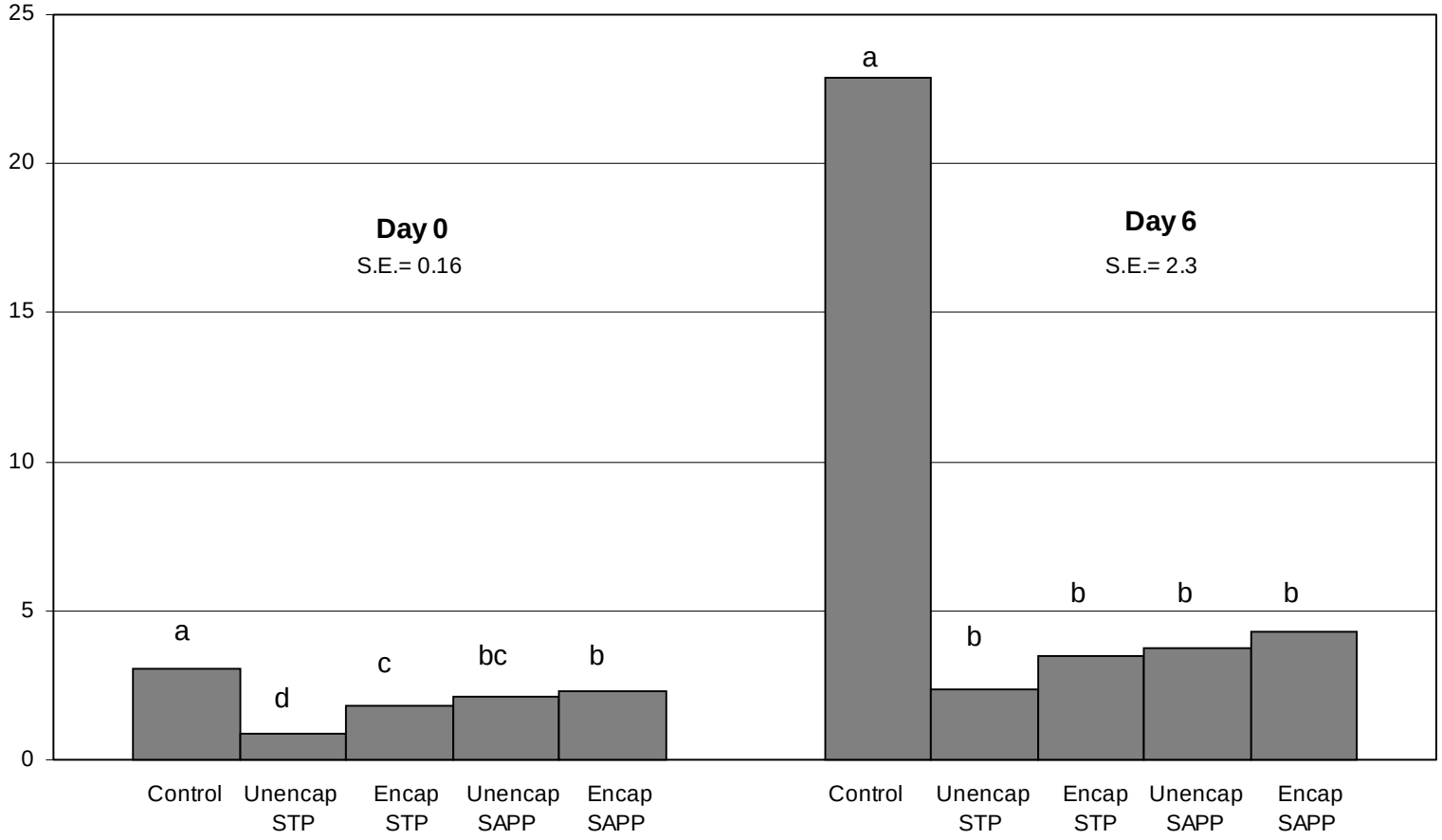


Figure 1-- Antioxidantive effect of phosphates in cooked, ground beef patties containing NaCl and stored for 0 and 6 days. Bars within a storage day with unlike letters are different (P<0.05).

Chapter 4

Reduction in Lipid Oxidation by Incorporation of Encapsulated Sodium Tripolyphosphate in Ground Turkey

4.1 Abstract

Ground turkey meat, with 1% NaCl, was incorporated with no sodium tripolyphosphate (STP; control), unencapsulated STP (0.3% or 0.5%), encapsulated STP (0.3% or 0.5% active), or a blend of unencapsulated (0.3%) and encapsulated (0.2% active) phosphate and stored at 3°C for 0, 5, and 10 days. Treatments were stored (4 and 24 hrs, 3°C) before cooking to two different endpoints (74°C and 79°C). An improvement of 77% and 80% in the reduction of TBARS was found with the 0.3% and 0.5% encapsulated STP, respectively, in comparison to the unencapsulated STP. The 4 hr pre-storage time resulted in lower ($P<0.05$) TBARS at 5 and 10 days compared to the 24 hr pre-storage time. A lower CIE a* value was found with an endpoint temperature of 79°C in comparison to the 74°C endpoint temperature. Meat manufacturing procedures that entail a delayed thermal processing step will benefit by an improvement in lipid oxidation control through the use of encapsulated phosphates.

4.2 Introduction

Today, health conscious consumers prefer to have meals which contain a higher percentage of unsaturated fatty acids than saturated fatty acids. Unfortunately, these unsaturated fatty acids are highly susceptible to lipid oxidation (Dugan, 1987). For every additional double bond in an unsaturated fatty acid, the oxidation rate increases by a

factor of two (Moerck and Ball, 1974). Poultry meat contains a relatively high amount of unsaturated fatty acids, thus, being highly oxidative in comparison with other meats (Dawson and Gartner, 1983). Therefore, a means to reduce the level of lipid oxidation is extremely beneficial to the food industry.

Natural antioxidants, synthetic antioxidants, and chelators are used to aid in controlling oxidation. Although all are effective, phosphates are predominately used in the meat industry (Dziezak, 1990). Phosphates have the capability to chelate free metals (Tims and Watts, 1958), increase pH (Miller et al., 1986), reduce phosphatase activity (McComb et al., 1979), and increase water-holding capacity (Claus et al., 1994). However, about 80% of added polyphosphate is lost by the time meat is cooked due phosphatase activity (Decker and Mei, 1996). Given an incorporation level of 0.5% (legal limit), suggests that only 0.10% polyphosphate (meat weight basis) remains after cooking. Furthermore, Li et al. (1993) demonstrated that 100% of STP was lost after one day of incorporation in raw turkey. Although, Decker and Mei (1996) found that an improvement in controlling oxidation during storage was achieved with the addition of sodium tripolyphosphate to meat after cooking. Unfortunately, the addition of phosphates after cooking is not feasible for the meat industry due to food safety issues and inefficiency in production.

Encapsulation of selected phosphates known for their strong antioxidative properties could be potentially beneficial by protecting the phosphates from the phosphatases during raw meat storage and the initial stages of cooking. This protection could provide time for some of the heat sensitive phosphatases to be inactivated prior to the release of the phosphate. However, the amount of time needed for the protection of

the phosphate to be of benefit is unknown. Also, the encapsulate melts at an endpoint temperature of 74°C, hence, giving reason to test the release of the phosphate and effects of temperature with endpoint temperatures of 74°C and 79°C. Therefore, the objective of this research was to determine the effect of various levels of encapsulated and unencapsulated phosphates (0.3%, and 0.5%, active phosphate) on the development of lipid oxidation during storage (0, 5, and 10 days), cooking loss, color, and pH. The significance of storage time before cooking (4 and 24 hrs) and endpoint temperature (74°C and 79°C) were determined.

4.3 Materials and Methods

4.3.1 Formulation and Processing

Fresh, boneless, skinless tom turkey breast muscles (*pectoralis major*) were obtained from a Virginia producer immediately following slaughter and stored at a temperature of 3°C. After 24 hr, the turkey breasts were cut into strips and coarse ground (Model 4532, Hobart Manufacturing Co., Troy, OH) through a 12.7-mm plate and then a 4.8-mm plate. The turkey was mixed for one minute in a bowl mixer with a dough hook attachment (Model A-200, Hobart Manufacturing Co., Troy, OH) between grinding. All treatments consisted of 1% NaCl (meat weight basis) and 400 g of ground turkey, and one of the following: no STP (control), unencapsulated STP (0.3% or 0.5%), encapsulated STP (0.3% or 0.5% active), or a blend of unencapsulated (0.3%) and encapsulated (0.2% active) phosphate. The amount of pure phosphate added to the meat is referred to as active. Rhodia Co. (Cranbury, NJ) provided the phosphates.

The STP was encapsulated by Balchem Inc. (Slate Hill, NY). The phosphates (STP-165-50) were encapsulated with hydrogenated vegetable oil designed to release the phosphate once the temperature reaches 74°C. The encapsulated ingredients were composed of 51.1-g active phosphate and 48.9-g encapsulating oil. The samples were mixed for 2 min with the ingredients at speed 1 using a hand mixer (Model KHM3WH-1, Kitchen Aid, St. Joseph, MI). The turkey was then stored in an unsealed bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at a temperature of 3°C in the dark. The storage time before cooking and after the addition of the phosphates was 4 and 24 hr.

The samples were cooked via sous vide method using a custom built circulating water bath (Virginia Polytechnic Institute and State University). Two sets of the six treatments, three centrifuge tubes per treatment, were cooked to an end point temperature of 74°C and 79°C. Each tube (50-mL 28x15 mm polypropylene tubes with screw plug seal, #05-539-9, Fisher Scientific, Pittsburgh, PA) contained a 45-g sample. The internal temperature was measured by four samples containing thermocouples attached to a datalogger (Model 5100, Electronic Controls Design, Milwaukie, OR). The thermocouples were placed in four tubes, which were distributed randomly throughout the other samples. The wire was placed at the center of the sample. The cooked samples were ground at speed 2 through a 4.8-mm plate (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI) after cooking loss was determined. The cooked ground turkey samples were then stored at 3°C in the dark until analyzed on days 0, 5, and 10, which are dependent on time of cooking.

4.3.2 pH

The pH of the raw and cooked ground turkey was measured (Model 340, pH meter, Corning Inc., Corning, NY) by placing 5 g of meat into a beaker and homogenizing at a speed of 40% (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) for 1 minute with 50-mL distilled water. The pH of all the sample treatments were measured for raw ground turkey at 24 hr after incorporation only and at 0, 5, and 10 days for cooked ground turkey. The raw ground turkey was measured to verify encapsulation stability.

4.3.3 Cooking loss

The cooking loss was determined by the equation: Cooking loss = (wt of raw turkey - wt of cooked turkey)/ (wt of raw turkey) * 100. The cooked weight was determined after the turkey was cooled to 25°C. The cooked turkey was removed from the centrifuge tube and patted dry with paper towels in order to absorb excess exudate.

4.3.4 Thiobarbituric Acid Reactive Substances (TBARS) determination

TBARS determination (modification of Spanier and Traylor, 1991) on a 5-g sample in triplicates was homogenized in a 250-mL beaker with 40 mL of distilled water, 0.1 mL of 10% sodium dodecyl sulfate (SDS), and 10 mL of solution III (0.05-g propylgallate and 0.10-g ethylenediaminetetraacetic acid, EDTA, dissolved 500-mL distilled water). The Macro Ultrafine generator (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) at a speed setting of 40% for one minute was used for homogenization. The homogenate volume was increased to 100 mL with distilled water and rehomogenized. All homogenates were maintained in an ice slush. A mixture of 0.8 mL of homogenate with 3.2 mL of solution I (0.375% TBA, 0.506% SDS, and 11.7% of

80% acetic acid for a final volume of 100 mL, diluted with distilled water) with a final pH of 3.4 was placed into a centrifuge tube. Spanier and Traylor (1991) used 0.4 mL of homogenate and 1.6 mL of solution I. These volumes were doubled to scale up to the available equipment. The centrifuge tube was placed into a 95°C water bath (Model 10-L, Fisher Scientific, Pittsburgh, PA) for 60 min. Samples were shaken every 15 min throughout heating. The tubes were then cooled in tap water to room temperature. After cooling, 1 mL (modified from 0.5 mL, Spanier and Traylor, 1991) of 4°C distilled water and 5 mL (modified from 2.5 mL, Spanier and Traylor, 1991) of solution II (1:15 ratio of pyridine to n-butanol) was added to the centrifuge tube and vortexed (Model G560, Vortex Genie 2, Scientific Industries Inc., Bohemia, NY) at maximum speed for 10 sec. The samples were centrifuged (Model PR-2, International Portable Refrigerated Centrifuge, International Equipment Company, Boston, MA) at room temperature (25°C) at 2180 x g for 15 min. The organic layer, located at the top of the tube, was then pipetted into a cuvette. Absorbance of the sample was measured at 532 nm (Model Spectronic 21D, Milton Roy Company, Rochester, NY).

