

To the Graduate Council:

I am submitting herewith a thesis by Cynthia Edith Adcox entitled "Measurement of Cholesterol, Cholesterol Oxides, and Fluorescent Products as Indicators of Lipid Oxidation in Precooked Dark and White Chicken Meat During Refrigerated (5-6°C) Storage for up to 10 Days." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Sharon L. Melton

Sharon L. Melton, Major Professor

We have read this thesis
and recommend its acceptance:

John R Mount
Genevieve Christen

Accepted for the Council:

Cowminkel

Associate Vice Chancellor
and Dean of The Graduate
School

FATTY ACID COMPOSITION AND MEASUREMENT (CHOLESTEROL
OXIDES, FLUORESCENT PRODUCTS AND TBA VALUE) OF LIPID
OXIDATION IN RAW AND COOKED WHITE AND DARK CHICKEN MEAT
DURING 5-6°C STORAGE

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee

Cynthia Edith Adcox
May, 1996

AD-VET-MED.

Thesis

96

.A337

ACKNOWLEDGEMENTS

The author expresses her sincere gratitude and deepest appreciation to Dr. Sharon Melton, major professor, for her expertise, patience, encouragement, guidance, and understanding throughout the entire project. Special thanks is expressed to Kay Trigiano, research associate, for her invaluable advice, assistance, friendship, and guidance throughout this research project.

Also, thanks and gratitude are extended to Sabina Silaula and Emayet Spencer for their time, friendship, and assistance on the moisture and TBA analyses.

Much appreciation is expressed to the faculty and staff of the Department of Food Science and Technology, The University of Tennessee, for making facilities available for the research, for their academic assistance and friendship, and for providing the teaching assistantship throughout the program.

Thanks are extended to Tyson's Foods, Springdale, Arkansas, for supplying the poultry samples used in this experiment.

Last, but not least, the author expresses her deepest love and appreciation to her parents for their encouragement, prayers, and support.

ABSTRACT

Chicken meat, white and dark, was treated with (1) salt, (2) phosphate, and (3) phosphate + antioxidant (rosemary oleoresin) while raw and then cooked and stored 0, 5, and 10 days at 5-6°C. The moisture, total lipid content, fatty acid composition of the total lipids, levels of cholesterol (CHOL), 7- α -hydroxycholesterol (ALPHA), 7- β -hydroxycholesterol (BETA), 7-ketocholesterol (KETO) and 3 polar, lipid soluble, fluorescent products, and the 2-thiobarbituric acid (TBA) value were determined in the meats. Compared with white meat, dark meat had less moisture (69.5 versus 66.6%), more lipid (13.76 versus 5.87%), lower concentrations of polyunsaturated fatty acids (22:5 and 22:6) and ALPHA (0.27 versus 0.85 $\mu\text{g}/100\text{ g meat}$) and higher CHOL (73 versus 43 $\text{mg}/100\text{ g meat}$), and KETO (5.8 versus 3.4 $\mu\text{g}/100\text{ g meat}$) levels.

Meat containing treatments of phosphate resulted in increased levels of moisture, and generally, in decreased oxidation during 10 days of 5-6°C storage. Levels of linoleic (18:2) and arachidonic acids decreased during cooking and with increasing storage of the chicken meats. In meat receiving salt treatment, levels of KETO and one fluorescent product increased with increasing storage time. TBA value also increased with increasing storage time of salt treated white meat and up to 5 days storage of salt treated dark meat. The TBA value was correlated ($p < 0.05$) with concentrations of 18:2 ($r = -0.54$) and KETO ($r = 0.47$). In combination with TBA value, KETO level may be an additional indicator of lipid oxidation in stored, cooked poultry meat.

Table of Contents

CHAPTER		PAGE
I.	Introduction.	1
II.	Literature Review.	5
	Poultry meat.	5
	Muscle types in poultry	5
	Lipid oxidation	6
	Health concerns from lipid oxidation	8
	Fatty acids degraded during lipid oxidation	9
	Phospholipids	11
	Warmed-over flavor.	12
	Heating effects on off-flavor formation	14
	Oxidative rancidity	15
	Cholesterol	16
	Separation and Quantitation of cholesterol and its oxides in muscle foods	17
	Malonaldehyde	19
	Fluorescent products.	20
	Antioxidants for meat	21
	"Natural" antioxidants.	22
	Phosphates in meats	24
	Antioxidant effects of phosphate. .	25
	Refrigeration of meat	25
	TBA analysis.	26
	Interferences and problems of TBA assay	27
III.	Materials and Methods	30
	Preparation of chicken meat and experimental design	30
	Materials and supplies.	33
	Equipment	34
	Lab sample preparation.	34
	Moisture determination and lipid extraction and quantitation .	34
	Preparation of fatty acid methyl esters (FAME) for fatty acid analysis.	36
	Gas chromatographic analysis of FAME.	37
	Cholesterol and cholesterol oxide sample preparation.	38
	Cholesterol and cholesterol oxides analyses.	39

CHAPTER		PAGE
	Fluorescent products.	40
	TBA method.	41
	Statistical analyses.	42
IV.	Results and Discussion	44
	Moisture.	44
	Lipid content	45
	Fatty acids	46
	Cholesterol content	57
	Cholesterol oxidation products. . .	59
	Fluorescent oxidation products. . .	63
	Thiobarbituric acid (TBA) values. .	67
	Relationship of TBA values with other measurements of oxidation	72
V.	Summary	75
	List of References.	78
	Appendices.	86
	Appendix A	87
	Appendix B	104
	VITA.	107

List of Tables

Table	Page
1 Gradient elution program for HPLC analysis of fluorescent oxidation products in raw and stored, cooked chicken meat.	41
2 Mean concentrations (%) of moisture in uncooked and cooked white and dark chicken meat receiving different treatments and stored at 5-6°C for up to ten days	45
3 Mean concentrations (%) of fatty acids in the total lipids of white and dark chicken meat averaged across meat treatments and states . .	48
4 Mean concentrations of fatty acids in total lipids of chicken meat receiving different treatments and averaged across meat type and state.	49
5 Mean concentrations of fatty acids in white and dark, uncooked chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days	51
6 Mean concentrations of fatty acids in white and dark chicken meat receiving different treatments as averaged across meat state . . .	52
7 Mean concentrations of fatty acids in uncooked and cooked chicken meat receiving different treatments and stored at 5-6°C for up to ten days after being cooked.	54
8 Mean concentrations of margaric acid (17:0) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat being stored up to ten days at 5-6°C	55
9 Mean concentrations of 9-heptadecenoic acid (17:1) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat stored up to ten days at 5-6°C. .	55
10 Mean concentrations of linoleic acid (18:2) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat stored up to ten days at 5-6°C	56
11 Mean concentrations of 5,8,11,14,17-eicosapentenoic acid (20:5n3) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat stored up to ten days at 5-6°C.	56

Table		Page
12	Mean cholesterol levels of white and dark chicken meats treated in different ways while uncooked and then cooked and stored up to ten days at 5-6°C.	58
13	Mean levels of 7-ketocholesterol in white and dark chicken meats treated in different ways while uncooked and then cooked and stored up to ten days at 5-6°C.	61
14	Mean concentrations of fluorescent oxidation products in white and dark chicken meat averaged across meat treatments and states . .	64
15	Mean concentrations of fluorescent oxidation products (FOPs) in chicken meat receiving different treatments and averaged across meat types and states	64
16	Mean concentrations of fluorescent oxidation products in uncooked chicken meat and in cooked chicken meat stored at 5-6°C up to ten days as averaged across meat type and treatment	65
17	Mean concentrations of FOP III in white and dark chicken meat treated differently while uncooked and then cooked and stored up to ten days at 5-6 °.	66
18	Mean 2-thiobarbituric acid (TBA) values of white and dark chicken meat treated differently while uncooked and then cooked and stored for up to ten days at 5-6°C.	69
A-1	Analyses of variance for total moisture of uncooked dark and white chicken meat and cooked dark and white chicken meat receiving different treatments and stored for up to ten days at 5-6°C	88
A-2	Analyses of variance for percentage of fat in uncooked dark and white chicken meat and cooked dark and white chicken meat receiving different treatments and stored at 5-6°C for up to ten days.	88
A-3	Analyses of variance for percentages of fatty acids in total lipids of uncooked dark and white chicken meat receiving different treatments and then stored at 5-6°C for up to ten days. .	89

Table	Page
A-4 Analysis of variance of cholesterol content of uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days.	99
A-5 Analysis of variance for concentration of 7- α -hydroxycholesterol in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days.	99
A-6 Analysis of variance for concentration of 7- β -hydroxycholesterol in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days.	100
A-7 Analysis of variance for concentration of 7-ketcholesterol in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days.	100
A-8 Analysis of variance for concentration of fluorescent oxidation product I (FOPI) in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days.	101
A-9 Analysis of variance for concentration of fluorescent oxidation product II (FOP II) in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days	101
A-10 Analysis of variance for concentration of fluorescent oxidation product III (FOP III) in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days.	102
A-11 Analysis of variance for 2-thiobarbituric acid (TBA) value in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days	102
A-12 Analyses of variance for TBA values of uncooked dark and white chicken meat and cooked dark and white chicken meat receiving different treatments and stored at 5-6°C for up to 10 days.	103
B-1 Mean concentrations of 7- α -hydroxycholesterol, 7- β -hydroxycholesterol, and total lipids for each type of chicken meat as averaged across treatment and state.	105

Table	Page
B-2 Mean concentrations of 7- α -hydroxycholesterol, 7- β -hydroxycholesterol, and total lipids for each treatment as averaged across type of meat and state.	105
B-3 Mean concentrations of 7- α -hydroxycholesterol, 7- β -hydroxycholesterol, and total lipids for each state of meat as averaged across type of meat and treatment.	105

Chapter I

Introduction

Fully cooked meat and poultry products are widely consumed, and an increasing number are refrigerated and/or frozen for later quick reheating and consumption. Many flavor components are generated during the cooking of poultry, and undesirable flavors from oxidation may occur during processing and storage of the meat including those generated by microbial degradation (Bailey et al., 1992). Lipid oxidation is a major cause of the deterioration of the quality of muscle foods (Rhee, 1988). The oxidation of lipids causes off-flavors and off-odors which result in unacceptable foods. Cooked meats are more susceptible to oxidation than uncooked meats (Igene and Pearson, 1979; Pearson and Gray, 1983; Rhee, 1988). Refrigerated and frozen storage also allow deleterious changes that result in oxidation of polyunsaturated fatty acids (PUFA), mostly in phospholipids (Brewer et al., 1992a; Gray and Pearson, 1987).