A standard curve was obtained by pipetting 1 mL of 5-mM tetramethoxypropane (TMP) into a test tube with 9-mL distilled water. Five standards of 0, 0.5, 1.0, 1.5, and 2.0 mL of 0.05-mM standard TMP solutions were made. The standards were treated as samples above with the absence of homogenization.

4.3.5 Instrumental Color Determination

The samples were analyzed for CIE L*a*b* readings immediately after cooking loss was determined by using a chroma meter (Model CR-200, Minolta Corp., Osaka, Japan). Once cooking loss was determined, the turkey cylinder was cut 1 cm from the

top. The cylinder was then ground through a 4.8-mm plate (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI) and placed in a bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC). The chroma meter was calibrated using standard Minolta calibration plates (white plate, No. 20933026, CIE L* 97.91, a* -0.68, b* +2.45; brown plate, No. 13433234, CIE L* 57.26, a* 9.45, b* 10.77; and red plate, No. 11533041, CIE L* 52.06, a* 42.13, b* 19.38) covered with a plastic bag. Three different readings were taken on each treated sample through the bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) on storage days 0, 5, and 10 after cooking.

4.3.6 Statistical Analysis

The design of the project was a 4x2x2 factorial with storage time representing the split plot design. Three replications were completed. The dependent variables that were analyzed on each phosphate treatment included: TBARS, pH (24 hr raw and all cooked), Minolta color (CIE L* a* b*), and cooking loss. Statistical analyses on the dependent variables were analyzed by the General Linear Model procedure (SAS, 1997). When significance ($P<0.05$) was determined in the model, the means were separated by the Least Significant Difference procedure of SAS (1997).

4.4 Results and Discussion

Cooking Loss

A difference ($P<0.05$) in cooking loss was found between the control and the 0.5% encapsulated STP, 0.5% unencapsulated STP, and blended samples (Table 1). These results agree with those of Craig et al. (1991) who also found a decrease in

cooking loss in ground turkey meat with unencapsulated STP in comparison to the control. The decrease in cooking loss was due to a higher concentration of phosphates and its effects on water-holding capacity (Li et al., 1993).

pH

The pH of the control was lower ($P<0.05$) than the other treated samples due to the alkaline properties of sodium tripolyphosphate (Table 1). Interactions ($P<0.05$) in pH were found between treatment and pre-storage time (Table 2), and pre-storage time and post-storage (Table 3). The turkey with the phosphate blend and pre-storage (4 hr) had the highest ($P<0.05$) pH (Table 2). This increase in pH is due to the amount of STP available pre- and post-cook. The 0.5% STP treatments were higher ($P<0.05$) in pH in comparison to the 0.3% STP treatments with 4 hr pre-storage. The 24 hr pre-storage turkey with the 0.5% active phosphate had a higher ($P<0.05$) pH than turkey formulated with 0.3% active phosphate. Overall, the pH with 24 hr pre-storage was higher ($P<0.05$) than the 4 hr pre-storage at 0, 5, and 10 days.

TBARS

The use of encapsulated phosphate affected the level of TBARS in comparison to the control (Table 1). Salih (1986) found that TBA values of 3.4 (distillation) were the threshold values for the detection of warmed-over flavor in cooked turkey breast by a sensory panel. Therefore, the lower TBARS values from the encapsulated phosphates would be more desirable. The use of 0.3% unencapsulated STP was not different ($P>0.05$) than the control. Furthermore, the 0.3% encapsulated STP was shown to be 77% more effective in the reduction of TBARS than the 0.3% unencapsulated STP. Similar results were seen between the 0.5% encapsulated STP and 0.5% unencapsulated

STP due to the encapsulated STP having an 80% improvement in the reduction of TBARS. The blend, 0.3% unencapsulated and 0.2% encapsulated, compared to 0.3% and 0.5% unencapsulated STP also exhibited an improvement in the reduction of TBARS of 69% and 63%, respectively. The instability of ground turkey meat is high in terms of TBARS with storage time which agreed with Whang and Peng (1987). Therefore, TBARS interactions were found between pre-storage and post-storage (Table 4), treatments and pre-storage (Table 5), treatments and endpoint temperature (Table 6), and treatments and post-storage (Table 7). The TBARS of 24 hr pre-storage samples were higher ($P<0.05$) after 10 days of post-storage in comparison to the 4 hr pre-storage (Table 4). TBARS was significant with an increase in post-storage time due to an increase in the rate of oxidation over time. The blend and encapsulated STP had lower ($P<0.05$) TBARS than the control and unencapsulated STP in pre-storage, 4 hr and 24 hr (Table 5). Furthermore, the 4 hr pre-storage was lower in TBARS with the encapsulated treatments in comparison to the 24 hr pre-storage. The 4 hr pre-storage time had less exposure to oxygen and enzymatic activity. Therefore, a pre-storage time of 4 hr is sufficient to reduce the level of TBARS. The reduction of TBARS by encapsulation may be due to the higher level of phosphate after cooking. The increase in phosphate concentration decreases the level of enzymatic activity by the chelation of metals (McComb et al., 1979). The endpoint temperature of 79°C was lower in TBARS in comparison to the endpoint of 74°C (Table 6). The melting point of the encapsulate may have an effect of the release of the phosphate. The treatments containing the encapsulated phosphate were significantly lower in TBARS on all post-storage days (Table 7). The control and unencapsulated treatments were 3 to 4 times higher in TBARS from day 0 to day 5. At

day 10, the blend was higher ($P<0.05$) in TBARS in comparison to the other encapsulated treatments. The increase in TBARS with the blend is due to the lower level of encapsulated phosphate (0.2%). Ang and Young (1989) also found that 0.5% STP was more beneficial in the reduction of oxidation in broiler breast patties than 0.2% STP.

CIE L*a*b* value

The means of CIE L*a*b* values are shown in Table 1. CIE L* values were lower ($P<0.05$) for treatments containing 0.5% active phosphate samples compared to the control. The presence of encapsulated STP produced higher ($P<0.05$) CIE a* values on day 5 and 10 than in the control and unencapsulated STP. The redness may be partially due to the increase in pigment stability due to the decrease in lipid oxidation (Akamittath et al., 1990). Trout (1984) also found an increase in pinkness due to the alkaline properties of STP forming pink hemochromes. CIE values were found to have interactions including treatment and temperature (CIE a* and b*, Table 8), treatment and post-storage (CIE L* and a*, Table 9), pre-storage and post-storage (CIE a* and b*, Table 10), and temperature and post-storage (CIE L* and a*, Table 11). In Table 8, the CIE a* value for endpoint temperature 79°C was lower than endpoint temperature 74°C due to the oxidative degradation of myoglobin (Faustman, 1994). A difference ($P<0.05$) in CIE b* was found with the control and encapsulated treatments at 74°C (Table 8). At 79°C, the CIE b* values were different between the 0.3% unencapsulated STP and the 0.3% encapsulated STP. At day 10 days, the CIE L* value was not different ($P>0.05$) with the treatments containing 0.5% STP (Table 9). Turkey containing only encapsulated phosphate was more ($P<0.05$) stable in CIE a* across post-cooking storage times than either the control or unencapsulated treatment (Table 9). Trout (1984) found that

oxidation of lipids is interrelated to the oxidation of meat pigments. Therefore, the increase in red pigment from the encapsulated STP may have been due to the decrease in lipid oxidation. The stability of CIE a* in post-storage is due to the higher level of phosphate to aid in the reduction of lipid oxidation. CIE a* values were not different ($P>0.05$) between the 4 and 24 hr samples at 0 days, however, the CIE a* values were lower ($P<0.05$) for the 24 hr pre-stored samples at 5 and 10 days (Table 10). The lower CIE a* values in the 24 hr compared to the 4 hr pre-stored turkey, in both 5 and 10 days post-storage, may have been associated with initial pre-cook bacterial growth and related oxidation which was not evaluated in this study. The CIE b* value was not different at 10 days of post-storage, regardless of pre-storage time. CIE a* value decreased with and increase in post-storage time in both endpoint temperatures (Table 11). Trout (1984) found that meat cooked below 76°C resulted in the incomplete denaturation of myoglobin. Therefore, samples cooked to a higher endpoint temperature appeared to have a greater decline in CIE a* value (Table 11).

4.5 Conclusions

Encapsulating all of the phosphate or utilizing a blend, in which a portion is fully encapsulated, has the potential to dramatically improve (63% to 80%) the ability to control lipid oxidation. Thus, processors that have or require a delayed thermal processing step after incorporation of adjuncts (e.g. unencapsulated phosphate) in the manufacture of uncured, cooked meat and poultry products could lower the rate of oxidation by incorporating encapsulated phosphate.

4.6 References

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513-1517.

Ang, C.Y.W. and Young, L.L. 1989. Factors relating to oxidative stability of cooked broiler breast patties treated with sodium tripolyphosphate. J. Food Sci. 54:1151-1154.

Bowers, J.A., Craig, J.A., and Perng, S.K. 1993. Sodium tripolyphosphate stability and effect in ground turkey meat. J. Food Sci. 58:501-504, 521.

Claus, J.R., Colby, J.W., and Flick, G.J. 1994. Processed meats/poultry/seafood. Ch.5 in Muscle Foods: Meat, Poultry, and Seafood Technology, D.M. Kinsman, A.W. Kotula, and B.C. Breidenstein (eds.). p.106-162. Chapman & Hall, New York.

Craig, J., Bowers, J.A., and Seib, P. 1991. Sodium tripolyphosphate and sodium ascorbate monophosphate as inhibitors of off-flavor development in cooked, vacuum-packaged, frozen turkey. J. Food Sci. 56:1529-1531, 1561.

Dawson, , L.E., and Gartner, R. 1983. Lipid oxidation in mechanically deboned poultry. Food Technol. 37:112-116.

Decker, E.A. and Mei, L. 1996. Oxidative Processes in Meat. Antioxidant mechanisms and applications in muscle foods. Proceedings from the 49th Reciprocal Meat Conference, Provo, UT. Published by the American Meat Science Assoc. Chicago, IL. 49:64-72.

Dugan, L.R., Jr. 1987. Meat Fats. Ch. 15 (part 1) in The Science of Meat and Meat Products,3rd ed., J.F. Price and B.S. Schweigert. Food & Nutrition Press, Inc., Westport, CT. p. 507-530.

Dziezak, J.D. 1990. Phosphates improve many foods. Food Technol. 44(4):80-92.

Faustman, C. 1994. Postmortem changes in muscle foods. Ch.3 in Muscle Foods: Meat, Poultry, and Seafood Technology, D.M. Kinsman, A.W. Kotula, and B.C. Breidenstein (Eds.). p. 63-78. Chapman & Hall, NY.

Li, W., Bowers, J.A., Craig, J.A., and Perng, S.K. 1993. Sodium tripolyphosphate stability and effect in ground turkey meat. J. Food Sci. 58:501-504, 521.

McComb, R.B., Bowers, G.N., Jr., and Posen, S (Ed.). 1979. Alkaline phosphatase. Plenum Press, New York.

Miller, M.F., David, G.W., Seidemann, S.C., Ramsey, C.B., and Rolan, T.L. 1986. Effects of various phosphates on the palatability, appearance, and storage traits of flaked and formed restructured beef steaks. J. Food Sci. 51:1435-1438.

Moerck, K.E., and Ball, H.R. 1974. Lipid oxidation in mechanically deboned chicken meat. J. Food Sci. 39:876-879.

Salih, A.M. 1986. Lipid stability in turkey meat as influenced by cooking, refrigerating, and frozen storage, salt, metal cations and antioxidants. Ph.D. dissertation, Michigan State Univ., East Lansing.

SAS Institute, Inc. 1997. SAS Users Guide to the Statistical Analysis System. SAS. Cary, NC

Spanier, A.M., and Traylor, R.D. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. J. Muscle Foods 2:165-176.

Tims, M.J., and Watts, B.M. 1958. Protection of cooked meats with phosphate. Food Technol. 12:240-243.