One of the secondary degradation products of lipid oxidation in meats is malonaldehyde. The 2-thiobarbituric acid (TBA) test quantifies the degree of oxidation by measuring the concentration of malonaldehyde in mg malonaldehyde/kg meat. One of the problems with the TBA test is that it measures not only concentrations of malonaldehyde (MA), but also other thiobarbituric acid reactive substances present (Igene et al., 1985). Also, because of the high reactivity of MA, it may further react with itself, proteins, or other nitrogen containing lipids such as phosphatidylethanolamine and

phosphatidylcholine as oxidation state increases in muscle foods, and the TBA value may actually decrease with increasing oxidation level.

In spite of disadvantages, the TBA-test is the most widely used method for assessing oxidation in meats (Melton, 1985). However, other tests which measure oxidation products that increase continually in concentration with increasing oxidation might prove more effective than the TBA-test. Two types of products which might fulfill this requirement are the cholesterol oxides and fluorescent oxidation products (Zheng, 1995). Zheng (1995) found, with continued oxidation of cooked, ground turkey, pork and beef stored at 5°C, increases in levels of 7-ketocholesterol, 7- α -hydroxycholesterol and 7- β -hydroxycholesterol as well as two fluorescent oxidation products. The rate of concentration increase of any one oxidation product was highly dependent on the type of meat (Zheng, 1995). If any of these products are to be used as the basis for oxidation assessment in meats, they must be evaluated in other meats and under different processing and storage conditions. In addition, several cholesterol oxidation products have detrimental health effects and knowledge of their concentration in all processed meats would be beneficial in assessing potential health risks (Finocchiaro and Richardson, 1983; Kumar and Singhal, 1991; Maerker, 1987; Pearson et al., 1983). Also, Zheng (1995) found that levels of some polyunsaturated fatty acids (PUFA) in the total lipids of the meats decreased steadily with increasing oxidation. Analysis of fatty acid composition of meats during oxidation not only might

prove to be a method for assessing oxidation but also would provide information which can be used for determining susceptibility of the meat to oxidation. Gas chromatographic (GC) analysis may be used to identify and quantify the fatty acid composition of meat lipids (AOCS, 1975).

Zheng (1995) used high performance liquid chromatography (HPLC) to detect and measure the cholesterol oxides and fluorescent oxidation products in meats after their isolation from nonpolar lipids and concentration using solid phase extraction (SPE). The isolation and determination of single cholesterol oxides are quite difficult due to the low concentrations (Maerker et al., 1988). HPLC in the normal phase appears to be one of the most effective approaches to separating and quantifying the cholesterol oxides (Maerker et al., 1988).

One solution to the partial prevention or delaying of lipid oxidation has been the use of antioxidants. Antioxidants inhibit free radical formation by donating a hydrogen atom to the free radical in fat to reorganize the molecule (Fennema, 1985). Various antioxidants have been used, with TBHQ, BHT, and BHA being the typical synthetic compounds selected, and spices and Maillard reaction products the primary "natural" antioxidants. The use of rosemary extract as an antioxidant in foods has been reported by many investigators to be an effective inhibitor of lipid oxidation (Ho et al., 1995; Liu et al., 1992).

In addition to the use of antioxidants for prevention of lipid oxidation and off-flavor production in stored meats, phosphates also provide some protection to lipid oxidation and

off-flavor production in stored, cooked meats (Brown, 1995). In fact, frozen, cooked meat entrees containing both phosphates and natural antioxidants are currently marketed for consumer consumption. Production and storage of such meat entrees using chicken, with and without the phosphate and/or antioxidants, would provide different levels of oxidation in which different methods of measuring increasing oxidation could be assessed in another species of muscle foods besides those studied by Zheng (1995). Therefore, the objectives of this study were (1) to measure the concentrations of fatty acids, cholesterol oxidation products, and lipid soluble, polar fluorescent products in uncooked, white and dark, chicken meat and in cooked, white and dark, chicken meat (with each meat treated with salt, phosphate or phosphate plus rosemary oleoresin) during 5-6°C storage and (2) to determine the relationship of the TBA test with other measurements of oxidation in cooked, white and dark chicken meat during 5-6°C storage.

Chapter II

Literature Review

Poultry meat

Meat has always been an important part of the human diet. Although poultry meat consists of that from turkeys, ducks, and geese, meat from chicken makes up the largest portion produced and consumed in the USA. Poultry meat is economical, easy to prepare and is higher in protein and lower in fat than red meat (Ensminger, 1980). Results of one study showed that breast meat lipid had a composition of 50.5% triglycerides, 45.5% phospholipids and 4.2% total cholesterol; the leg lipid contained 74.5% triglycerides, 21.7% phospholipids and 3.8% total cholesterol (Pikul et al., 1984a). The percentage of fatty acids in chicken lipids was reported as lauric acid (12:0), 1.9%; myristic acid (14:0), 2.5%; palmitic acid (16:0), 36%; palmitoleic acid (16:1), 8.2%; stearic acid (18:0), 2.4%; oleic acid (18:1), 48.2%; and linoleic acid (18:2), 0.8% (deMan, 1980).

Muscle types in poultry

Differences in muscle types within any animal are apparent in the differences in color of muscles. For example, the breast of the chicken is white muscle and the leg is dark muscle (Fennema, 1985). Compared with white muscle, the dark muscle consists of more red muscle fibers which contain larger amounts of myoglobin, receive more blood and contain more fat. Thus, the red muscle is used for prolonged activity and contains more energy (Ensminger, 1980). The white fibers contain very little

myoglobin, less fat, but more glycogen for anaerobic activity. Miller et al. (1994) reported that since red muscle is composed of more fibers per unit area, it contains more cell membranes rich in phospholipids. However, because there are higher lipid levels in leg than in breast meat, overall the phospholipid content is greater in the leg than the breast meat.

Pikul et al. (1984a) found that the concentration of free malonaldehyde in fat from chicken breast was more than double that in fat from the leg. This is due to the fat from the breast meat containing twice the proportion of phospholipid fraction than the fat from the leg muscle. Also, the fatty acids in phospholipids are more unsaturated than those in triglycerides, and therefore, more susceptible to oxidation. However, the calculated TBA number of leg meat reported by Pikul et al. (1984a) was higher than that of the breast meat because the leg contained more than twice as much total fat as the breast meat.

Lipid oxidation

Lipid oxidation in meat products is initiated when PUFA, particularly polyenoic fatty acids, react with molecular oxygen, through a free radical chain mechanism forming peroxides (Allen and Foedeging, 1981; Gray, 1978). The primary autoxidation is succeeded by secondary reactions which lead to degradation of the peroxides. A combination of aldehydes, alcohols, acids, and other compounds, which are responsible for oxidative rancidity in meat is formed. These oxidative changes are initiated mainly from the membrane components of muscle.

Lipid oxidation during cooking plays an important role in developing a complexity of pleasing volatile aromas in meat. However, oxidation of lipids (autoxidation) can occur readily in cooked meats at low temperatures during storage which leads to the formation of undesirable off-flavors (Ahn et al., 1993; Igene et al., 1979).

Oxidative changes in lipids can become a problem in uncooked meats when they are submitted to freeze-thawing, temperature abuse, and long term storage (Rhee, 1988). In uncooked red meat, metmyoglobin and hydrogen peroxide interact to generate an active metmyoglobin that is capable of initiating oxidation in lipids (Rhee, 1988). The hydrogen peroxide activates and releases the nonheme iron from the metmyoglobin (Lee and Hendricks, 1995). The oxidation of oxymyoglobin to metmyoglobin is a common occurrence in cut-up stored red meat. It is assumed that hydrogen peroxide can be produced from the pigment oxidation in abundant quantities to allow metmyoglobin-hydrogen peroxide mediated initiation of lipid oxidation (Rhee, 1988). Raw meat has been found to be more stable to oxidation than cooked meats because the enzyme, catalase, is present in raw meat. This enzyme partially interferes with the activation of metmyoglobin.

Enzyme systems can also catalyze lipid oxidation (Rhee, 1988), but cofactors are needed. The enzymic lipid peroxidation in muscle microsomes is dependent on nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), and requires adenosine diphosphate (ADP) and iron for a higher rate of lipid oxidation to occur (Rhee, 1988).

Mitochondrial enzymic lipid peroxidation systems also play a role in lipid degradation in raw muscles. In fish the mitochondrial peroxidation is similar to the actions and requirements of microsomal lipid peroxidation. However, an analogous system has not been demonstrated in pork, beef, and poultry (Rhee, 1988).

In summary, current literature indicates that enzymes and possible metmyoglobin activation are accountable for initiation of lipid oxidation in raw muscles and metal catalysts (nonheme iron in red meats) encourages lipid oxidation in raw muscles through their particular part in the increase of free radicals in the oxidation process.

Health concerns from lipid oxidation

Oxidative reactions can decrease the nutritional quality of food, and certain products of oxidation can be potentially toxic or carcinogenic to humans (Ajuyah et al., 1993; Finocchiaro and Richardson, 1983; Kumar and Singhal, 1991; Maerker, 1987; Pikul et al., 1984a). Oxidative processes are involved in both initiation of carcinogenesis and the advancement of tumor formation (Block and Langseth, 1994). Oxidants may contribute to carcinogenesis by causing mutations and by stimulating cellular division (Block and Langseth, 1994). The mutagenicity of cooked foods is an area of much research activity with several heterocyclic mutagenic amines already isolated from cooked foods. Three new mutagens from cooked meat extracts are as follows: a hydroxyphenyl imidazopyridine (4-OH-PhIP), a carboxymethyl derivative of dimethylimidazo-quinoxaline

(4-CH₂OH-8-MeIQx), and a trimethylimidazoquinoxaline (7,9-DiMeIgQx) (O'Brien and Labuza, 1994). Oxidation is also believed to advance cardiovascular disease by the oxidative changes in blood lipoprotein exhibiting a role in the long term development of atherosclerosis (Steinberg et al., 1989).

Fatty acids degraded during lipid oxidation

Various foods particularly muscle foods, are found to undergo lipid oxidation. Lipid oxidation is a major cause of the deterioration of the quality of meats (Asghar et al., 1988; Rhee, 1988). Polyunsaturated fatty acids are largely responsible for lipid (per)oxidation (Allen and Foegeding, 1981; Dillard and Tappel, 1971; Pikul et al., 1984a). The primary unsaturated fatty acids in the lipids in foods are oleic, linoleic, linolenic, and arachidonic acids. The primary site for the oxidative attack is the methylene group adjacent to a double bond, with the beginning step involving removal of hydrogen from this allyl methylene group. The resulting radical can undergo a rearrangement before a reaction with oxygen that propagates a number of different hydroperoxides. GC analysis of natural occurring animal lipids, both polar and nonpolar has resulted in extensive knowledge of their fatty acid composition. Greater levels of PUFA, such as linoleic (18:2) and arachidonic acid (20:4), in the polar than the nonpolar lipids result in a more rapid rate of oxidation for polar lipids (Bailey et al., 1992; Igene et al., 1980).