Trout, G.R. 1984. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. J. Food Sci. 49:687-694.

Whang, K. and Peng, I.C. 1987. Lipid oxidation in ground turkey skin and muscle during storage. Poultry Sci. 66:458-466.

4.7 Tables

Table 1- Means[1] for various physical and chemical traits in cooked ground turkey breast.

Treatments[2]	Cooking Loss (%)	pH	TBARS (mg/kg)	CIE Values L*	a*	b*
Control	16.8[a]	6.09[c]	12.50[a]	76.5[a]	1.50[b]	16.1[a]
Unencap STP, 0.3%	15.3[ab]	6.25[b]	11.73[a]	75.3[ab]	1.75[b]	16.1[a]
Unencap STP, 0.5%	13.3[b]	6.32[a]	9.66[b]	74.7[b]	1.97[b]	15.9[a]
Encap STP, 0.3%	15.8[ab]	6.27[b]	2.61[cd]	75.4[ab]	3.37[a]	15.6[a]
Encap STP, 0.5%	14.6[b]	6.33[a]	1.97[d]	74.6[b]	3.66[a]	15.6[a]
Unencap, 0.3% & Encap, 0.2%	13.6[b]	6.34[a]	3.62[c]	74.1[b]	3.38[a]	15.8[a]
Standard Error	0.49	0.01	0.37	0.39	0.16	0.25

[1]Means were pooled over pre-storage time (4 hr, 24 hr), endpoint temperature (74°C, 79°C), and post-storage (0, 5, 10 days).
[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis
[a-d]Means bearing unlike superscripts within each trait are different (P<0.05).

Table 2- Treatment interaction means[1] with storage in pH in cooked ground turkey formulated with different antioxidant ingredients.

	Pre-Storage	
Treatments[2]	4 hr	24 hr
Control	6.07[e]	6.11[d]
Unencap STP, 0.3%	6.23[d]	6.27[c]
Unencap STP, 0.5%	6.31[c]	6.34[a]
Encap STP, 0.3%	6.24[d]	6.29[b]
Encap STP, 0.5%	6.32[bc]	6.35[a]
Unencap, 0.3% & Encap, 0.2%	6.34[a]	6.33[a]
Standard Error	0.004	0.004

[1]Means were pooled over endpoint temperature (74°C, 79°C) and post-storage (0, 5, 10 days).

[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.

[a-e]Means bearing unlike superscripts within each column are different (P<0.05).

Table 3- Pre-Storage interaction means[1] with Post-Storage on pH of cooked ground, turkey.

	Post-Storage		
Pre-Storage	0 Days	5 Days	10 Days
4 hr	6.20[bz]	6.24[by]	6.32[bx]
24 hr	6.26[ay]	6.32[ax]	6.27[ay]
Standard Error	0.01	0.01	0.01

[1]Means were pooled over ingredient treatments and endpoint temperature (74°C, 79°C).

[ab]Means within Post-Storage column bearing unlike superscripts are different (P<0.05).

[x-z]Means within Pre-Storage row bearing unlike superscripts are different (P<0.05).

Table 4- Pre-Storage interaction means[1] with Post-Storage on TBARS (mg/kg) of cooked, ground turkey.

	Post-Storage		
Pre-Storage	0 Days	5 Days	10 Days
4 hr	2.81^{az}	8.71^{ax}	7.96^{by}
24 hr	2.59^{az}	9.47^{ay}	10.55^{ax}
Standard Error	0.25	0.25	0.25

[1]Means were pooled over ingredient treatments and endpoint temperature (74°C, 79°C).
[ab]Means within Post-Storage column bearing unlike superscripts are different (P<0.05).
[x-z]Means within Pre-Storage row bearing unlike superscripts are different (P<0.05).

Table 5- Storage effect on TBARS (mg/kg) means[1] of cooked, ground turkey formulated with different antioxidant ingredients.

	Pre-Storage	
Treatments[2]	4hr	24 hr
Control	12.62^{a}	12.37^{a}
Unencap STP, 0.3%	11.76^{ab}	11.70^{ab}
Unencap STP, 0.5%	10.02^{b}	9.30^{b}
Encap STP, 0.3%	1.57^{c}	3.66^{c}
Encap STP, 0.5%	1.28^{c}	2.66^{c}
Unencap, 0.3% & Encap, 0.2%	1.71^{c}	5.52^{c}
Standard Error	0.65	0.65

[1]Means were pooled over endpoint temperature (74°C, 79°C) and post storage (0, 5, 10 days).
[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[a-c]Means bearing unlike superscripts within each column are different (P<0.05).

Table 6- Temperature effects on TBARS (mg/kg) means[1] of cooked, ground turkey formulated with different antioxidant ingredients.

	End-point Temperature	
Treatments[2]	74°C	79°C
Control	13.06[a]	11.93[a]
Unencap STP, 0.3%	12.29[ab]	11.17[ab]
Unencap STP, 0.5%	10.50[b]	8.82[b]
Encap STP, 0.3%	2.91[cd]	2.31[c]
Encap STP, 0.5%	2.32[c]	1.62[c]
Unencap, 0.3% & Encap, 0.2%	4.18[d]	3.06[c]
Standard Error	0.60	0.60

[1]Means were pooled over pre-storage (4 hr, 24 hr) and post storage (0, 5, 10 days).
[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[a-d]Means bearing unlike superscripts within each column are different (P<0.05).

Table 7- Post-storage effect on TBARS (mg/kg) means[1] of cooked, ground turkey formulated with different antioxidant ingredients.

	Post-Storage		
Treatments[2]	0 days	5 days	10 days
Control	5.30[a]	17.13[a]	15.07[a]
Unencap STP, 0.3%	4.12[a]	15.44[b]	15.63[a]
Unencap STP, 0.5%	2.66[b]	13.16[c]	13.16[b]
Encap STP, 0.3%	1.47[bc]	2.94[de]	3.43[d]
Encap STP, 0.5%	1.29[c]	2.04[e]	2.58[d]
Unencap, 0.3% & Encap, 0.2%	1.37[c]	3.83[d]	5.65[c]
Standard Error	0.44	0.44	0.44

[1]Means were pooled over pre-storage (4hr, 24hr) and endpoint temperature (74°C, 79°C).
[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[a-e]Means bearing unlike superscripts within each column are different (P<0.05).

Table 8- Cooking temperature effect on CIE (a* and b*) means[1] of cooked, ground turkey formulated with different antioxidant ingredients.

	CIE values			
	a*		b*	
Treatments[2]	74°C	79°C	74°C	79°C
Control	1.69^{d}	1.31^{c}	15.9^{a}	16.3^{ab}
Unencap STP, 0.3%	1.95^{d}	1.54^{bc}	15.8^{ab}	16.4^{a}
Unencap STP, 0.5%	2.26^{c}	1.68^{b}	15.5^{b}	16.2^{ab}
Encap STP, 0.3%	3.82^{b}	2.91^{a}	15.1^{c}	16.0^{b}
Encap STP, 0.5%	4.12^{a}	3.20^{a}	15.1^{c}	16.1^{ab}
Unencap, 0.3% & Encap, 0.2%	3.85^{ab}	2.92^{a}	15.3^{bc}	16.3^{ab}
Standard Error	0.10	0.10	0.10	0.10

[1]Means were pooled over pre-storage (4hr, 24hr) and post-storage (0, 5, 10 days).
[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[a-d]Means bearing unlike superscripts within each column are different (P<0.05).

Table 9- Post-storage effect on CIE (L* and a*) means[1] of cooked, ground turkey formulated with different antioxidant ingredients.

	CIE values					
	L*			a*		
Treatments[2]	0 Days	5 Days	10 Days	0 Days	5 Days	10 Days
Control	74.9^{a}	77.4^{a}	77.1^{a}	4.36^{ab}	0.09^{e}	0.05^{f}
Unencap STP, 0.3%	73.6^{ab}	76.4^{b}	75.9^{b}	4.46^{a}	0.49^{d}	0.30^{e}
Unencap STP, 0.5%	72.8^{c}	75.9^{bc}	75.3^{c}	4.43^{ab}	0.88^{c}	0.59^{d}
Encap STP, 0.3%	74.4^{ab}	75.9^{bc}	75.8^{bc}	4.30^{b}	3.18^{b}	2.63^{b}
Encap STP, 0.5%	74.0^{b}	74.6^{c}	75.2^{c}	4.32^{ab}	3.61^{a}	3.06^{a}
Unencap, 0.3% & Encap, 0.2%	73.3^{c}	73.9^{d}	75.0^{c}	4.37^{ab}	3.33^{b}	2.48^{c}
Standard Error	0.19	0.19	0.19	0.06	0.06	0.06

[1]Means were pooled over pre-storage (4hr, 24hr) and endpoint temperature (74°C, 79°C).
[2]Treatments- percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[a-f]Means bearing unlike superscripts within each column are different (P<0.05).

Table 10- Pre-storage interaction means[1] with Post-storage on CIE (a* and b*) value of cooked, ground turkey.

	CIE values					
	a*			b*		
	Post-Storage			Post-Storage		
Pre-Storage	0 Days	5 Days	10 Days	0 Days	5 Days	10 Days
4 hr	4.34^{ax}	2.03^{ay}	1.70^{az}	14.9^{ax}	15.9^{ay}	17.2^{az}
24 hr	4.41^{ax}	1.82^{by}	1.32^{bz}	14.5^{bx}	15.4^{by}	17.1^{az}
Standard Error	0.03	0.03	0.03	0.09	0.09	0.09

[1]Means were pooled over ingredient treatments and endpoint temperature (74°C, 79°C).
[ab]Means within Post-Storage column bearing unlike superscripts are different (P<0.05).
[x-z]Means within Pre-Storage row bearing unlike superscripts are different (P<0.05).

Table 11- Cooking temperature interaction means[1] with Post-storage on CIE (a*) value of cooked, ground turkey.

	CIE a* values		
	Post-Storage		
Cooking Temperature	0 Days	5 Days	10 Days
74°C	4.50^{ax}	2.36^{ay}	1.99^{az}
79°C	4.25^{bx}	1.50^{by}	1.03^{bz}
Standard Error	0.03	0.03	0.03

[1]Means were pooled over ingredient treatments and endpoint temperature (74°C, 79°C).
[ab]Means within Post-Storage column bearing unlike superscripts are different (P<0.05).
[xyz]Means within Cooking Temperature row bearing unlike superscripts are different (P<0.05).

Chapter 5

Antioxidative Efficacy of Encapsulated Sodium Tripolyphosphate compared to Lemo-fos and Freez-Gard FP-15 in Cooked, Ground Turkey

5.1 Abstract

Ground turkey meat, with 1% NaCl, was incorporated with no adjunct (control), 0.5% unencapsulated sodium tripolyphosphate (STP), 0.5% active encapsulated STP, 0.5% Lemo-fos, or 0.5% Freez-Gard FP-15. The pH was higher ($P<0.05$) with the treated samples in comparison to the controls. No difference ($P>0.05$) in cooking loss was found between the samples. The encapsulated phosphate was lower ($P<0.05$) in Thiobarbituric Reactive Acid Substances (TBARS), 3.5 mg/kg, in comparison to the other treatments which ranged from 15.6 to 20.4 mg/kg. The encapsulated STP was higher ($P<0.05$) in CIE a* value and lower ($P<0.05$) in
CIE b* value in comparison to the other treatments.

5.2 Introduction

The control of oxidation is of prime interest to the poultry and seafood industry. High levels of unsaturated fatty acids are contained in seafood and poultry meat (Allen and Foegeding, 1981). These unsaturated fatty acids are highly susceptible to oxidation and cause rapid deterioration of the product (Dawson and Gartner, 1983). For each additional double bond contained in an unsaturated fatty acid, the rate of oxidation increases by a factor of two (Moerck and Ball, 1974). Therefore, a technique to reduce the rate of oxidation would be extremely beneficial to the industry.

Many additives are used to aid in the cessation of oxidation such as natural antioxidants, synthetic antioxidants, and chelators. The application of phosphates to retard lipid oxidation and increase shelf stability is ubiquitous to the food industry (Dziezak, 1990). Sodium tripolyphosphate (STP) is the most common of the alkaline phosphates used in the industry (Lindsay, 1996). The many functional properties of phosphates include the ability to chelate free metals (Tims and Watts, 1958), increase pH (Miller et al., 1986), reduce phosphatase activity (McComb et al., 1979), and increase water-holding capacity (Claus et al., 1994). However, about 80% of added polyphosphate is lost by the time meat is cooked by phosphatase activity (Decker and Mei, 1996). Li et al. (1993) demonstrated that 100% of STP was lost by one day after incorporation in raw, ground turkey. Furthermore, added phosphate is limited to 0.5% by the USDA. Although, Decker and Mei (1996) reported that the addition of STP to meat after cooking resulted in tremendous improvement in controlling oxidation during storage, this would not be a practical application for the meat industry due to concerns with post-processing contamination.

Encapsulation of selected phosphates known for their strong antioxidant property could be potentially beneficial by protecting the phosphates from the phosphatases during raw meat storage and the initial stages of cooking. This protection would provide time for some of the heat sensitive phosphatases to be inactivated prior to the release of the phosphate. The objective of the research was to compare the antioxidant effects of 0.5% active encapsulated STP to 0.5% unencapsulated STP, Lemo-Fos, and Freez-Gard FP-15. Lemo-Fos and Freez-Gard FP-15 are commercial ingredient blends that include STP and are predominately used in the seafood industry (Rhodia Co., Cranbury, N.J.).

5.3 Materials and Methods

5.3.1 Formulation and Processing

Fresh, boneless, skinless tom turkey breast muscles (*pectoralis major*) were obtained from a Virginia processor immediately following slaughter and stored at a temperature of 3°C. After 24 hr, turkey breasts were cut into strips and coarse ground (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI) at speed 2 through a 12.7-mm plate and then a 4.8-mm plate. The turkey was mixed for one minute between grinding in a bowl mixer with a dough hook attachment (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI). All treatments consisted of 1% NaCl and 400 grams of ground turkey and a test ingredient such as: 0.5% unencapsulated STP, 0.5% active encapsulated STP, 0.5% Lemo-fos, or 0.5% Freez-Gard FP-15. In reference to the encapsulated phosphate, the amount of pure phosphate added to the meat is referred to as active. Rhodia Co. (Cranbury, NJ) provided the ingredients. The Freez-Gard FP-15 contained a blend of STP, lemon juice solids, and rosemary extract. A STP and lemon juice concentrate blend compiled the Lemo-fos. The STP was encapsulated by Balchem Inc. (Slate Hill, NY). Phosphates (CAP-SHURE® STP-165-50) were encapsulated with hydrogenated vegetable oil designed to release the phosphate once the temperature reached 74°C. The encapsulated ingredients were composed of 51.1-g active phosphate and 48.9-g encapsulating oil. The samples, with the ingredients, were mixed in a bowl mixer with a dough hook attachment (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI) for 2 min at speed 1. The turkey was then

stored in an unsealed bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at 3°C in the dark for 24 hr.

The samples were cooked via sous vide method in centrifuge tubes (50-mL 28x15 mm polypropylene tubes with a screw plug seal, #05-539-9, Fisher Scientific, Pittsburgh, PA), 45 grams per tube, using a water bath (Model 10-L, Fisher Scientific, Pittsburgh, PA). The samples were cooked to an end point temperature of 79°C. A cooking thermometer was used to measure the internal temperature. The temperature was taken from samples randomly throughout the water bath. The cooked samples were ground at speed 2 through a 4.8-mm plate (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI) after cooking loss was determined. The cooked ground turkey samples were then stored at 3°C in the dark until analysis.

5.3.2 pH

The pH was measured (Model 340, pH meter, Corning Inc., Corning, NY) by placing 5 g of meat into a beaker and homogenizing at a speed of 40% (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) for 1 minute with 50-mL of distilled water. The pH was analyzed for all cooked sample treatments stored for 10 days.

5.3.3 Cooking loss

The cooking loss was determined by the equation: Cooking loss = (wt of raw turkey - wt of cooked turkey)/ (wt of raw turkey) x 100. The cooked weight was determined after the turkey was cooled to 25°C. The cooked turkey was removed from the centrifuge tube and patted dry with paper towels in order to absorb excess cooking juice.

5.3.4 Thiobarbituric Acid Reactive Substances determination

TBARS determination (modification of Spanier and Traylor, 1991) on a 5-g sample in triplicates was homogenized in a 250-mL beaker with 40 mL of distilled water, 0.1 mL of 10% sodium dodecyl sulfate (SDS), and 10 mL of solution III (0.05-g propylgallate and 0.10-g ethylenediaminetetraacetic acid, EDTA, dissolved 500-mL distilled water). The Macro Ultrafine generator (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) at a speed setting of 40% for one minute was used for homogenization. The homogenate volume was raised to 100 mL with distilled water and rehomogenized. All homogenates were maintained in an ice slush. A mixture of 0.8 mL of homogenate with 3.2 mL of solution I (0.375% TBA, 0.506% SDS, and 11.7% of 80% acetic acid for a final volume of 100 mL, diluted with distilled water) with a final pH of 3.4 was placed into a centrifuge tube. Spanier and Traylor (1991) used 0.4 mL of homogenate and 1.6 mL of solution I. These volumes were doubled for analysis to scale up to the available equipment. The centrifuge tube was placed into a 95°C water bath (Model 10-L, Fisher Scientific, Pittsburgh, PA) for 60 min. Samples were shaken every 15 min throughout heating. The tubes were then cooled in tap water to room temperature. After cooling, 1 mL (modified from 0.5 mL, Spanier and Traylor, 1991) of 4°C distilled water and 5 mL (modified from 2.5 mL, Spanier and Traylor, 1991) of solution II (1:15 ratio of pyridine to n-butanol) was added to the centrifuge tube and vortexed (G 560, Vortex Genie 2, Scientific Industries Inc., Bohemia, NY) at maximum speed for 10 sec. The samples were centrifuged (Model PR-2, International Portable Refrigerated Centrifuge, International Equipment Company, Boston, MA) at room temperature (25°C) at 2180 x g for 15 min. The organic layer, located at the top of the

tube, was then pipetted into a cuvette. Absorbance of the sample was measured at 532 nm (Model Spectronic 21D, Milton Roy Company, Rochester, NY).

A standard curve was obtained by pipetting 1 mL of 5-mM tetramethoxypropane (TMP) into a test tube with 9-mL of distilled water. Five standards of 0, 0.5, 1.0, 1.5, and 2.0 mL of 0.05-mM standard TMP solutions were made. The standards were treated as samples above with the absence of homogenization.

5.3.5 Instrumental Color Determination

The samples were analyzed for CIE L*a*b* readings immediately after cooking loss was determined by using a chroma meter (Model CR-200, Minolta Corp., Osaka, Japan). Once cooking loss was determined, the turkey cylinder was cut 1 cm from the top. The cylinder was then ground through a 4.8-mm plate (Model K45SS Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI) and placed into a bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC). The chroma meter was calibrated using standard Minolta calibration plates (white plate, No. 20933026, CIE L* 97.91, a* -0.68, b* +2.45; brown plate, No. 13433234, CIE L* 57.26, a* 9.45, b* 10.77; and red plate, No. 11533041, CIE L* 52.06, a* 42.13, b* 19.38) covered with a plastic bag. Three different readings were taken on each treated, cooked turkey sample through the bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) after 10 days of storage.

5.3.6 Statistical Analysis

The experimental design incorporated was a completely randomized block design. Three replications were completed. The dependent variables analyzed were pH, cooking loss, TBARS, and Minolta color (CIE L* a* b*). Statistical analyses for the dependent

variables were analyzed by the Proc mixed procedure (SAS, 1997). When significance ($P<0.05$) was determined in the model, the means were separated by the Least Significant Difference procedure of SAS (1997).

5.4 Results and Discussion

pH and Cooking Loss

The treatment effects on pH, cooking loss, TBARS, and CIE L*a*b* are shown in Table 1. The treated samples were higher ($P<0.05$) in pH than the controls due to the alkaline properties of the phosphates. The lack of significance in pH between the commercial blends, Lemo-fos and Freez-Gard FP-15, and the 0.5% unencapsulated STP may indicate that a high level of STP was incorporated into the commercial blends. No difference ($P>0.05$) in cooking loss was found between treatments. However, previous studies found a decrease in cooking loss with the treated samples in comparison to the controls (Sickler et al., 2000). The lack of significant difference in cooking loss may be due to a high standard error. Li et al. (1993) found that the WHC was higher in samples with STP added prior to cooking than samples with added STP after cooking. Although not statistically different, the tendency for a lower water-holding capacity of the encapsulated STP in the study may have been related to the delayed release of the phosphate during thermal processing. The phosphate in the encapsulate would not have been available to function in myofibrillar protein extractability during mixing.

TBARS

The level of oxidation was reduced ($P<0.05$) by the encapsulated STP at day 10. The use of encapsulation was shown to be 83% more effective than for the control

samples. Whereas, the TBARS were 24%, 20%, and 18% lower at day 10, than the control with Lemo-fos, unencapsulated STP, and Freez-Gard FP-15, respectively. In contrast, many studies have shown a high increase in TBARS with an increase of storage time (Dawson and Schierholz 1976; Whang and Peng 1987). However, the Lemo-fos was lower ($P<0.05$) than the control at day 10. Lemo-fos was more effective due to the blend of STP and lemon juice, which are known for their antioxidative properties. Trout and Dale (1990) also found a decrease in TBARS using a similar commercial blend of lemon juice and STP.

CIE L*a*b* values

Freez-Gard FP-15 was lower ($P<0.05$) in CIE L* value in comparison to the controls and Lemo-fos. The encapsulated STP samples were higher ($P<0.05$) in CIE a* values than the other treatments by a 10 fold factor. The higher CIE a* values may be related to the preservation of reduced myoglobin as a result of overall reduction in oxidative conditions in the meat because of the improved antioxidative properties of the phosphate. Akamittath et al. (1990) stated that lipid oxidation was interrelated to pigment oxidation. The high temperature and increase in oxidation with the other treatments resulted in a low level of pigmentation. The other treatments were extremely low with the control having a CIE a* value of –0.12. Furthermore, the encapsulated STP was lower ($P<0.05$) in CIE b* values than the other treatments.

5.5 Conclusions

In comparison to the commercial blends and unencapsulated STP, the encapsulated phosphate demonstrated the ability to be the most effective in the reduction

of TBARS. Although an increase in CIE a* value was detected, further research should be conducted to determine the effect on the overall appearance. The use of the encapsulated phosphate could provide meat processors with the ability to produce a higher quality, more shelf stable product.

5.6 References

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513-1517.

Allen, C.E. and Foegeding, E.A. 1981. Some lipid characteristics and interactions in muscle food. A review. Food Technol. 35:253-257.

Dawson, L.E., and Gartner, R. 1983. Lipid oxidation in mechanically deboned poultry. Food Technol. 37:112-116.

Dawson, L.E. and Schierholz, K. 1976. Influence of grinding, cooking and refrigerated storage on lipid stability in turkey. Poultry Sci. 55:618-622.

Dziezak, J.D. 1990. Phosphates improve many foods. Food Technol. 44(4):80-92.

Decker, E.A. and Mei, L. 1996. Oxidative Processes in Meat. Antioxidant mechanisms and applications in muscle foods. Proceedings from the 49th Reciprocal Meat Conference, Provo, UT. Published by the American Meat Science Assoc. Chicago, IL. 49:64-72.

Li, W., Bowers, J.A., Craig, J.A., and Perng, S.K. 1993. Sodium tripolyphosphate stability and effect in ground turkey meat. J. Food Sci. 58:501-504, 521.

Lindsay, R.C. 1996. Food Additives. Ch. 12 in Food Chemistry, 3rd ed., O.R. Fennema (ed). p. 17-94. Marcel Dekker, Inc., N.Y.

Moerck, K.E., and Ball, H.R. 1974. Lipid oxidation in mechanically deboned chicken meat. J. Food Sci. 39:876-879.

SAS Institute, Inc. 1997. SAS Users Guide to the Statistical Analysis System. SAS. Cary, NC.

Sickler, M.L., Claus, J. R., Eigel, W.N., Marriott, N.G., and Wang, H. 2000. Inhibition of lipid oxidation with phosphates in muscle foods. MS. thesis, Virginia Polytechnic Instit. & State Univ., Blacksburg.

Spanier, A.M., and Traylor, R.D. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. J. Muscle Foods 2:165-176.

Trout, G.R. and Dale, S. 1990. Prevention of warmed-over flavor in cooked beef: effect of phosphate type, phosphate concentration, a lemon juice/ phosphate blend, and beef extract. J. Agric. Food Chem. 38:665-669.