Measuring the loss of PUFA in muscle foods has allowed many researchers to follow oxidation. Several PUFA in the

phospholipid fraction decreased in concentration approximately 10% during frozen storage of beef (Melton, 1985). The fatty acids with substantial decreases in concentration were 18:1, 18:2, linolenic (18:3), and 20:4. Therefore, Melton (1983) concluded that "one of the best ways to follow lipid oxidation in muscle foods was to analyze the fatty acid composition of the muscle phospholipids". Also, the changes in the fatty acid composition of lipids can provide an indirect measure of susceptibility to lipid oxidation. Fatty acids containing three or more double bonds are highly inclined to oxidation during refrigeration of meats, and this inclination increases with the number of double bonds in the fatty acid. Brewer et al. (1992a) reported that the oxidation of 18:1, 18:2, and 20:4 make notable contributions to increased oxidative rancidity in cooked beef.

However, the decrease in levels of PUFA in poultry during refrigerated or frozen storage has been found to be quite small, or in fact, absent (Melton, 1983). The change in the fatty acid composition in muscle foods during storage differ, and for this reason, other methods are necessary to observe the extent of oxidation during refrigeration (Melton, 1985).

Lipid oxidation can be measured by absorption of oxygen, the 2-thiobarbituric acid (TBA) test, and measurement of fluorescent products. Lipid oxidation is one of the most common sources for off-flavor development in food products and can directly affect color, flavor, nutritive value (essential fatty acids and fat soluble vitamins), and safety (Ajuyah et al., 1993; Pearson et al., 1983). The process of lipid oxidation results in the formation of primary and secondary products such

as hydroperoxides, free radicals, epoxides, alkanes, alkenes, aldehydes, ketones, alcohols, hydrocarbons, and acids, all of which are associated with warmed-over flavor development (Bailey et al., 1980; Smith and Alfawaz, 1995). Aldehydes are the major contributors to off-flavors due to their high reactivity and low flavor thresholds (Simic et al., 1992). Ketones and alcohols have high flavor thresholds and are less common causes of off-flavors than aldehydes (Wu and Brewer, 1994).

Phospholipids

Phospholipids include those lipids that contain phosphoric acid as part of their structure and are the main lipids associated with oxidative rancidity of meat because of their high levels of PUFA (Bailey et al., 1980; Igene and Pearson, 1979). Despite the higher content of triglycerides in meat than phospholipids, these lipids are less important than phospholipids in the development of off-flavors (Bailey et al., 1992; Igene and Pearson, 1979; Pikul et al., 1984a). This is especially apparent for the phospholipid, phosphatidylethanolamine, which contains polyunsaturated C-20 and C-22 acids. Studies have confirmed that phospholipids are the primary contributors to warmed-over flavor in cooked meats (Igene and Pearson, 1979; Pearson and Gray, 1983). Igene et al. (1979) found that the level of phospholipids remained relatively constant during frozen storage for up to 18 months. However, during cooking there is an increase in the proportion of phosphatidylethanolamine in meat; phosphatidylethanolamine is least stable of all phospholipids to oxidation and this may

account for faster oxidation in cooked meat during frozen storage (Bailey et al., 1992).

Initially during cooking, disruption and dehydration of the cell membranes releases membrane phospholipids allowing them to become more inclined to oxidation, which is catalyzed by traces of iron. This iron is made more available by the degradation of heme pigments during the cooking process (Ang and Lyon, 1990).

Warmed-over flavor

The primary products of lipid oxidation are hydroperoxides which can break down to form secondary products such as aldehydes, which result in off-flavors in foods or rancidity. The concentration of hydroperoxides in heated lipids always remains small due to their instability at elevated temperatures while at lower temperatures they are more stable and can increase in substantial concentrations before degradation into volatile compounds (Bailey et al., 1987). Several volatiles identified in association with off-flavors such as "warmed over" flavor, are pentanal, hexanal, heptanal, and 2,3-octanedione (Fennema, 1985). The term "warmed-over" flavor was first used by Tims and Watts (1958) to describe the oxidative rancidity detected in cooked meat after refrigeration. Ang and Lyon (1990) found that refrigerated, precooked red meat and poultry develop off-flavors upon reheating and up to 5 days in storage (2°C). Other factors, which have been found to be responsible for the formation of other off-flavors in meats, include frozen storage (Igene et al., 1980), cooking time and temperature (Igene et al., 1979; Su et al., 1991), and exposure to oxygen

(Pikul et al., 1984b). Studies have shown that the main phospholipid responsible for the development of warmed-over flavor is phosphatidylethanolamine (Igene et al., 1979). Other studies have shown that phospholipids are the major contributors to warmed-over flavor development in beef, turkey and chicken (Wilson et al., 1976), and that triglycerides play only a minor role (Igene and Pearson, 1979). Lipids can oxidize readily in air, at elevated temperatures, in the presence of free radical initiators, or light, or a combination of any of these factors (Maerker, 1987). However, Brewer et al. (1992b) reported that light was not required to initiate lipid peroxidation, and even in the presence of light, the absence of oxygen limited lipid oxidation. Warmed-over flavor can develop in restructured fresh meats as a result of the disruption of the tissue membrane and the exposure of the lipids to limited oxygen (Gray and Pearson, 1987).

Muscles contain iron, a known prooxidant (Wu and Brewer, 1994), and the distribution of iron in muscles changes during processing and storage (Decker et al., 1993). It is now believed that heme compounds increase lipid oxidation and that warmed-over flavor in cooked meats is the result of the catalytic oxidation of unsaturated fatty acids by iron (Pearson et al., 1977). However, specific mechanisms are not fully understood. Since red muscle contains higher amounts of heme iron, it consistently endures more warmed-over flavor development than white muscle from the same species. Various forms of nonheme iron such as lactoferrin and ferritin, have been found to enhance lipid oxidation in meats (Decker and

Hultin, 1990). Iron-catalyzed lipid oxidation is believed to be a major cause of the deterioration of muscle (Ang and Lyon, 1990; Igene et al., 1979; Pearson et al., 1977). Iron can be associated with low and high molecular weight compounds, and the low molecular weight compounds are thought to be responsible for the catalysis of lipid oxidation in muscle tissue (Decker et al., 1993). High molecular weight iron is associated with membrane lipids and proteins such as myoglobin, and some of these compounds also are believed to directly catalyze lipid oxidation (Decker et al., 1993).

In spite of the many publications on the mechanism of lipid peroxidation, several questions remain unanswered. For instance, the need exists to clarify more fully the contribution of the iron present in muscle to lipid peroxidation since some researchers believe hemoproteins are prooxidants in muscles, while others believe nonheme iron compounds are the major contributors to lipid oxidation (Lee and Hendricks, 1995). Nonheme iron is distinguished from heme iron on the basis of solubility (Igene et al., 1979). Iron exists in a number of different complexes including ferritin, hemoglobin, and low molecular weight fractions. Therefore, to follow the iron content transition during cooking may be more beneficial than just measuring the nonheme iron.

Heating effects on off-flavor formation

Cooking affects the distribution of iron in muscle foods and iron is released from ferritin by heating, which results in an increase in lipid oxidation (Decker and Welch, 1990; Decker

et al., 1993). It is generally accepted that heme compounds hasten lipid oxidation in cooked meat. Heat denatures proteins which increases the development of warmed-over flavor, by increasing the prooxidant activity of hemoproteins (Lee and Hendricks, 1995). This increase in prooxidant activity is due to the unfolding of the protein which "allows a greater exposure of heme groups to the substrate" (Bailey et al., 1992). Igene et al. (1979) reported that the levels of free Fe(II) increase during cooking and acts to accelerate lipid oxidation in cooked meat. Myoglobin served as a source of Fe(II), which is broken down during cooking and is then available to catalyze autoxidation (Bailey et al., 1980; Decker et al., 1993).

Oxidative rancidity

Lipid oxidation is one of the major causes of food spoilage. It is a concern to the food industry because it leads to the development of off-flavors and off-odors (generally called oxidative rancidity) which renders these foods unacceptable or reduces their shelf life (Fennema, 1985; Poste et al., 1986). Various elements are responsible for the degree of oxidation including the number, position, and geometry of double bonds of the fatty acids in lipids. Oxidative rancidity of meats is a major concern, and chicken is especially susceptible due to the high degree of polyunsaturation of the fat. Several researchers hypothesize that the membrane-bound lipids in meats are the principal element in the production of oxidative rancidity in fresh and cooked muscle foods during storage (Asghar et al., 1988). The membrane-bound lipids

identified with the cell of the muscle, such as the mitochondria and sarcoplasmic reticulum, are particularly susceptible to oxidation by advantage of the extreme content of PUFA in the phospholipids (Asghar et al., 1988; Pearson et al., 1977).

The 2-thiobarbituric acid (TBA) assay is typically the technique used to measure this rancidity (Marion and Woodroof, 1965). This test relies on measuring the quantity of malonaldehyde in the meat (Sinnhuber and Yu, 1958).

Oxygen and heat are the main factors that initiate lipid oxidation in muscle foods and cooking may cause an increase in the oxidation of the fat. Another belief is that frozen storage of cooked meat may also lead to oxidative rancidity (Igene et al., 1979). When comparing muscle types in chicken with oxidative changes after cooking and refrigeration, the largest increases in TBA values were found in breast meat followed by leg meat, skin, and depot fat (Ang, 1988).

Cholesterol

The tendency of cholesterol to oxidize has caused investigators to separate and quantitate oxidized cholesterol products for many reasons. Such oxidation products have been found in many foods and these oxidation products have been linked to adverse human health effects (DeVore, 1988; Finocchiaro and Richardson, 1983; Kumar and Singhal, 1991; Maerker et al., 1988; Pearson et al., 1983). Foods that are exposed to elevated temperatures and air during cooking render cholesterol more vulnerable to oxidation (DeVore, 1988). Some of the oxidation products (for example 7-ketocholesterol, and α -

epoxides) have been implicated as angiotoxins, cytotoxins, mutagens, and carcinogens (Finocchiaro and Richardson, 1983). Other factors such as light, radiation, and free radicals are known also to increase the oxidation of cholesterol (Csallany et al., 1989).

Separation and quantitation of cholesterol and its oxides in muscle foods

The quantitation of cholesterol oxides (COS) is quite difficult not only because they occur in low concentrations but also because their isolation is restricted by large amounts of triglycerides, phospholipids, and other lipid soluble components (Csallany et al., 1989; Maerker, 1987). Prior to any type of analysis, the COS must be extracted from the muscle and isolated and concentrated from the other lipid material. Many researchers who have measured COS in muscle foods extracted the total lipids and then used an overnight saponification procedure to remove the nonpolar and phospholipids (Park and Addis, 1987; Sander et al., 1989). A dry-column extraction method followed by some type of chromatographic separation of COS from neutral lipids may also be used (Csallany et al., 1989; Zubilliga and Maerker, 1991). Zheng (1995) isolated COS from ground meats by extracting total lipids using a modified chloroform-methanol method of Melton et al. (1979) followed by solid phase extraction in which cholesterol and COS were separated from the nonpolar and polar lipids.

After COS have been isolated and concentrated, they may either be analyzed by gas chromatographic (GC) procedures or by

HPLC methods. To be analyzed by GC procedures, COS as well as cholesterol are usually derivatized to silyl ethers. However, without derivitization, Zubigilla and Maerker (1991) analyzed 7-ketocholesterol, cholesterol $5\beta,6\beta$ -epoxide and cholesterol $5\alpha,6\alpha$ -epoxide by direct on-column injection into the GC. Without direct on-column injection, COS decompose before volatilizing (Melton, 1995). In HPLC analysis, COS may be analyzed directly using normal phase chromatography with gradient elution (Devore, 1988; Maerker et al., 1988). Maerker et al. (1988) used a flame ionization detector in HPLC analysis of COS. However, the flame ionization detector for the HPLC is no longer available commercially limiting the detection of COS in HPLC. Since concentrations of COS are so minute, detection by universal detectors such as evaporative light scattering mass detectors or refractive index detectors is not possible because they are not sensitive enough. An ultraviolet (UV) detector is sensitive enough to measure COS but only three COS (7-ketocholesterol, 7- α -hydroxycholesterol and 7- β -hydroxycholesterol) absorb in the UV region.

COS, including the three previously listed, have been quantified in several different meats. Zubilliga and Maerker (1991) quantified 7-ketocholesterol, cholesterol $5\beta,6\beta$ -epoxide and cholesterol $5\alpha,6\alpha$ -epoxide in irradiated, raw veal, beef, pork and chicken muscle tissues using methods previously described. Other researchers such as Csallany et al. (1989) and DeVore (1988) extracted, separated and concentrated COS, as well as cholesterol, from muscle tissue but used HPLC for their quantification. Csallany et al. (1989) were successful at

measuring 7-ketocholesterol, 7- α -hydroxycholesterol and 7- β -hydroxycholesterol in pork muscle at the ppm level using HPLC methods with UV detection. DeVore (1988) was successful in quantifying 7-ketocholesterol in cooked beef after 2 days storage using normal phase HPLC analysis.

Malonaldehyde

One of the secondary degradation products of lipid oxidation is malonaldehyde. Malonaldehyde develops from the decomposition of prostaglandin-like endoperoxides produced during the oxidation of PUFA (Fennema, 1985). Malonaldehyde has been associated with human cancer and heart disease (Pearson et al., 1983). Formation of malonaldehyde is the basis for TBA measurement of lipid oxidation, since the majority of malonaldehyde is produced during the distillation step of the TBA procedure (Ajuyah et al., 1993).

Researchers have investigated the effect of cooking on the malonaldehyde content of poultry. Results have shown that the cooking process does indeed increase malonaldehyde content of chicken by 5 to 20 fold when compared to raw chicken samples (Siu and Draper, 1978). This increase in malonaldehyde has caused great concern, since meat is exposed to heat and oxygen during the cooking process. Also, during the TBA analysis, this increase in malonaldehyde may be due to sample autoxidation (Pikul et al., 1984a). To prevent this possibility, antioxidants are often used to prevent lipid oxidation. Many times in TBA analysis BHT is added in small amounts to samples before they are blended and heated (Rhee, 1978). This practice

has resulted in a 6-fold reduction in the TBA number (Pikul et al., 1983).

Fluorescent products

Fluorescent products also may be useful in the measurement of lipid oxidation (Melton, 1983) and for the quantitation of peroxidation damage to muscle tissue (Pearson et al., 1983). Fluorescent compounds are derived from the reaction of proteins, amino acids and phospholipids with malonaldehyde and other carbonyl compounds from oxidized lipids. The fluorescent products are a family of compounds with the structure $R-N=C-C=N-R$ (Dillard and Tappel, 1971), and their production is enhanced by the presence of oxygen. Also, with an increase in age of tissue, the amount of fluorescent products also increased, and they are assumed to be derived from the reaction of oxidized lipids and proteins (Chio and Tappel, 1969). Even though accumulation of fluorescent products indicates lipid oxidation, the mechanism of their formation is uncertain.

One advantage to using fluorescence measurement as an indicator of oxidation is that it is highly sensitive. Fluorescence measurement allows determination of the fluorescent Schiff base product to the degree of one part per billion (Dillard and Tappel, 1971). Another advantage is that the development of fluorescence is correlated to the absorption of oxygen even in the last phase of peroxidation. The measurement of fluorescent products has been useful in the quantification of peroxidation damage to living muscle tissue and was suggested as being useful in the

assessment of lipid oxidation in meat during storage (Melton, 1983).

Pikul et al. (1984a) found that meat that had been frozen for six months had an 11% increase of fluorescence per g fat, and a 31% increase of fluorescence per μ gram protein. However, after 4 days of refrigerated storage (4°C), the fluorescence of cooked meat increased 62% per g fat, but only 25% per μ gram protein (Pikul et al., 1984a).

Antioxidants for meat

Oxidation reactions can be minimized by the reduction of oxygen through vacuum packaging or the addition of antioxidants (Empson et al., 1991). Antioxidants inhibit or disrupt free radical formation due to their phenolic structure. They donate a hydrogen atom to the fat free radical to reorganize the fat molecule or they donate a hydrogen to a peroxide free radical to form a hydroperoxide and a stable antioxidant (Fennema, 1985).

Exogenous antioxidants have been added to meat products to preserve the quality (Wheeler et al., 1990). Butylated hydroxytoluene (BHT) has become the choice of the phenolic synthetic antioxidants, possibly because it is soluble in fat, stable at high temperatures, and has synergistic actions with other antioxidants such as propyl gallate and citric acid. Lipid oxidation has been inhibited also in restructured cooked beef-pork steaks by the addition of phenolic antioxidants such as BHA and tertiary-butylhydroxyquinone (TBHQ) (Chastain et al., 1982). Lipid oxidation has also been limited in ground beef patties when they were treated with metal chelators, free

radical scavengers, rosemary, or sodium alginate (Shahidi et al., 1987). Antioxidants which were extracted from soybean flour, concentrate or isolate and added to beef, were found to decrease lipid oxidation (Rhee et al., 1981; Sato et al., 1973). Wu and Brewer (1994) studied the effects of a soy protein isolate antioxidant on peroxidation of membrane fatty acids in ground beef stored at 4°C. They found that the soy protein isolate prevented beef lipid peroxidation products from forming, but the mechanism of its action was unclear (Wu and Brewer, 1994). The major phenolic compounds identified in soy flour were believed to act as free radical scavengers by the reaction with unpaired electrons from the lipid molecule, or as chelators by complexing with Fe^{2+} (Wu and Brewer, 1994). The conclusion drawn by Wu and Brewer (1994) was that the soy protein isolate was an effective antioxidant in meats under limited storage conditions.

"Natural" antioxidants

Although poultry meat consists of relatively high levels of unsaturated fatty acids, levels of natural antioxidants such as tocopherols are fairly low and this causes poultry products to become susceptible to off-flavors from lipid oxidation (Ajuyah et al., 1993). The addition of natural antioxidants in poultry diets may supplement the antioxidant effects at the cellular level.

The popularity of natural antioxidants results from the belief that natural food ingredients are safer and healthier than synthetic (Dorko, 1994). Many herbs and spices (such as

rosemary and rosemary extract) exhibit antioxidant properties in fat systems (Dorko, 1994). The use of rosemary extract as an antioxidant in foods has been reported by several researchers. Naturally occurring compounds in rosemary extracts exhibit antioxidant attributes better than BHA and equal to BHT (Wu et al., 1982). Extracts of rosemary have been found to be very effective against lipid oxidation in muscle foods (Ho et al., 1995). Rosemary oleoresin contains high amounts of essential oil which contains α -pinene, camphene, 1,8-cineole, camphor, borneol, and geraniol. Many of these are cyclic alcohols that have antioxidant activity. Liu et al. (1992) looked at the efficiency of rosemary oleoresin combined with sodium tripolyphosphate in preventing lipid oxidation in restructured pork steaks during cooking and subsequent refrigeration, and in raw meat during frozen storage. Rosemary oleoresin acted as an antioxidant but the addition of rosemary oleoresin to samples containing sodium tripolyphosphate did not display any increased benefits in preventing lipid oxidation (Liu et al., 1992).

Maillard reaction products (MRP) encompass a group of antioxidants regarded as natural because the reactions occur normally in foods (Smith and Alfawaz, 1995). Bailey et al. (1987) used MRPs from histidine and glucose in cooked ground beef and found them to be effective inhibitors of oxidative rancidity. Smith and Alfawaz (1995) reported that the use of MRPs in ground beef that had been cooked and stored (4°C) for 8 days had an antioxidative activity, which prevented oxidative deterioration in the beef. Phytic acid, a naturally occurring antioxidant, and abundant plant constituent, was found to be an

effective agent for inhibiting oxidation in food products (Empson et al., 1991). Phytic acid has iron chelating ability rendering iron catalytically inactive (Lee and Hendricks, 1995). Empson et al. (1991) investigated the use of phytic acid in cooked, refrigerated chicken and concluded that iron-induced oxidation in foods can be inhibited with small amounts (<1 millimole) of phytic acid. Empson et al. (1991) also demonstrated the inhibitory effects of phytic acid on malonaldehyde development during refrigeration (4°C) of cooked chicken, and Lee and Hendricks (1995) found that the addition of small amounts (<20 millimoles) of phytic acid to muscle tissue may inhibit lipid oxidation by the acceleration of autoxidation of ferrous to ferric ion and by the formation of catalytically inactive iron chelates. It is presumed that phytic acid removes myoglobin-derived iron from the negatively charged phospholipids preventing their autoxidation (Lee and Hendricks, 1995).

Phosphates in meats

The addition of phosphates to raw and cooked meats increases the water-holding capacity and decreases the drip loss in poultry (Fennema, 1985). The mechanism by which the phosphate enhances meat hydration is not fully understood. It is believed that a phosphate-induced solubilization occurs between the protein and the phosphate which takes place primarily on the surface of fish and poultry (Fennema, 1985). A layer of coagulated protein is formed during the cooking process and helps to improve moisture retention.