Whang, K. and Peng, I.C. 1987. Lipid oxidation in ground turkey skin and muscle during storage. Poult. Sci. 66:458-466.

5.7 Table

Table 1. Effects of adjuncts on various physical and chemical properties of cooked ground turkey breast stored for 10 days at 3°C.

Treatment[1]	pH	Cooking Loss (%)	TBARS (mg/kg)	CIE Values L*	a*	b*
Control	6.19^{c}	20.4^{a}	20.4^{a}	76.4^{a}	-0.12^{c}	17.5^{a}
0.5% Unencapsulated STP	6.41^{a}	17.9^{a}	16.3^{ab}	75.4^{ab}	0.32^{b}	17.6^{a}
0.5% Encapsulated STP	6.35^{b}	19.0^{a}	3.5^{c}	75.3^{ab}	2.98^{a}	16.3^{b}
0.5% Lemo-Fos	6.38^{ab}	17.7^{a}	15.6^{b}	75.9^{a}	0.25^{bc}	17.7^{a}
0.5% Freez-Gard FP-15	6.40^{ab}	16.5^{a}	16.7^{ab}	74.3^{b}	0.07^{bc}	17.4^{a}
Standard Error	0.018	1.3	1.3	0.40	0.12	0.13

[a-c] Means bearing unlike superscripts within each column are different (P<0.05).

[1]Treatment - all treatments have 1% NaCl and were cooked to an internal temperature of 79°C. Percentages are amounts of the ingredient added on the meat weight basis, except encapsulated STP. The 0.5% for the encapsulated STP reflects the amount of STP (active) added to the meat.

Chapter 6

Efficacy of TBARS Determination by Use of Rapid Extraction and Cryogenic Freezing

6.1 Abstract

The effects of freezing in liquid nitrogen and time of particle size reduction on lipid oxidation (TBARS) were determined on uncooked and sous vide cooked (74°C, internal temperature) ground turkey breast samples containing 1.0% NaCl. The treatments were: control (no freezing or storage), frozen with immediate particle reduction (2-4 mm particle size) and subsequent frozen storage (0, 14, and 33 days at -80°C), and storage of frozen intact samples (14 and 33 days at -80°C). Freezing alone did not affect (P>0.05) Thiobarbituric Acid Reactive Substances (TBARS). Samples stored intact were not different (P>0.05) than those that were first reduced in particle size before storage. Samples that were frozen intact and stored or immediately reduced in particle size before frozen storage for up to 33 days were not different (P>0.05) in TBARS than non-stored samples. Based on these results, the samples can be frozen in liquid nitrogen and stored (reduced in particle size or intact) for approximately one month without significant changes in TBARS.

6.2 Introduction

At times, it is not feasible to measure lipid oxidation using Thiobarbituric acid reactive substances (TBARS) analysis on a large number of samples in one day. Fortunately, the use of cryogenic freezing can completely freeze products in less than five

min, depending on the thickness and rate of movement of a product (Henry et al., 1995). Furthermore, cryogenic freezing minimizes the dislocation of water, reduces textural change, decreases the fluid loss during freezing and thawing, and reduces chemical degradation (Sebranek, 1982). These effects are a result from the presence of small intracellular ice crystals caused by the fast freezing capabilities of liquid nitrogen (Tomas et al., 1990). For example, Lind et al. (1971) reported increased tenderness and decreased cooking loss in lamb chops frozen in liquid nitrogen when compared with slow freezing.

The oxidation of meat during frozen storage is due to the changes in the triglyceride fraction and low water activity (van Laack, 1994). The level of susceptibility to lipid oxidation varies from species to species. Meats which contain mostly saturated fat, such as beef and lamb, are much more resistant to lipid oxidation deterioration than are those containing more unsaturated fats, such as pork and turkey (Dugan, 1987). Moerck and Ball (1974) state that for each additional bond contained in an unsaturated fatty acid, the rate of oxidation increases by a factor of two. The objective of this experiment was to determine the effects of cryogenic freezing, particle reduction, and storage time on lipid oxidation (TBARS) in uncooked and cooked ground turkey breasts. Turkey was chosen because of its high polyunsaturated fatty acid content (Allen and Foegeding, 1981), and decrease in storage stability in comparison to chicken (Wilson et al., 1976).

6.3 Materials and Methods

6.3.1 Formulation and Processing

Pectoralis major muscles (PMM), from Tom turkeys, were obtained fresh on the day of processing from a Virginia plant and stored in a cooler at a temperature of 3°C. Six replications consisted of different PMM using only the right lobe. Skinless whole turkey breasts were cut into strips 2.5 cm thick and coarse ground through a 9.5-mm plate (Model K45SS Kitchen Aid™ Classic, Kitchen Aid Inc., St. Joseph, MI). The ground turkey (800-g) was mixed with NaCl (1% of meat weight) for 2 min at speed 1 using a dough hook attachment (Model K45SS, Kitchen Aid™ Classic, Kitchen Aid Inc., St. Joseph, MI). The turkey was ground through a 4.8-mm plate (Model K45SS, Kitchen Aid™ Classic, Kitchen Aid Inc., St. Joseph, MI). The raw samples for Day 0 were analyzed immediately after treatment. The raw samples at 5 days were stored without light exposure in an unsealed bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at 3°C.

The ground turkey was cooked by a sous vide method using a water bath (Model 10-L, Fisher Scientific, Pittsburgh, PA). A total of six 50-mL centrifuge tubes (50-mL 28x15 mm polypropylene tubes with screw plug seal, #05-539-9, Fisher Scientific, Pittsburgh, PA), each containing 45 g of turkey, were used for each treatment. The samples were placed into an 80°C water bath and cooked to an internal temperature of 74°C. A cooking thermometer, inserted into the center of the tube, measured the internal sample temperature. The temperature was taken from samples randomly throughout the water bath. The cooked turkey samples were then mixed at speed 1 (Model K45SS

Kitchen Aid™ Classic, Kitchen Aid Inc., St. Joseph, MI) and ground through a 4.8-mm plate (Model K45SS Kitchen Aid™ Classic, Kitchen Aid Inc., St. Joseph, MI). The cooked samples for Day 5 were stored in an unsealed bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at a temperature of 3°C in the dark.

Four freezing techniques were applied to uncooked and cooked samples. Treatment 1 was the control, which did not use liquid nitrogen. Treatment 2 consisted of freezing a 10-g sample contained in a 6-oz bag (B679, Nasco Whirl-pack, Fisher Scientific, Pittsburgh, PA) with liquid nitrogen for 40 sec on Day 0. Upon removal, the ground turkey sample was immediately reduced into 2 to 4-mm particles and 5 g was used for TBARS determination. Reduction was achieved by repeated force onto the sample. Treatment 3 consisted of placing a 10-g sample contained in a 6-oz bag (B679, Nasco Whirl-pack, Fisher Scientific, Pittsburgh, PA) in liquid nitrogen for 40 sec and storing the intact sample in an ultralow freezer (-80°C). On day 14 (treatment 3) and 33 (treatment 4), the intact samples were reduced into 2 to 4-mm particles and placed into TBARS solutions. Treatment 4 was prepared by reducing the frozen (10 grams, 40 sec in liquid nitrogen) sample into 2 to 4- mm particles immediately after removal from the liquid nitrogen. Treatment 4 samples were stored in an ultralow freezer (-80°C) and analyzed at 14 days (treatment 5) and 33 days (treatment 6).

6.3.2 Thiobarbituric Acid Reactive Substances determination

TBARS determination (modification of Spanier and Traylor, 1991) on a 5-g sample in triplicate was homogenized in a 250-mL beaker with 40 mL of distilled water, 0.1 mL of 10% sodium dodecyl sulfate (SDS), and 10 mL of solution III (0.05-g propylgallate and 0.10-g ethylenediaminetetraacetic acid (EDTA) dissolved 500-mL

distilled water). The Macro Ultrafine generator (Virtishear 225318, The Virtis Company Inc., Gardiner, NY) at a speed setting of 40% for one minute was used for homogenization. The homogenate volume was raised to 100 mL with distilled water and rehomogenized. All homogenates were maintained in ice slush. A mixture of 0.8 mL of homogenate with 3.2 mL of solution I (0.375% TBA, 0.506% SDS, and 11.7% of 80% acetic acid to 100 mL with distilled water) with a pH of 3.4 was placed into a centrifuge tube. This was similar to Spanier and Traylor (1991) who used 0.4 mL of sample and 1.6 mL of Solution I. These volumes were doubled for analysis to scale up to the available equipment. The centrifuge tube was placed into a 95°C water bath (Model 10-L, Fisher Scientific, Pittsburgh, PA) for 60 min. Samples were shaken every 15 min throughout heating. The tubes were cooled in tap water to room temperature. After cooling, 1 mL (modified from 0.5 mL, Spanier and Traylor, 1991) of 4°C distilled water and 5 mL (modified from 2.5 mL, Spanier and Traylor, 1991) of solution II (1:15 ratio of pyridine to n-butanol) was added and vortexed (G560, Vortex Genie 2, Scientific Industries Inc., Bohemia, NY) at maximum speed for 10 sec. The samples were centrifuged (Model PR-2, International Equipment Company, Boston, Mass.) at room temperature (25°C) at 2180 x g for 15 min. The organic layer, located at the top of the tube, was then pipetted into a cuvette. The samples were spectrophotometrically measured for absorbancy at a wavelength of 532 nm (Model Spectronic 21D, Milton Roy Co., Rochester, NY).

A standard curve was obtained by pipetting 1 mL of 5.0-mM tetramethoxypropane (TMP) into a test tube with 9-mL distilled water. Five standards of 0, 0.5, 1.0, 1.5, and 2.0 mL of 0.05-mM standard TMP solutions were made. The standards were then treated as samples above with the absence of homogenization.

6.3.3 Statistical analysis

A randomized complete block design composed of six replications was used for statistical analysis. The dependent variable that was interpreted is TBARS. The statistical analysis was determined by the Proc mixed procedure (SAS, 1997). When a significant difference was detected, means were separated by the Least Significant Difference procedure of SAS (1997).

6.4 Results & Discussion

The uncooked treatments were low in TBARS which assured that samples were fresh regardless of freezing (Figure 1), particle reduction (Figure 2), or storage time (Figure 3). Usually a threshold of 1.0 mg/kg indicates freshness, although TBARS may vary due to functions of meat fatty acid composition, nonmeat ingredients, or by TBA method used (Decker, 1998). TBARS results were similar (less than 1.0 mg/kg) to those reported by Akamittath et al. (1990) for uncooked restructured turkey steaks frozen for 2 weeks at -10°C. An increase in lipid oxidation in the cooked samples may be partially attributed to the release of heme-iron upon cooking (Sato and Hegarty, 1973). Wilson et al. (1976) found similar results with TBARS in a comparison of the uncooked and cooked turkey samples.

The cooked samples had higher ($P<0.05$) TBARS than the uncooked samples (data not shown). Salih et al. (1989) also found that cooked turkey meat, breast and thigh, was higher in TBARS value in comparison to the raw turkey meat. The increase in TBARS with the cooked samples is due to the nonheme iron, a major prooxidant, released from heme pigments during the heat treatment (Schricker and Miller, 1983;

Chen et al., 1984). There were no differences (P>0.05) between the control and frozen and immediately reduced, in uncooked and cooked samples, demonstrating that freezing alone did not effect TBARS (Figure 1). Unlike cryogenically frozen samples, refrigerated samples oxidize rapidly throughout storage (Salih et al., 1989). The effects of cryogenic freezing and ultralow temperature storage with TBARS are not significant due to absence of chemical degradation and microbiological activity (Sebranek, 1982). Samples that were frozen intact and stored or immediately reduced in particle size before frozen storage for up to 33 days were not different (P>0.05) in TBARS than non-stored samples (Figure 2). However the immediate reduced particle reduction technique appeared to be the most efficient for TBARS analysis. Regardless of storage time, the samples were not different (P>0.05) (Figure 3). Schlimme et al. (1991a) also found no difference in TBARS with turkey frankfurters blast-frozen and stored at -9.4°C and -18°C for 16 months. The stability of the turkey frankfurters was most likely associated with the addition of nitrite. Schlimme et al. (1991b) found a significant difference in with TBARS with the storage duration time of blast-frozen turkey breast rolls stored at –9.4°C and -18°C for 12 months. The increase in TBARS and decrease in sensory quality beyond six months of storage indicated the presence of lipid oxidation.