Antioxidant effects of phosphate

Researchers are in agreement that phosphate has an antioxidant, flavor preservation effect (Goll et al., 1992; Tims and Watts, 1958), due to its chelating ability (Ahn et al., 1993). Goll et al. (1992) found a decrease in TBA value and warmed-over flavor in restructured, pre-cooked beef roasts that had phosphates and salt added. They also believed that the phosphate may have subdued the prooxidant effects of the salt (Goll et al., 1992). Another study demonstrated the use of sodium tripolyphosphate combined with sodium ascorbate greatly reduced TBA values in pork during the refrigeration (4°C) for 35 days (Shahidi et al., 1986). Brewer et al. (1992a) found the use of 0.2% sodium tripolyphosphate in beef patties that were frozen stored for up to 12 weeks and cooked at 190°C prior to analysis inhibited lipid oxidation as evident by lower TBA values. Another study illustrated the use of tripolyphosphate in precooked turkey patties after 7 days in refrigerator storage by the lowering of TBA values (Ahn et al., 1993).

Refrigeration of meat

Refrigeration is a widely used method in the preservation of meat. Refrigeration helps to preserve meat by preventing the growth of microorganisms and slowing several chemical and enzymatic reactions (Fennema, 1985; Mount, 1993). In the USA, refrigeration is a popular method for the short-term storage of cooked foods including meat.

Refrigeration, however, has been linked to off-flavor development in precooked muscle foods (Ang and Lyon, 1990).

Refrigerated, precooked poultry developed off-flavors after reheating and short term refrigeration (Ang and Lyon, 1990). The distribution of iron in muscle foods changes during refrigerator storage resulting in increased lipid oxidation (Decker et al., 1993), and in formation of detectable warmed-over flavor (Tims and Watts, 1958).

TBA Analysis

The 2-thiobarbituric acid (TBA) test is widely used for measurement of oxidative degradation of polyunsaturated fatty acids in muscle foods (Fennema, 1985; Gray, 1978). This test more specifically measures the quantity of malonaldehyde (MA) (Sinnhuber and Yu, 1958). The condensation of two molecules of TBA with one molecule of malonaldehyde produces a reddish color reaction. In the performance of the TBA test, the sample is blended and distilled. The distillate is filtered, the TBA reagents added and the solution mixed and the color reaction allowed to proceed. The color reaction may be speeded by heating the samples in a boiling water bath for 30 to 60 minutes (Tarladgis et al., 1960) or allowing it to continue at a slower rate by storing samples in the dark for approximately 20 hours (Brewer et al., 1992a). The absorbance of the colored complex is then read at 530nm on a spectrophotometer and the concentration of malonaldehyde and TBA reacting substances can be calculated using a standard curve (Brewer et al., 1992a). TBA numbers are expressed in mg malonaldehyde/kg sample.

Pikul et al. (1984a) reported that in both chicken breast and leg meat stored frozen for 6 months, the TBA numbers

increased after refrigeration (4°C) and reheating. They also reported that no differences were found in TBA values between convection and microwave oven upon reheating (Pikul et al., 1984a).

Interferences and problems of TBA assay

Various compounds have been found to interfere with the TBA test, and decreasing TBA values with increasing oxidation have been found since malonaldehyde reacts with proteins, phospholipids and itself. Other aldehydes and sugars can interfere with TBA test for malonaldehyde and increase absorption of the colored complexes at 530-570 nm (Gray, 1978; Melton, 1985). Other aldehydes react with thiobarbituric acid to form a red complex that also absorbs at 532 nm. Many reports have referred to the TBA numbers as TBA-RS(reactive substances) to accentuate that other substances are included in the measurement (Ang and Lyon, 1990; Igene et al., 1985; Lyon et al., 1988; Melton, 1983). Some components, for example, sugar and compounds in wood smoke, also give a red color upon reaction with TBA. However, in many instances, the TBA test is applicable for comparison of samples of a single material at different oxidation states (Fennema, 1985). Ajuyah et al. (1993) considered malonaldehyde to represent a small percentage of the compounds produced from lipid oxidation and hypothesized that other compounds react with TBA. Ajuyah et al. (1993) also believed that the majority of the malonaldehyde that reacts with TBA is produced from the decomposition of hydroperoxides during the distillation step of TBA analysis. The method used in this

study as modified by Tarladgis et al. (1960) produces higher TBA numbers than other methods (Melton, 1985), because lipid oxidation occurs during the blending and distillation steps (Melton, 1985). The addition of propyl gallate and ethylenediaminetetraacetic acid (EDTA) to the sample during the blending gives proof that oxidation did occur (Melton, 1985; Rhee, 1978). It has been reported that the addition of BHT results in a 6-fold reduction in the TBA value (Pikul et al., 1984b).

Another problem with the analysis of TBA is that of sample autooxidation. Investigators have used antioxidants to protect against this (Pikul et al., 1983; Pikul et al., 1984b). The addition of BHT, an antioxidant, to samples before blending and before heating in the TBA assay was helpful in eliminating sample autooxidation (Ang and Lyon, 1990; Pikul et al., 1983; Su et al., 1991).

Several investigators have found that after chill storage of broiler chicken, the TBA values were higher in the dark meat than the white meat (Igene et al., 1985; Wilson et al., 1976). However, other investigators reported that the TBA values changed more rapidly in the chicken breast compared to the thigh and skin (Ang and Lyon, 1990).

Although problems exist in using TBA values to measure the extent of lipid oxidation in muscle foods, it is still the preferred method because of its simplicity and inexpensive equipment necessary for analysis. Determining TBA changes in value by a single procedure can still estimate the amount of lipid oxidation occurring during a storage period (Melton,

1985). It is however, not advisable to compare TBA values directly across studies and various meat types as an evaluation of oxidation, since many factors can affect the TBA values. TBA numbers differ among species, within a species, and among muscles, and they are also affected by cooking, methods of cooking, and refrigeration and freezer storage (Melton, 1985).

Even with the indigenous differences in TBA values among muscles and species, and malonaldehyde reacting with other food components, it is the most widely used method for measuring oxidation in meat systems. Many procedures and modifications of the TBA method found in the literature help to make the TBA method the choice for measuring oxidation in muscle foods, but not necessarily to imply that it is absolutely the best method (Melton, 1985). Perhaps, as Zheng (1995) concluded, to get a complete picture of lipid oxidation in meats during processing and storage, several methods of measuring lipid oxidation should be used.

Chapter III

Materials and Methods

Preparation of chicken meat and experimental design

Frozen, uncooked and cooked chicken white (boneless, skinless breast meat) and dark (boneless, skinless thigh meat) meats with different treatments were obtained from Tyson Foods, Inc. (Springdale, AR). Enough of each type of uncooked chicken meat was obtained from Tyson at one time to complete all of the planned experiment. For the control or salt treatment, 10.89 kg of each type of chicken meat separately were ground through a 0.635 cm plate and blended (10.66 kg meat + 0.68 kg marinade) for seven min with 6% marinade containing by weight, 96.67% water and 3.33% NaCl. The marinated meat therefore contained 0.20% NaCl by weight.

For each meat type with salt (control) treatment, the marinated meat was chilled using liquid CO₂ to a forming temperature of approximately -1 to -2°C before being formed into meat nuggets. A 0.908 kg sample was selected from the uncooked nuggets, divided into two 0.454-kg samples with each sample placed in a polyethylene freezer bag, sealed, and frozen (-38°C). The remaining meat nuggets were fully cooked to an internal temperature of 73.9°C before being divided into 0.454 kg samples which were placed in individual polyethylene freezer bags and frozen (-18°C).

For the phosphate treatment, a second 10.89 kg batch of each type of chicken meat was ground through a 0.635 cm plate

and blended (10.66 kg meat with 0.68 kg marinade) for seven min with 6% of marinade which contained by weight, 90.00% water, 3.33% NaCl, and 6.67% phosphate. The marinated meat contained by weight 0.20% NaCl and 0.40% phosphate. From this point each type of phosphate treated chicken meat was treated in the same manner as the control meat.

For the phosphate plus antioxidant treatment, again 10.89 kg of each type of chicken meat was selected and ground through a 0.635 cm plate. The ground meat was blended (10.66 kg meat plus 0.68 kg marinade) for seven min with 6% marinade. For the white meat the marinade contained by weight, 89.98% water, 3.33% NaCl, 6.67% phosphate and 0.0156% Herbalox W, a water soluble rosemary oleoresin antioxidant from Kalsec, Inc., (Kalamazoo, MI). The resulting marinated white meat contained 0.20% NaCl, 0.40% phosphate and 0.075% antioxidant per percent fat content which was based on a standard fat content of 1.29% in the boneless skinless breast meat. For the dark meat, the marinade contained by weight, 89.95% water, 3.33% NaCl, 6.67% phosphate and 0.049% of the Herbalox W. The marinated dark meat contained by weight 0.20% salt, 0.40% phosphate and 0.075% antioxidant per percent fat content based on a standard 3.92% fat content in the dark meat. From this point, both types of the phosphate plus antioxidant treated chicken meat were treated as the control treated meat.

Packaged frozen samples were placed in large polyethylene bags, flushed with nitrogen, sealed, and placed in styrofoam containers containing dry ice. These containers of meat were shipped for overnight delivery to The University of Tennessee

Department of Food Science and Technology, Knoxville, TN.

Upon receipt, the uncooked (UNC) samples from each treatment were randomly assigned to replication one or two and stored at -18°C no longer than one month for analysis. Six 0.454 kg packages of the cooked samples of each type chicken were divided evenly and assigned to either replication one or two. The packages of cooked meat were refrigerated at $5-6^{\circ}\text{C}$ in non-overlapping layers on shelves for 16 hr to thaw. After thawing, for each type of chicken meat from each treatment and replication, a package was randomly selected as a 0-day sample. Each package of meat was opened, the meat pieces were arranged in a single layer on a paper towel in a General Electric Model microwave oven (441 watts), and the meat was microwave heated for 1 min on high power (95% maximum). After being microwave heated, each sample was sealed in a labeled 3.79-L size heavy duty polyethylene freezer bag with zipper closure (Glad Lock Freezer bag, size 25.4 x 1.43 x 27.54 cm, First Brands Corporation, Danbury, CT) and returned to frozen storage (-18°C) until analyzed. These samples were designated as cooked and stored zero day or C0 samples.

After five days storage at $5-6^{\circ}\text{C}$, for each type of chicken meat from each treatment and replication, a package was randomly selected as a five day or C5 sample. Each package was opened, microwave heated, packaged and refrozen as described for the C0 samples.

On the tenth day of $5-6^{\circ}\text{C}$ storage, the last set of samples for each treatment, meat type and replication were removed, microwave heated, packaged and refrozen in the same manner as

the C0 samples. The samples stored for ten days were designated as C10 samples. The uncooked, C0, C5, and C10 samples represent successively advancing states or stages of lipid oxidation in the each type chicken meat from each treatment with the phosphate treated and the phosphate plus antioxidant treated representing increasing levels of protection against oxidation.

The experimental design of this experiment was a split plot design; the whole plot was the meat type (light or dark) which was split into subplots of three treatments and four oxidation states for a single replication. Two replications were run. There were three treatments: 1 (salt), 2 (phosphate), and 3 (phosphate + antioxidant), and four oxidation states (UNC, C0, C5 and C10). A total of 48 chicken meat samples were evaluated during this experiment.

Materials and supplies

HPLC grade solvents (chloroform, methanol, hexane, diethyl ether, isopropanol, and water), sodium hydroxide, sodium chloride, and sodium sulfate were obtained from Baxter Scientific Products (Stone Mountain, GA). Standards for cholesterol, cholesterol oxides (7-ketocholesterol, 7- α -hydroxycholesterol, 7- β -hydroxycholesterol), tetraethoxypropane and 2-thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO). Individual and mixtures of fatty acid methyl ester standards and boron trifluoride (14%) in methanol were obtained from Alltech, Inc. (Deerfield, IL) or Supelco, Inc. (Bellefonte, PA). Sources of other chemicals and supplies are given under the procedures in which they were used.

Equipment

A high performance liquid chromatograph equipped with a U6K injector, two M6000A pumps, a Model 480 UV detector (Water's Associates, Inc., Milford, CT) and a fluorescence HPLC Monitor RF-530 (Shimadzu Scientific Instruments, Inc., Columbia, MD) was used for analysis of cholesterol, cholesterol oxides (COS) and fluorescent oxidation products (FOP). A Shimadzu C-R2AX chromatographic data processor (Shimadzu Scientific Instruments, Inc., Columbia, MD) was used for HPLC data processing. A μ Porasil 10 μ m silica HPLC column (3.9 o.d. x 300 mm long) (Phenomenex, Torrance, CA) was used for the HPLC analyses of cholesterol, COS and FOP. A Shimadzu Model UV-160A UV-VIS spectrophotometer was used for the determination of TBA values. Other instruments used in analyses are described under the specific methods in which they are used.

Lab sample preparation

Each uncooked and stored, cooked chicken meat sample was powdered with liquid nitrogen in a 1-L stainless steel cylinder by a Waring Blender at high speed, and the powdered samples were placed in 0.9-L Glad Lock Freezer bags and stored in a freezer (-18°C) until analyzed. Samples were analyzed within one month of being powdered.

Moisture determination and lipid extraction and quantitation

Moisture level was determined on each meat sample according to the AOAC (1980) vacuum oven drying method. From each chicken

meat sample, total lipids and lipid soluble material, cholesterol oxides and fluorescent oxidation products, were extracted by chloroform-methanol extraction (Melton et al., 1979), modified by the addition of butylated hydroxytoluene (0.1200g/915mL) to the chloroform. Forty g of powdered sample were homogenized with 130-mL methyl alcohol for 5 min in a Waring blender. Sixty-five mL of a 0.013% solution of butylated hydroxytoluene (BHT) in chloroform solution were then blended with the methanolic homogenate for 5 min; then 65-mL of the BHT-chloroform solution was added to the mixture and blended for 20 sec. Next, 65-mL of an aqueous solution containing 1.5 g zinc acetate in water was added to the homogenate and it was reblended for 10 sec. The mixture was filtered through a Whatman #1 filter paper in a Buchner funnel into a 1-L filter flask using a vacuum. The solid residue and the filter paper from the Buchner funnel were then blended with 100-mL BHT-chloroform solution for 5 min, and filtered as described previously; and the filtrate was added to the first extract. Any residual sample in the blender was rinsed with 75-mL chloroform and filtered into the filter flask with the extract. The solvent extract was then poured into a 500-mL graduated cylinder. The filter flask containing any remaining extract was rinsed with 25-mL methanol and the contents added to the graduated cylinder. The graduated cylinder containing all the extract was then flushed with nitrogen, covered tightly, and stored at 5-6°C overnight. The volume of the chloroform layer (bottom layer) in the cylinder was recorded the next day, and the extract transferred to a 500-mL separatory funnel. The

contents were allowed to separate in the funnel for 4 hrs at 5-6°C. The chloroform layer was drained into a 500-mL round bottom flask, nitrogen flushed, covered securely, and stored at 5-6°C for further analysis.

For the determination of lipid content in each sample, 50-mL beakers were washed, oven dried for 2 hrs (90-100°C), cooled in a desiccator and weighed. Ten mL of the chloroform extract from each sample was transferred to a single beaker and evaporated overnight under a hood. Duplicate measurements were made for each meat sample. The lipid residue left in the beaker was dried further in an oven (90-100°C) for 30 min. The weight of the lipid residue was determined by weighing the beaker after cooling and subtracting the empty weight beaker. The lipid content was calculated by the following formula:

$$\text{Total lipid (\%)} = [(\text{lipid wt} \times \text{vol chloroform}) / (40\text{g} \times 10\text{mL})] \times 100$$

The lipid extract for each sample was dried on a rotary evaporator at 52-53°C under vacuum. The residue was dissolved in 25-mL chloroform, poured into a 25-mL Erlenmeyer flask, nitrogen flushed, sealed securely, and stored at -18°C for further analysis.

Preparation of fatty acid methyl esters (FAME) for fatty acid analysis

The weight of total lipids per mL of the concentrated extract of each sample was determined, and the volume of extract

containing 0.1000 g of total lipids from each sample was placed into a 125-mL Erlenmeyer flask with a ground glass 24/40 top. The extract in the flask was dried by rotary evaporation at 52-53°C under vacuum and converted to FAME by AOCS (1975) procedure.

The lipid residue in each 125-mL Erlenmeyer flask had 4-mL of 0.5N sodium hydroxide in methanol, several glass boiling beads, and 1-mL of an internal standard solution containing 1 mg of tridecanoic acid (13:0) and 1 mg of uneicosanoic acid (21:0) per mL CH_3Cl . The flasks were placed on a hot plate and attached to condensers and the contents allowed to boil for 10 min under reflux. Five mL boron trifluoride (14% by volume) in a methanol solution were added to the flask contents, and the contents allowed to boil under reflux for an additional 2 min. The flasks were cooled and 8-mL pentane was added through the condenser. The flask contents were boiled under reflux for 1 min, removed from heat, and cooled thoroughly. The flasks were removed from the condensers, and the pentane layer containing the FAME was brought to the neck of the flask by the addition of a saturated sodium chloride solution to the flask contents. The FAME layer was transferred to a vial containing a small amount of sodium sulfate which absorbed any residual water from the FAME. The vial was concentrated to 1-mL under a nitrogen flow, sealed, and stored at -18°C until gas chromatographic analysis.

Gas chromatographic analysis of FAME

Fatty acid methyl esters (FAME) in each sample were quantified using a Shimadzu Model GC-9A gas chromatograph

equipped with an automatic injection system Model AOC-9 and a flame ionization detector (Shimadzu Scientific Instruments, Inc., Columbia, MD). The GC conditions were as follows: a 0.25mm i.d. by 30m long fused silica SP-2230 column (Supelco, Inc., Bellefonte, PA) using helium as a carrier gas with a flow rate of 50 mL/min and a split ratio of 1:30. The injection temperature was maintained at 250°C. The column oven was programmed to start at 130°C and then increase to a final temperature of 220°C at 2°C/min. The peak areas of the individual FAME and their relative retention times were recorded by a Shimadzu data processor, Chromatopac Model CR501 interfaced with an IBM personal computer. All chromatograms were stored using the Chromatopac Data Archive Utility version 3.1 software (Shimadzu Scientific Instruments, Inc., Columbia, MD).

The fatty acids identified and analyzed included those with 12 to 22 carbon atoms in a straight chain and those which were saturated to those that contained as many as six double bonds in their carbon chain. The concentration of each identified fatty acid was determined as the weight percentage of the total fatty acids present according to the AOCS (1975) method. Fatty acids were identified by matching retention times of known fatty acid methyl esters with retention times of sample FAME when both were analyzed under the same gas chromatographic conditions.

Cholesterol and cholesterol oxide sample preparation

The separation of cholesterol and cholesterol oxides (COS) was conducted using solid phase extraction as reported by Zheng (1995). From each concentrated lipid

extract of a meat sample, a portion containing 1.000 g of lipids were loaded onto a 3 g silica BondElut (Phenomex, Analytichem, CA), and the neutral lipids were then eluted with 40-mL of 8% diethyl ether by volume in hexane for fraction 1. Cholesterol and polar compounds (free fatty acids, tocopherols, mono- and di-acylglycerols) were eluted by 60% diethyl ether by volume in hexane for fraction 2. Cholesterol oxides were eluted with 20-mL diethyl ether, followed by 40% isopropyl alcohol by volume in hexane for fraction 3. Elution with 40-mL methanol (MeOH) recovered the polar, lipid soluble, fluorescent compounds for fraction 4. Fractions 2, 3, and 4 were dried by rotary evaporation at 52-53°C under vacuum, and the residues dissolved and brought to volume as follows: Fraction 2 in 4-mL of 6% isopropyl alcohol by volume in hexane; Fraction 3 in 5-mL of 4% isopropyl alcohol by volume in hexane; and Fraction 4 in 3-mL of 20% MeOH by volume in chloroform. They were stored under nitrogen at -18°C in sealed glass vials until analysis by HPLC.

Cholesterol and cholesterol oxides analyses

Cholesterol was analyzed on the μ Porasil column attached to a Waters HPLC, detected at wavelength 207nm and eluted from the column at a flow rate of 2.5mL/min by 4% IPA by volume in hexane. Different amounts of a cholesterol standard were also run under the same conditions as the samples. A standard curve of amount (μ g) of cholesterol versus HPLC peak area was constructed and the concentration of cholesterol in mg/100 g meat sample on a wet basis was calculated using the amount of cholesterol determined from the sample peak area and the

equation of the standard curve.

Cholesterol oxide standards: 7-ketocholesterol (3 β -hydroxycholest-5-en-7-one), 7- α -hydroxycholesterol (cholest-5-ene-3 β ,7 α -diol) and 7- β -hydroxycholesterol (cholest-5-ene-3 β ,7 α -diol) (Sigma, St. Louis, MO) were analyzed on the μ Porasil column attached to the Water's HPLC under the same conditions as described for the cholesterol. Standards containing appropriate concentration ratios of the COS above were prepared as follows. One milligram 7-ketocholesterol was dissolved in 10-mL 4% IPA by volume in hexane. Six milligrams 7- β -hydroxycholesterol was dissolved in 100-mL 4% IPA by volume in hexane and then diluted 1:10. Four milligrams 7- α -hydroxycholesterol was dissolved in 100-mL 4% IPA by volume in hexane and diluted 1:10.

Portions (μ L) of each COS standard or diluted COS standard were analyzed under the same HPLC conditions as portions (μ L) of fraction 3 from each meat sample. Standard curves of amounts (μ g) of each COS standards analyzed versus their HPLC peak areas were constructed and used to calculate the amount of each COS in each sample in terms of μ g COS/100 g meat on a wet basis.