Overall, within either uncooked or cooked samples, there were no differences (P>0.05) in TBARS, regardless of particle reduction or storage application. The procedure in which the samples were frozen and immediately reduced in particle size appeared to be the most convenient procedure. Based on these results, the samples can be frozen in liquid nitrogen and stored up to 33 days without significant changes in TBARS.

6.5 Conclusions

Regardless of freezing, particle size, or storage up to 33 days, the TBARS were not different. Therefore, freezing samples in liquid nitrogen facilitates the determination of lipid oxidation (TBARS) when an excessive number of samples cannot be analyzed immediately.

6.6 References

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55:1513-1517.

Allen, C.E. and Foegeding, E.A. 1981. Some lipid characteristics and interactions in muscle food. A review. Food Technol. 35:253-257.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H., and Ku, P.K. 1984. Some factors influencing the nonheme content of meat and its implications in oxidation. J. Food Sci. 49:581-584.

Claus, J.R., and Marriott, N.G. 1995. Influence of vitamin E on improving meat colour and lipid stability. Meat Focus Internat. 4(Part 3):109-112.

Decker, E.A. 1998. Personal communication. Dept. of Food Science, Univ. of Mass., Amherst, MA.

Dugan, L.R., Jr. 1987. Fats. Ch. 3 (part 2) in The Science of Meat and Meat Products, 3rd ed., J.F. Price and B.S. Schweigert. p. 103-114. Food & Nutrition Press, Inc., Westport, CT.

Henry, L.K., Boyd, L.C., and Green, D.P. 1995. Cryoprotectants improve physical and chemical properties of frozen blue crab meat (*Callinectes sapidus*). J. Sci. Food Agric. 69:15-20.

Lind, M.L., Harrison, D.L., and Kropf, D.H. 1971. Freezing and thawing rates of lamb chops: effects on palatability and related characteristics. J. Food Sci. 36:629-633.

Moerck, K.E., and Ball, H.R. 1974. Lipid oxidation in mechanically deboned chicken meat. J. Food Sci. 39:876-879.

Salih, A.M, Price, J.F., Smith, D.M., and Dawson, L.E. 1989. Lipid oxidation in turkey meat as influenced by salt, metal cations, and antioxidants. J. Food Qual. 12:71-83.

SAS Institute, Inc. 1997. SAS Users Guide to the Statistical Analysis System. SAS. Cary, NC.

Sato, K. and Hegarty, G.R. 1973. Warmed-over flavor in cooked meats. J. Food Sci. 38:398-403.

Schlimme, D.V., Smith, M.A., and Ali, L.M. 1991a. Influence of freezing rate, storage temperature, and storage duration on the quality of turkey frankfurters. ASHRAE Transactions 97:214-220.

Schlimme, D.V., Smith, M.A., and Ali, L.M. 1991b. Influence of freezing rate, storage

temperature, and storage duration on the quality of cooked turkey breast roll. ASHRAE Transactions 97:221-227.
Schricker, B.R., and Miller, D.D. 1983. Effects of cooking and chemical treatment on heme and nonheme iron in meat J. Food Sci. 48:1340.
Sebranek, J.G. 1982. Use of cryogenics for muscle foods. Food Technol. 36(4):120-127.
Spanier, A.M., and Traylor, R.D. 1991. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. J. Muscle Foods 2:165-176.
Tomas, M.C. and Anon, M.C. 1990. Study on the influence of freezing rate on lipid oxidation in fish (salmon) and chicken breast muscles. Int. J. Food Sci. and Technol. 25:718-721.
van Laack, R.L.J.M. 1994. Spoilage and preservation of muscle foods. Ch. 14 in *Muscle Foods: Meat, Poultry, and Seafood Technology*, D.M. Kinsman, A.W. Kotula, and B.C. Breidenstein (Ed.), p.378-405. Chapman & Hall, NY.
Wilson, B.R., Pearson, A.M., and Shorland, F.B. 1976. Effect of total lipids and phospholipids on warmed-over-flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. J. Agric. Food Chem. 24 (1):7-11.

6.7 Figures

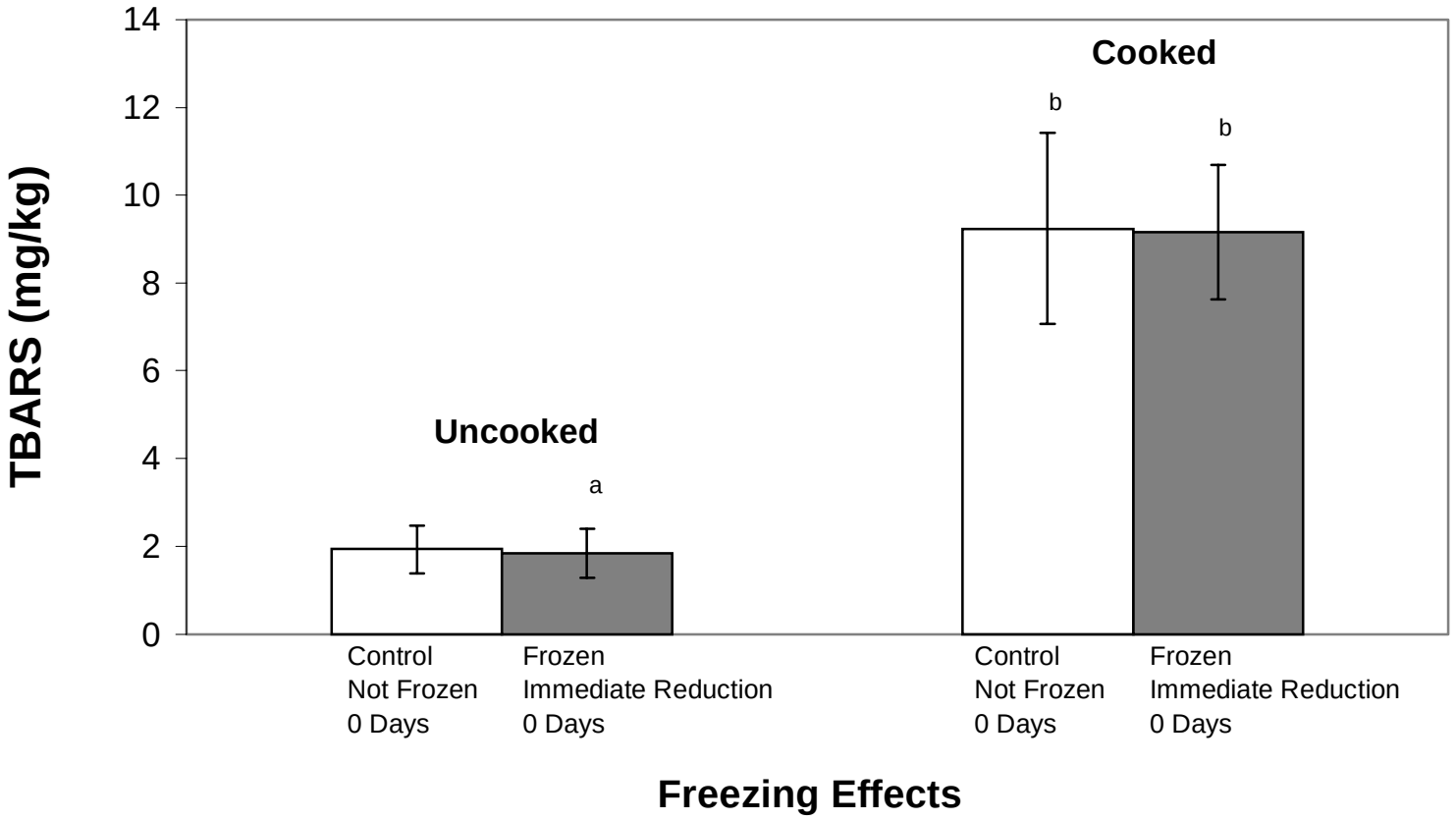


Figure 1- Freezing effects on TBARS of nonstored, uncooked and cooked, ground turkey breast. Frozen turkey was submerged into liquid nitrogen and immediately reduced. Paired bars with unlike letters are different (P<0.05).

Vertical lines are standard deviations.

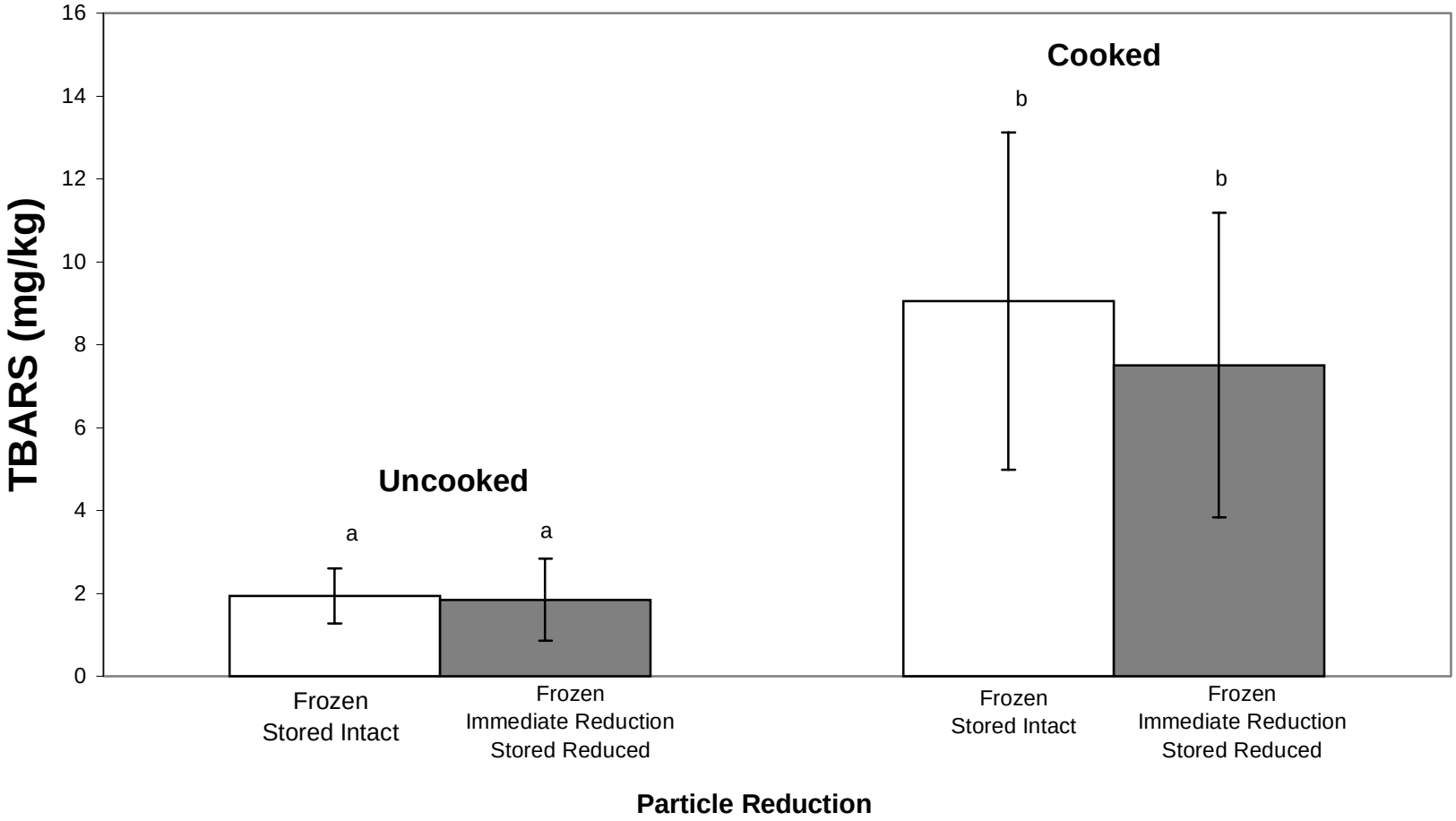


Figure 2- Particle reduction effects on TBARS of uncooked and cooked ground turkey breast frozen in liquid nitrogen and stored in an ultralow temperature intact and immediately reduced. Paired bars with unlike letters are different (P<0.05). Vertical bars are standard deviations.