Fluorescent products

Fluorescent oxidation products (FOP) from fraction 4 of each sample were analyzed on a μ Porasil column attached to a Water's HPLC equipped with a fluorescence detector at an excitation wavelength of 360nm and an emission wavelength of 440nm. The FOP were eluted from the column by a gradient elution program (Table 1) at a solvent flow rate of 1.2 mL/min. A vitamin A (retinol) standard was prepared by dissolving 212 mg

of t-retinol in 100-mL of 20% methanol by volume in chloroform. Two (2.0) mL of this solution was transferred into a 10-mL volumetric flask and diluted to 10-mL with 20% methanol in chloroform by volume. Different amounts (ng) of vitamin A were analyzed under similar conditions as fraction 4 of each sample was analyzed. From analyses of different amounts of the vitamin A standard, a standard curve

Table 1-Gradient elution program for HPLC analysis of fluorescent oxidation products in raw and stored, cooked chicken meat

Time	% Solvent A ^a	% Solvent B ^b	Change
Initial	100	0	
1.00 min	100	0	Linear
1.10 min	90	10	None
6.00 min	90	10	Linear
6.50 min	50	50	None
16.00 min	50	50	Linear
19.00 min	100	0	None
30.00 min	100	0	

^{ab}Solvent A is pure hexane and Solvent B is 20% by volume methanol in chloroform

was constructed of the amounts of vitamin A injected versus HPLC peak area. Areas of selected FOP peaks in a sample chromatogram were converted to ng vitamin A equivalents/g lipids using the standard curve to relate peak area to amount of FOP.

TBA method

The TBA analysis was carried out by a modified distillation method of Tarladgis et al. (1960). This method involved homogenizing 10 g of sample with 40-mL of water, 0.5% solution

of propyl gallate in 95% ethanol (0.5 g propyl gallate in 100-mL ethanol by volume), and 0.5 g Na₂EDTA in 100-mL water by volume. After homogenation, 47.5-mL water and 5-mL of 6N HCl were added to the flask before the distillation. The distillate (50-mL) was collected and 5.0-mL portions of the distillate was used for formation of the colored complex in combination with 5.0-mL TBA reagent. The absorption of the colored complex was determined at a wavelength of 530nm.

At the same time samples were distilled, malonaldehyde standards were distilled also. Appropriate concentrations of the distilled standard were reacted with TBA reagent, and the absorbance of the resulting complex was determined. The equation of the standard curve was as follows:

$$\text{Concentration (moles MA)} = (\text{Absorbance} + 0.00312) / 96974.3$$

The linear correlation coefficient of this standard curve was 0.996. Levels of TBA reactive substances or TBA value (mg MA/kg meat) were calculated using the standard equation and appropriate dilution and concentration factors.

Statistical analyses

All dependent variables: moisture content (%), level of total lipids (%), concentration (wt. %) of each fatty acid, amount (mg/100 g wet meat) of cholesterol, concentrations of each COS (μg/100 g wet meat) and FOP (μg vitamin A equiv./g lipid), and TBA values (mg malonaldehyde/kg meat) were analyzed statistically as a function of meat type (MEAT), replication

(REP), MEAT x REP, treatment (TRT), oxidation state of meat (STATE), and MEAT x TRT, MEAT x STATE, TRT x STATE and MEAT x TRT x STATE interactions. MEAT and REP were tested for significance by the MEAT x REP interaction and other main effects and interactions by the residual error mean square. The General Linear Models (PROC GLM) of SAS Institute, Inc. (1989) was used to run the analyses of variances. When a dependent variable was significantly ($p < 0.05$) affected by an independent variable, the means were separated by the Student Newman Keul's (SNK) test (SAS Institute, Inc., 1989). An interaction was deemed significant at $p < 0.10$ except for the three way interaction for TBA value which was deemed significant at $p < 0.11$. If a significant interaction existed for a dependent variable, it was analyzed statistically by one of the independent variables using PROC GLM, and least square means for the other dependent variable(s) were separated by the PDIFF option of SAS Institute, Inc. (1989). For example, if a dependent variable had a significant three way interaction (MEAT x TRT x STATE), it was analyzed statistically for each type of meat, and significantly ($p < 0.05$) different means (TRT*STATE) were separated for each meat across TRT and STATE.

Chapter IV

Results and Discussion

Moisture

The moisture content for each type of chicken meat from each treatment and state are presented in Table 2. The moisture was affected ($p < 0.05$) by type of meat (MEAT), treatment (TRT), STATE (uncooked or cooked stored zero, five or ten days at 5-6°C), several two way interactions and the three way interaction of MEAT x TRT x STATE (Appendix A, Table A-1). White chicken meat on the average, contained ($p < 0.05$) a higher level of moisture than dark meat (69.5 versus 66.6%), and meat treated with phosphate had 68.4% moisture which was higher than the 67.9% in meat receiving salt treatment and 67.8% for phosphate plus antioxidant treated meat. Uncooked meat also contained ($p < 0.05$) a higher percentage moisture than any of the stored cooked meats, therefore, cooking the meat resulted in a significant decrease in moisture level. Further refrigerated storage of the cooked meat did not change moisture content significantly (Table 2). The lack of significant changes in the moisture content during refrigerated storage agrees with the results of Ang and Lyon (1990) who found no significant changes in moisture content of any tissue during a five day storage at 5°C for breast, thigh and skin of chicken meat.

Table 2-Mean concentrations^{ab} (%) of moisture in uncooked and cooked white and dark chicken meat receiving different treatments and stored at 5-6°C for up to ten days

Meat type	State ^d	Treatment ^c		
		1	2	3
White	UNC	75.42a	74.49a	74.58a
	C0	66.89ef	69.32b	66.75ef
	C5	67.84cde	68.46bcd	66.68f
	C10	67.64def	68.83bc	66.99ef
Dark	UNC	72.35a	71.78ab	71.19b
	C0	64.34e	65.20cde	65.97c
	C5	64.35e	64.47de	64.69de
	C10	64.36e	64.95de	65.47cd

^an = 2; percent on a wet matter basis.

^bFor a single meat type, means for moisture concentration followed by unlike letters differ (p<0.05).

^c1=salt; 2=phosphate, 3=phosphate + antioxidant.

^dUNC=uncooked; C0=cooked and stored zero day at 5-6°C; C5=cooked and stored five days at 5-6°C and C10=cooked and stored ten days at 5-6°C.

Lipid content

The analysis of variance for lipid content of chicken meat is presented in Appendix A, Table A-2. Lipid content was affected (p<0.05) only by the type of meat (light versus dark). Dark chicken meat contained more than twice as much lipid as the white chicken meat samples (13.76 versus 5.87%). This is in agreement with Pikul et al. (1984a) who stated that dark chicken meat contains more than twice as much fat as breast meat. Also, there were no changes in lipid content during cooking and subsequent storage or among the treatments. These latter

results agree with that of other researchers who observed no change ($p < 0.05$) in fat content of cooked chicken meat over several days storage at 2-5°C (Ang and Lyon, 1990; Kregel et al., 1986). The higher moisture content and lower fat contents in white chicken meat (breast) compared with dark meat are supported by the findings of Kregel et al. (1986) who found that moisture content in uncooked chicken meat was inversely proportional to the fat content.

Fatty acids

The following fatty acids were identified in chicken meat: lauric (12:0), myristic (14:0), myristoleic (14:1), palmitic (16:0), palmitoleic (16:1), margaric (17:0), 9-heptadecenoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), 11-eicosenoic (20:1), 8,11,14-eicosatrienoic (20:3), arachidonic (20:4), 5,8,11,14,17-eicosapentenoic (20:5n3), 10,13,16,19-docosatetraenoic (22:4n3), 7,10,13,16-docosatetraenoic (22:4n6), 7,10,13,16,10-docosapentaenoic (22:5) and docosahexenoic (22:6) acids. The analysis of variance for each of these acids are presented in Appendix A, Table A-3. The acids, 14:0, 16:0, 16:1, 18:0, 18:1, 20:3, and 20:4, were affected ($p < 0.05$) by meat type (MEAT). Treatment (TRT) affected levels of 17:0, 18:0, 18:1, 18:2, 18:3, 20:3, 20:4, 22:4n3, 22:4n6, 22:5 and 22:6. Cooking and refrigerated storage up to ten days (STATE) influenced ($p < 0.05$) levels of 12:0, 14:0, 17:0, 17:1, 18:2, 20:5n3, and 22:4n3. Concentrations of 12:0, 16:0, 16:1, 18:2, 20:3, 20:4, and 22:4n3 had a significant ($p < 0.10$) MEAT x TRT interaction while only

18:2 had a significant MEAT x STATE interaction. Other fatty acids (12:0, 17:1, 18:2, 20:3, 20:4, 20:5n3, 22:4n3, and 22:4n6) had a significant TRT x STATE interaction. Only a few acids (17:0, 17:1, 18:2, and 20:5n3) had a three-way interaction ($p < 0.10$). The mean levels of each fatty acid averaged across TRT and STATE for each type of meat are presented in Table 3.

The fatty acids that had no two or three way interactions ($p < 0.10$) but had differences between meat types were 14:0, 18:0, and 18:1 (Table 3). The white meat samples had higher concentrations of 14:0 fatty acid while the dark chicken meat had higher levels of 18:0, and 18:1. Oleic acid (18:1) was in the highest concentration of all fatty acids in both meat types, however 18:1 concentration was lower than the reported value of 48.2% from Deman (1980). Lauric acid, (12:0) was lower than the reported value of 1.9% by deMan (1980). High levels of fatty acids (16:0, 16:1, 18:0, 18:1 and 18:2) were found in chicken meat while low levels of polyunsaturated fatty acids (18:3, 20:3, 20:4, 20:5n3, 22:4n3, 22:4n6, 22:5 and 22:6) were found (Table 3). These findings agree with the data of Jantawat and Dawson (1980) for poultry meat.

Among those fatty acids not affected ($p > 0.10$) by any interactions but affected ($p < 0.05$) by treatment were 18:0, 18:1, 18:3, 22:5 and 22:6 (Table 4). Meat receiving salt treatment had higher concentrations ($p < 0.05$) of 18:0 and 18:1 than meat receiving other treatments. Meat receiving phosphate treatment had highest concentrations of 18:3, 22:5 and 22:6 of all treatments (Table 4). The fatty acids, 14:0, 14:1, 20:0 and 20:1 were not affected by treatment or any interaction.

Table 3-Mean concentrations^{ab} (%) of fatty acids in the total lipids of white and dark chicken meat averaged across meat treatments and states

Fatty acid	Type of meat	
	White	Dark
12:0 ^c	0.020	0.019
14:0	0.651a	0.548b
14:1	0.235	0.260
16:0 ^c	24.471a	23.547b
16:1 ^c	9.232b	9.906a
17:0 ^d	0.041	0.032
17:1 ^d	0.074	0.076
18:0	5.113b	5.192a
18:1	40.990b	41.630a
18:2 ^{cde}	15.874	16.149
18:3	0.620	0.651
20:0	0.063	0.082
20:1	0.441	0.487
20:3 ^c	0.278a	0.184b
20:4 ^c	0.878a	0.554b
20:5n3 ^d	0.092	0.085
22:4n3 ^{cf}	0.165	0.096
22:4n6 ^f	0.281	0.161
22:5	0.159	0.103
22:6	0.265	0.192

^an=24; weight percentage of fatty acids.