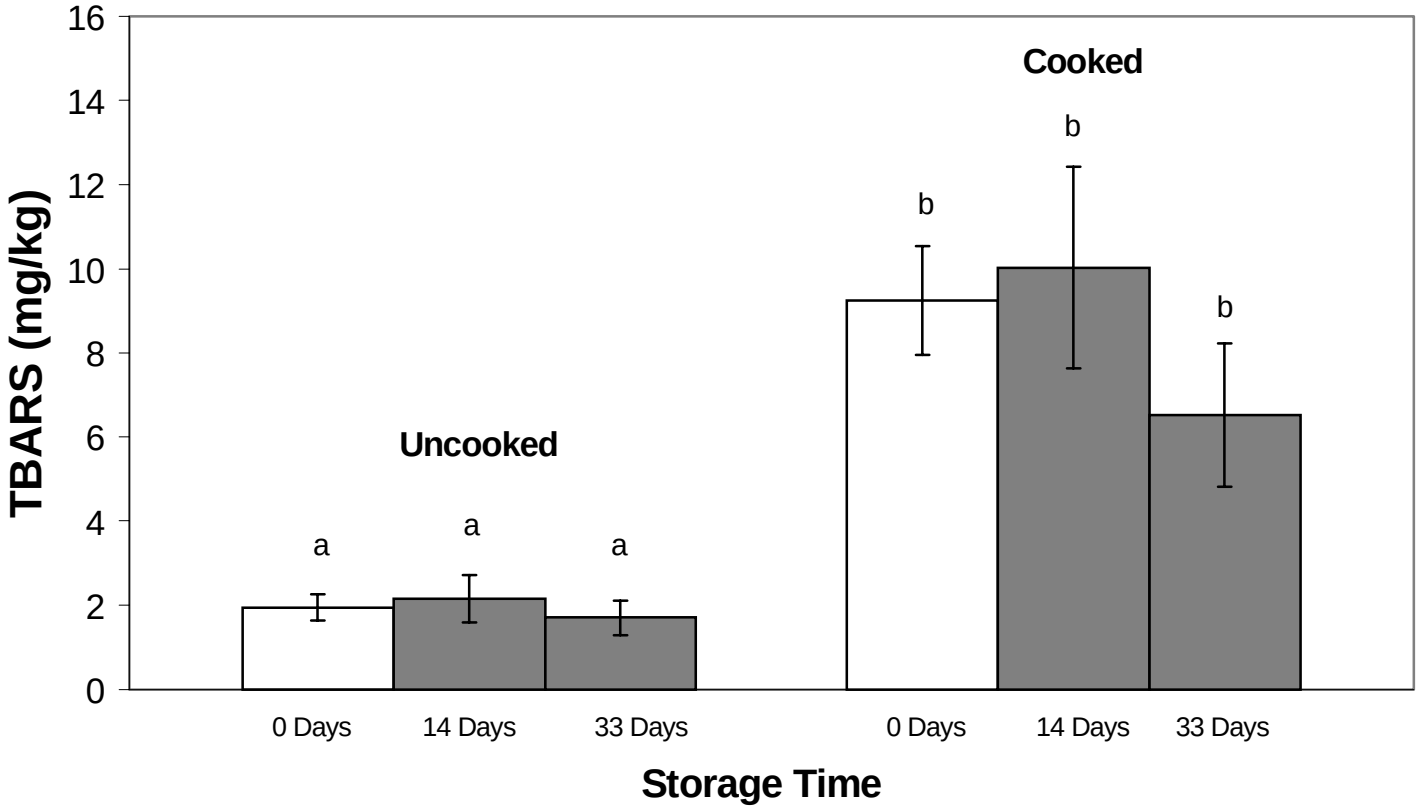


Figure 3- Storage effects on TBARS of uncooked and cooked, ground turkey breast frozen in liquid nitrogen and stored in ultralow temperature for 0, 14, and 33 days. Paired bars with unlike letters are different (P<0.05). Vertical lines above bars are standard deviations.

Chapter 7

Summary and Conclusions

Many factors appear to affect the ability of encapsulated phosphate to control lipid oxidation. Some of these factors include the: duration of time between when the phosphate is added and thermal processing, type of phosphate encapsulated, blend of encapsulated and unencapsulated phosphates, end point temperature, storage time after cooking, and amount of total active phosphate used.

A comparison in the use of SAPP and STP in ground beef patties favored the incorporation of STP to further decrease the rate of oxidation. Although the encapsulated STP and SAPP reduced oxidation, neither of the encapsulated phosphates was more effective than the unencapsulated phosphates due to the lack of storage time prior to heat processing. The lack of storage time did not allow sufficient time for the phosphatases to breakdown the available phosphates. However, with a storage time of 24 hrs pre-cook, 0.1% encapsulated STP was essentially as effective as 0.2% unencapsulated STP in the reduction of oxidation.

In cooked ground turkey, the use of completely encapsulated or a blend of encapsulated and unencapsulated STP gave improvements of 63% to 80% in controlling lipid oxidation when compared to the product containing only unencapsulated STP. However, stored ground turkey containing encapsulated phosphates had higher CIE a* values. Although, an increase in endpoint temperature reduced the level of CIE a* in all treatments and over storage time. The increase in temperature may have decreased the concentration of myoglobin, which is associated with redness. However, the use of encapsulation decreased the level of oxidation which could have preserved the reduced

state of myoglobin. The most desirable results in cooked ground turkey were seen when encapsulated phosphate was used with a 4 hr storage time prior to cooking to an end point temperature of 79 °C.

Encapsulated STP was compared to various commercial antioxidant blends and unencapsulated STP in ground turkey. Encapsulated STP resulted in the greatest (83%) inhibition of lipid oxidation followed by Lemo-fos (24%), unencapsulated STP (20%), and Freez-Gard FP-15 (18%) when compared to the control. However, the CIE a* was higher in the turkey containing encapsulated phosphate possibly due to a higher concentration of reduced myoglobin from a decrease in lipid oxidation.

At higher levels of available phosphate, the cooking loss was decreased in comparison to the control. This was in direct correlation to the level of pH. The higher pH was more effective in the retention of moisture in the samples.

A methodology to possess the ability to analyze large numbers of TBARS samples was investigated. The use of cryogenic freezing with raw and cooked ground turkey did not affect the rate of oxidation. Samples stored intact in comparison to those immediately reduced in particle size after freezing did not have any effect on lipid oxidation. The length of ultralow temperature storage of the samples did not effect TBARS due to the immobilization of oxidants at these low temperatures. Thus, cryogenic freezing and ultralow temperature storage can be used in research and industry without affecting lipid oxidation.

The necessity of a means to reduce lipid oxidation in muscle foods is immense. The use of sodium tripolyphosphate has been effective in the reduction of lipid oxidation. However, using current technology, encapsulated phosphates can extend the benefits of

phosphates in muscle foods. Although these results are promising, further research involving the encapsulation effects on controlling lipid oxidation is needed to evaluate the degree of encapsulation, types of phosphates, applications in other food substances, and effects on sensory characteristics.

Appendix

8.1 Thiobarbituric Acid Reactive Substances (TBARS) Determination Lab Procedure

REAGENTS

1. 10% sodium dodecyl sulfate (SDS). Dissolve 5.0 g of SDS in distilled water and bring up to 50 mL. Need 0.1 mL per sample.
2. Solution I. 0.375% TBA (thiobarbituric acid), 0.506% SDS, and 9.370% acetic acid. Dissolve 0.375 g of TBA, 0.506 g of SDS in distilled water, add 11.9 mL of 80% acetic acid, and bring up to 100 mL. Adjust pH to 3.4 with HCl or NaOH. Need 3.2 mL per sample. May be refrigerated but must be re-mixed at least two hr prior to experiment. Do not store for over three days.
3. Solution II. Mix n-butanol and pyridine at the ratio of 15:1. Make fresh daily under a hood and store in an amber bottle. Need 5.0 mL per sample. Used under a hood.
4. Solution III. Antioxidant and chelator solution. Dissolve 0.05 g of propylgallate and 0.10 g of EDTA (ethylenediaminetetraacetic acid) in distilled water and bring up to 500 mL. Prepare fresh daily. Need 10 mL per sample.
5. 5.0 mM MDA (TMP) stock solution. For calculation purposes, 1 mole of TMP standard was treated as equivalent to 2 moles of reacting equivalents of MDA. Transfer 0.829 mL of TMP (99% tetramethoxypropane, Sigma Chemical Company) standard solution into a 1000 mL volumetric flask, add distilled water, and bring up to the mark (1000 mL). The concentration of this TMP stock solution is 5.0 mM. Turn the flask upside down several times to mix the solution.

STANDARD CURVE

1. Pipet 1.0 mL of 5.0 mM TMP stock solution into a test tube and add 9.0 mL of distilled water. The TMP concentration of this standard solution is 0.5 mM.
2. If estimated TBARS values are high (cooked meat), pipet 0.0 (blank), 0.5, 1.0, 1.5, and 2.0 mL of 0.5 mM standard TMP solution to 100 mL volumetric flasks, respectively. If estimated TBARS values are low (raw meat), pipet 0.0 (blank), 0.05, 0.1, 0.15, and 0.2 mL of 0.5 mM standard TMP solution to 100 mL volumetric flasks, respectively.
3. Add 0.1 mL of 10% SDS and 10 mL of solution III to each flask and bring the standard solution up to the mark (100 mL) with distilled water. Mix the solutions by turning the flask upside down several times. For samples with a high TBARS value, the TMP concentration in the standard solutions are 0.0 (blank), 2.5, 5.0, 7.5, and 10.0 μM. For samples with low TBARS value, The TMP concentration in the standard solutions are 0.0 (blank), 0.25, 0.50, 0.75, and 1.0 μM.
4. Mix 0.8 mL of each standard TMP solution with 3.2 mL of solution I in a glass centrifuge tube. Continue the rest of the step 4 in the procedure. Follow steps 5 through 7 in the procedure.

PROCEDURE

1. Turn on the power to the water bath and set the temperature to 95°C. Turn the safety switch clockwise to the end. When the temperature reaches 95°C, set the safety switch to control the temperature.
2. Homogenize 5.0 g of meat sample in a 250 mL beaker with 40.0 mL of distilled water, 0.1 mL of 10% SDS, and 10.0 mL of solution III, using a Virtis Homogenizer

in short bursts. The Macro Ultrafine generator should be used on the homogenizer and the speed setting should be 70%.

3. Clean the generator and bring the homogenate volume to 100 mL with distilled water. Maintain all homogenate in ice slush. Also, place a beaker of distilled water in ice for step 5.

4. Transfer 0.8 mL of homogenate into a glass centrifuge tube, add 3.2 mL of solution I, and mix with a Vortex mixer. Then, cap the tubes loosely with snap caps and incubate the mixture in a 95 ^{o}C water bath for 60 min. Mix the solution every 15 min.

   Do the same to the solutions for the standard curve (see next page).

5. Cool the tubes in tap water. Then add 1.0 mL of cold distilled water (4 ^{o}C) and 5.0 mL of solution II to each tube under hood and mix on vortex mixer at maximum speed for 10 sec under hood.

6. Centrifuge the solution at room temperature (25 ^{o}C) at 3500 rpm for 15 min. Transfer about 4.0 mL organic solution (top layer) to cuvettes.

7. Read the absorbance of the organic solution at 532 nm within 1 hr. Let spectrophotometer warm up for 1 min. Do not leave spectrophotometer on for a long period of time.

CALCULATION OF SAMPLE TBARS:

1. Calculate the equivalent TBARS* value of standard TMP solutions

In order to simplify the calculation, the equivalent TBARS* value of each standard TMP solution is calculated first. The equivalent TBARS* value of the standard TMP solution is defined as the TBARS values of a meat sample (5.0 g) if their absorbance are the same.

The equivalent TBARS* value of each standard TMP solution is calculated as following:

$$\text{TBARS}^* = \frac{V_i \times \mathbf{C_o} \times 10^{-6} \text{ mol} \times 72.3 \text{ g/mol} \times 10^6 \text{ mg/kg}}{5.0 \text{ g}}$$

$$= 14.46 \times V_i \times \mathbf{C_o} \text{ mg/kg}$$

where $\mathbf{C_o}$ = TMP concentration of the stock solution, 0.5 mM for this procedure.

V_i = Volume (mL) of stock solution in each standard solution.

The results are listed in the following table:

Cooked Meat Sample		Raw Meat Samples	
Standard TMP (μM)	Eql. TBARS* (mg/kg)	Standard TMP (μM)	Eql. TBARS* (mg/kg)
0.0	0.0	0.00	0.00
0.25	3.6	0.25	0.36
0.50	7.2	0.50	0.72
0.75	10.8	0.75	1.08
1.00	14.4	1.00	1.44

2. Conduct regression on absorbance and Equivalent TBARS* of the standard TMP solutions. Determine K_s for the following equation:

$$\text{TBARS}^* = K_s \times A_s$$

where A_s = absorbance of the standard solutions.

3. Calculate TBARS value for samples:

Sample TBARS = K_s x A

where K_s is the slope and A is the absorbance of the samples.

4. A spreadsheet can be used to calculate TBARS value.

8.2 Antioxidative Effect of Encapsulated Sodium Tripolyphosphate Added to Ground Beef Patties and Stored (24 hr) Before Cooking

Introduction

This research was an extension of the preliminary studies performed in ground beef patties. Due to the results obtained from the previous studies, this experiment was conducted to investigate the length of storage time needed for the encapsulated samples to reduce lipid oxidation before cooking. The increase in storage time before cooking should hypothetically give time for the natural phosphates to be depleted. Upon cooking the encapsulate will melt thus, replenishing phosphate levels to aid in the reduction of lipid oxidation. Therefore, the objectives were to evaluate the effects of added encapsulated phosphates on cooked ground beef patty cooking loss, cooked pH, and TBARS values (3 and 11 days post-cook storage) for patties formed 24 hr prior to cooking.

Materials and Methods

Formulation and Processing

A beef *semimembranous* muscle was obtained from Virginia Polytechnic Institute and State University Meat Laboratory. The *semimembranous* muscle was removed after 24 hr postmortem from a bull carcass stored at 4°C. The muscle was trimmed of external fat and sliced into 2.5 cm strips. The strips were then coarse ground (Model 4532, Hobart Manufacturing Co., Troy, OH) through a 12.7-mm plate and mixed in a bowl

mixer using dough hook attachment (Model A-200, Hobart Manufacturing Co., Troy, OH) for 1 min.

The ground meat was then divided into seven treatments. All treatments consisted of 1% NaCl (based on meat weight) and 400 grams of ground beef and one of the following: no STP (control), the addition of unencapsulated STP (0.05%, 0.10%, or 0.20% active) or encapsulated STP (STP-165-50) (0.025%, 0.05%, or 0.10% active) based on the meat weight. Active refers to the amount of pure phosphate added to the meat. The phosphates were provided by Rhodia Co. (Cranbury, NJ). The STP was encapsulated by Balchem Inc. (Slate Hill, NY). Encapsulated phosphates were encapsulated with hydrogenated vegetable oil designed to release the phosphate once the temperature reached 74°C. The encapsulating ingredients were composed of 51.1% phosphate and 48.9% encapsulating oil. The samples were mixed for 2 min at speed 1 and ground through a 4.8-mm plate (Model K45SS Kitchen Aid Classic Mixer™, Kitchen Aid Inc., St. Joseph, Michigan). The treated samples were vacuum packed into an oxygen permeable bag and stored for 24 hr at 4°C.

The treated samples were formed into 85-g patties with a stainless steel hand hamburger patty press (11.4 cm diameter, Twoyco, Taiwan, R.O.C). Two patties per treatment were formed for Day 3 and Day 11 storage after cooking. Patties were randomly placed on aluminum broiler pans and heated at 163°C in an oven (Model Mark V, Blodgett Inc., Burlington, VT) to an internal temperature of 76°C. The internal temperature was measured by thermocouples placed into the center of the patty (AMSA, 1995). Thermocouples were placed randomly and attached to a datalogger (Model 5100, Electronic Controls Design, Milwaukie, OR). Day 0 patties were analyzed immediately

after cooking. The patties were stored in the dark in an unsealed bags (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at a temperature of 3°C. Before analysis, the patties were ground through a 4.8-mm plate (Model K45SS Kitchen Aid Classic Mixer™, Kitchen Aid Inc., St. Joseph, MI).

Chemical Determinations

Raw and cooked samples were analyzed for pH and TBARS following the procedures outlined in Chapter 3. The pH of the raw, and cooked samples were measured on Day 0, and Day 3 and Day 11 of storage, respectively.

Results and Discussion

No differences were detected between treatments in the pH of the raw and cooked samples regardless of storage time. Generally, cooking loss percentages decreased as phosphate incorporation increased. Encapsulation tended to increase cooking loss in the 0.10% active encapsulated STP over the unencapsulated STP. The antioxidant ability of encapsulated STP (0.05% active) was improved by 24.8% in day 3 and 18.8% on day 11 in comparison to the control. In the patties containing 0.10% active STP, there was a 30.0% improvement on day 3 and 74.5% improvement at day 11 when compared to the control, as a result of encapsulation. Furthermore, 0.10% active, encapsulated phosphate was essentially as effective as 0.20% unencapsulated phosphate at both day 3 and 11 days.

Conclusions

These results support the theory that the encapsulation process can be an effective means of protecting the phosphate from phosphatases during raw meat storage. Thus, the

added phosphate protection improves the control of lipid oxidation. Since this research was only preliminary, a similar experiment should be designed and conducted to include several replications in order to statistically validate these observations.

Table

Table 1. Means for raw[1] and cooked[2] ground beef patties after the addition of phosphates .

	pH			Cooking Loss	TBARS values (mg/kg)	
		Cooked			Cooked	
Treatments[3]	Raw	Day 3	Day 11	(%)	Day 3	Day 11
0.0% control	5.1	5.7	5.7	31.7	10.86	24.27
0.025% encapsulated	N/D[4]	5.8	5.8	31.18	9.57	20.74
0.05% unencapsulated	N/D[4]	5.8	5.8	29.53	11.03	21.47
encapsulated	N/D[4]	5.8	5.8	29.13	8.3	17.44
0.10% unencapsulated	N/D[4]	5.8	5.8	24.16	7.04	16.67
encapsulated	5.2	5.8	5.9	29.12	4.91	4.25
0.20% unencapsulated	5.4	5.8	5.9	22.16	4.23	4.04

[1]Raw- immediately analyzed after treatment.
[2]Cooked- stored 24hr after treatment prior to cooking.
[3]Treatments-additional 1% NaCl was added and percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[4]N/D- not determined.

8.3 Encapsulated Sodium Tripolyphoshate (STP) Effects on Raw and Cooked Ground Turkey Meat

Introduction

Preliminary tests were analyzed to determine if encapsulating phosphate would improve the antioxidative properties of the phosphate in stored, ground, cooked turkey. It was theorized that encapsulation could potentially be beneficial by protecting the phosphates from the phosphatases during raw meat storage and the initial stages of thermal processing. This protection can provide time for some of the heat sensitive phosphatases to be inactivated prior to the release of the phosphate. At this point, this was not a replicated study in the evaluation of this technology. The main objective of this research was to determine the effects of various levels of encapsulated STP (0.1% and 0.25%, active phosphate) and unencapsulated STP (0.2% and 0.5%, active phosphate) on raw and cooked ground turkey.

Material and Methods

Formulation and Processing

Fresh, boneless, skinless tom turkey breast muscles (*pectoralis major*) were obtained from a Virginia processor immediately following slaughter and stored at 3°C. To maintain consistency, the right lobe of the turkey breast was used. Turkey samples were cut into 2.5 cm thick strips and coarse ground (Model 4532, Hobart Manufacturing Co., Troy, OH) through a 12.7-mm plate and then a 4.8-mm plate. The turkey was mixed

in a bowl mixer for one minute using a dough hook attachment (Model A-200, Hobart Manufacturing Co., Troy, OH) between grinding.

The ground meat was allocated to six treatments. All treatments consisted of 400 g of ground turkey in addition to one of the following ingredients: no STP and NaCl (control), 1% NaCl and unencapsulated STP (0.20%, or 0.50%, active phosphate) or 1% NaCl and encapsulated STP (0.10%, or 0.25%, active phosphate). Active refers to the amount of pure phosphate added to the meat. The phosphates were provided by Rhodia Co. (Cranbury, NJ.). Balchem Inc. (Slate Hill, NY) encapsulated the STP. Encapsulated phosphates (STP-165-50) were encapsulated with hydrogenated vegetable oil designed to release the phosphate once the temperature reached 74°C. The encapsulated ingredients are composed of 51.1-g active phosphate and 48.9-g encapsulating oil. The samples were mixed for 2 min at speed 1(Model K45SS, Kitchen Aid™ Classic Mixer, Kitchen Aid Inc., St. Joseph, MI). The treated samples were stored in an unsealed bag (8x14, 90053, Cryovac Division W.R. Grace & Co., Duncan, SC) at a temperature of 3°C in the dark. Raw turkey samples were analyzed immediately after the ingredients were incorporated.

After 48 hr of storage, the samples were cooked via sous vide method using a water bath (Model 10-L, Fisher Scientific, Pittsburgh, PA). A total two centrifuge tubes (50-mL 28x15 mm polypropylene tubes with screw plug seal, #05-539-9, Fisher Scientific, Pittsburgh, PA), each containing 42.5 grams per tube, were used for each treatment. The samples were placed into an 82°C water bath and cooked to an end point temperature of 76°C. A cooking thermometer, inserted into the center of the tube, measured the internal sample temperature. The temperature was taken from samples randomly throughout the water bath. The samples were placed into a 6-oz bag (B679,

Nasco Whirl-pack, Fisher Scientific, Pittsburgh, PA) and mixed by hand to reabsorb the liquid extracted by cooking. The cooked ground turkey samples were stored for 48 hr at 3°C in the dark until analysis. Before analysis, the turkey was re-ground at speed 2 through a 4.8-mm plate (model K45SS, Kitchen Aid™Classic Mixer, Kitchen Aid Inc., St. Joseph, MI).

Chemical determinations

Raw and cooked turkey samples were analyzed for pH and TBARS following procedures outlined in Chapter 4. The pH and TBARS of the samples were measured for raw, day 1, and cooked turkey, day 5.

Results and Discussion

Means of pH and TBARS values for raw and cooked ground turkey are shown in Table 1. The pH of the raw product was higher in the phosphate added treatments compared to the control due to the alkaline properties of the phosphates. The treated cooked samples were higher than the control. All treatments of the raw ground turkey had low TBARS values indicating that the product used was fresh prior to treatment. The TBARS value was lower in the treated samples. The encapsulated STP was comparable to two times that of the unencapsulated STP. The higher levels of phosphates were more effective in the reduction of TBARS.

Conclusions

Overall, the incorporation of phosphates in the cooked, ground turkey resulted in a decrease in lipid oxidation, regardless of level of the phosphate or the presence of encapsulation. The activity of the encapsulated phosphate has been shown to be equal to

approximately twice that of the unencapsulated phosphate. Since this research only represented preliminary testing and no replications were performed, subsequent testing needs to be conducted to validate these results. In addition, future research should investigate the effect of various combinations of unencapsulated and encapsulated phosphates.

Table

Table 1. Means for raw[1] and cooked[2], ground turkey breast treated with various ingredients.

	pH		TBARS (mg/kg)	
Treatments[3]	Raw	Cooked	Raw	Cooked
Control	6.01	5.97	0.90	15.34
0.2% unencapsulated	6.40	6.27	0.97	8.82
0.5% unencapsulated	6.38	6.44	0.69	6.54
0.1% encapsulated	N/D[4]	6.16	N/D[4]	9.37
0.25% encapsulated	N/D[4]	6.32	N/D[4]	5.53

[1]Raw- immediately analyzed after treatment.
[2]Cooked- stored 48hr after treatment prior to cooking and analyzed 48hr after cooking.
[3]Treatments-additional 1% NaCl was added (except control) and percentage of phosphate represents amount of pure phosphate added to product on a meat weight basis.
[4]N/D- not determined.

VITA

On November 19th, 1973, Marsha Lynn Sickler was born into the family of Glenden Gene and Roberta Jean Sickler in New London, Connecticut. Marsha attended school in North Stonington, Connecticut with her older brother John until the age of thirteen. At that time, Marsha and her family moved to Chesapeake, Virginia. There she received a high school diploma from Great Bridge High School in 1991. Marsha continued her studies at the Tidewater Community College, where she achieved an Associate's degree in Science in 1993. Furthering her studies, she enrolled in the Animal Science program at Virginia Tech where she earned a Bachelor's degree in the winter of 1995. In the fall of 1996, Marsha enrolled in the Food Science and Technology graduate program. There she was voted the outstanding graduate student in 1997 and 1998. During her time as a graduate student, Marsha attended the 51st Annual Reciprocal Meat Conference where she placed second in the Graduate Student Research Poster Competition. On September 21, 1998 Marsha began employment with Boar's Head Provisions Co., Inc.