^bMeans in a row followed by unlike letters differ (p<0.05).

^cHad a meat x treatment interaction (p<0.10).

^dHad a meat x treatment x state interaction (p<0.10).

^eHad a meat x state interaction (p<0.10).

^fTentatively identified.

Table 4-Mean concentrations^{ab} of fatty acids in total lipids of chicken meat receiving different treatments and averaged across meat type and state

Fatty acid	Treatment		
	Control (salt)	Phosphate	Phosphate + antioxidant
12:0 ^{de}	0.019	0.019	0.019
14:0	0.592	0.619	0.612
14:1	0.243	0.254	0.246
16:0 ^e	24.113	23.778	24.136
16:1 ^e	9.535	9.589	9.583
17:0 ^f	0.039a	0.034b	0.035b
17:1 ^{df}	0.077	0.075	0.074
18:0	5.225a	5.107b	5.125b
18:1	41.889a	40.943b	41.088b
18:2 ^{def}	15.460c	16.425a	16.150b
18:3	0.547b	0.701a	0.660ab
20:0	0.071	0.093	0.053
20:1	0.494	0.435	0.464
20:3 ^{de}	0.208b	0.241a	0.241a
20:4 ^{de}	0.630b	0.771a	0.747a
20:5n3 ^{df}	0.084	0.091	0.089
22:4n3 ^{deg}	0.245a	0.070b	0.070b
22:4n6 ^{dg}	0.201b	0.242a	0.221ab
22:5	0.102b	0.162a	0.130ab
22:6	0.196b	0.281a	0.205b

^an=16; weight percentages of fatty acids.

^bMeans in a row followed by unlike letters differ ($p < 0.05$).

^c1=salt; 2=phosphate; 3=phosphate + antioxidant.

^dHad a treatment x state interaction ($p < 0.10$).

^eHad a meat x treatment interaction ($p < 0.10$).

^fHad a meat x treatment x state interaction ($p < 0.10$).

^gTentatively identified.

The mean levels of the fatty acids for each meat oxidation state are presented in Table 5. Fatty acids which were not affected ($p>0.05$) by state of meat or any interaction of state with other independent variables include 14:1, 16:0, 16:1, 18:0, 18:1, 20:0, 20:1, 22:5, and 22:6 (Table 5). Cooking the chicken meat reduced levels of 14:0, but further refrigerated storage had no effect ($p>0.05$) on 14:0 concentration (Table 5). The concentrations of fatty acids not affected ($p>0.05$) by state but with significant state interactions with other independent variables were 18:3, 20:3, 20:4, and 22:4n6. The levels of the fatty acids, 12:0, 17:0, 17:1, 18:2, 20:5n3, and 22:4n3, were influenced by state ($p<0.05$) and interactions of state with other independent variables ($p<0.10$).

The mean levels of the following fatty acids, 12:0, 16:0, 16:1, 20:4 and 22:4n3 for each type of meat x treatment combination are given in Table 6. These fatty acids which had higher levels in white than dark chicken meat also were affected ($p<0.10$) by the MEAT x TRT interaction (Table 3). For any one of these fatty acids, the manner in which the concentration changed with treatment was different for white chicken meat than for dark chicken meat resulting in the significant MEAT x TRT interaction. For example, the salt treated white chicken meat had higher levels of 16:0 than phosphate treated white meat, but no difference existed in the 16:0 level between salt treated and phosphate treated dark chicken meat (Table 6). For 20:4, phosphate treated white chicken meat had a lower amount than the phosphate plus antioxidant white meat; while the reverse was true in dark chicken meat. The significantly higher 20:4

Table 5-Mean concentrations^{ab} of fatty acids in white and dark, uncooked chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

Fatty acid	State ^c			
	UNC	C0	C5	C10
12:0 ^d	0.018b	0.019ab	0.020a	0.020a
14:0	0.654a	0.602b	0.594b	0.580b
14:1	0.245	0.247	0.250	0.248
16:0	23.885	23.783	24.116	24.252
16:1	9.558	9.455	9.608	9.656
17:0 ^f	0.030b	0.032ab	0.039a	0.041a
17:1 ^{df}	0.056b	0.082a	0.080a	0.081a
18:0	5.119	5.181	5.147	5.162
18:1	41.039	41.536	41.262	41.388
18:2 ^{def}	16.507a	16.108b	15.849c	15.585d
18:3 ^d	0.579	0.650	0.697	0.606
20:0	0.037	0.111	0.103	0.066
20:1	0.472	0.437	0.486	0.464
20:3 ^d	0.222	0.236	0.244	0.218
20:4 ^d	0.721	0.740	0.720	0.684
20:5n3 ^{df}	0.074b	0.080b	0.091b	0.112a
22:4n3 ^{dg}	0.066b	0.081b	0.178a	0.189a
22:4n6 ^{dg}	0.210	0.224	0.231	0.219
22:5	0.150	0.111	0.115	0.149
22:6	0.277	0.176	0.214	0.249

^an=12; weight percentages of fatty acids.

^bMeans in a row followed by unlike letters differ (p<0.05).

^cUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; C10=cooked meat stored ten days at 5-6°C.

^dHad a treatment x state interaction (p<0.10).

^eHad a meat x state interaction (p<0.10).

^fHad a meat x treatment x state interaction (p<0.10).

treatment or meat or with both treatment and meat (Table 5).

^gTentatively identified.

Table 6-Mean concentrations^{ab} of fatty acids in white and dark chicken meat receiving different treatments as averaged across meat state

Fatty acid	Meat type	Treatment ^c		
		1	2	3
12:0	White	0.020a	0.019b	0.020a
	Dark	0.019b	0.019b	0.019b
16:0	White	24.919a	24.247b	24.245bc
	Dark	23.307d	23.308d	24.027c
16:1	White	9.230b	9.332b	9.134b
	Dark	9.841a	9.845a	10.030a
20:3	White	0.246b	0.282a	0.299a
	Dark	0.169d	0.201c	0.182cd
20:4	White	0.775b	0.918a	0.942a
	Dark	0.485d	0.625c	0.552cd
22:4n3 ^d	White	0.324a	0.079b	0.082b
	Dark	0.167b	0.066b	0.059b

^an=8; weight percentage of fatty acids

^bFor any one acid, means followed by unlike letters differ (p<0.05).

^c1=salt; 2=phosphate; 3=phosphate + antioxidant.

^dTentatively identified.

concentration in white than dark chicken meat concurs with findings of other researchers (Jantawat and Dawson, 1980; Pikul et al., 1984a). Jantawat and Dawson (1980) stated that the most obvious differences among phospholipids from different types of tissue was in the quantities of arachidonic (20:4) acid.

The following fatty acids had a TRT x STATE interaction ($p < 0.10$): 12:0, 20:3, 20:4, 22:4n3 and 22:4n6 but no three way interaction (MEAT X TRT X STATE). The concentration means for these fatty acids are presented for each treatment-state combination in Table 7. For each of these acids, the manner in which the concentration changed across state differed among the three treatments resulting in the TRT x STATE interaction. For example, the concentrations of 20:4 and 22:4n6 generally decreased in the salt treated meat during cooking and subsequent 5-6°C storage (Table 6), however, this trend was not found in the treated meats. For 22:4n3, for unknown reasons, the concentration was highest in salt treated meat at 5 and 10 days storage at 5-6°C.

A three way interaction of MEAT x TRT x STATE ($p < 0.10$) was found for the following fatty acids: 17:0, 17:1, 18:2 and 20:5n3 (Appendix A, Table A-3). The concentrations means of each of these acids for each type of meat, treatment and state are presented in Tables 8 through 11. Generally in salt treated white meat, levels of 17:0 and 17:1 increased (Tables 8 and 9) and levels of 18:2 decreased (Table 10) during cooking and subsequent ten days storage at 5-6°C. In the treated white meats and in dark chicken meat from all treatments, no consistent trend was observed in the concentration changes of

Table 7-Mean concentrations^{ab} of fatty acids in uncooked and cooked chicken meat receiving different treatments and stored at 5-6°C for up to ten days after being cooked

Fatty acid	Treat- ment ^d	State ^e			
		UNC	C0	C5	C10
12:0	1	0.0184c	0.0190bc	0.0199abc	0.0202ab
	2	0.0184bc	0.0190bc	0.0185bc	0.0200abc
	3	0.0181c	0.0193abc	0.0208a	0.0184bc
20:3	1	0.213cde	0.231bcd	0.206de	0.181e
	2	0.208cde	0.245abc	0.271a	0.242abcd
	3	0.244abc	0.230bcd	0.255ab	0.233bcd
20:4	1	0.720ab	0.696ab	0.596ab	0.508b
	2	0.704ab	0.785a	0.810a	0.788a
	3	0.739ab	0.739ab	0.754ab	0.758ab
22:4n3 ^e	1	0.115b	0.061b	0.387a	0.419a
	2	0.065b	0.066b	0.082b	0.067b
	3	0.063b	0.072b	0.065b	0.080b
22:4n6 ^e	1	0.250ab	0.199ab	0.196ab	0.158b
	2	0.222ab	0.212ab	0.293a	0.239ab
	3	0.201ab	0.218ab	0.204ab	0.260ab

^an=8; weight percentages of fatty acids.

^bFor a single fatty acid, means followed by unlike letters differ (p<0.05).

^cUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; and C10=cooked meat stored ten days at 5-6°C.

^d1=salt; 2=phosphate; 3=phosphate + antioxidant.

^eTentatively identified.

Table 8-Mean concentrations^{ab} of margaric acid (17:0) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat being stored up to ten days at 5-6°C

Meat type	Treat-ment ^c	State ^d			
		UNC	C0	C5	C10
White	1	0.0333bc	0.0426bc	0.0481b	0.0642a
	2	0.0372bc	0.0300bc	0.0409bc	0.0325c
	3	0.0000d	0.0403bc	0.0339bc	0.0375bc
Dark	1	0.0209cd	0.0294bcd	0.0453a	0.0335abc
	2	0.0000d	0.0124d	0.0331abc	0.0370abc
	3	0.0262bcd	0.0304abcd	0.0317abcd	0.0391ab

^an=2; weight percentages of fatty acids.

^bFor any one meat, means followed by different letters differ (p<0.05).

^c1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^dUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; and C10=cooked meat stored ten days at 5-6°C.

Table 9-Mean concentrations^{ab} of 9-heptadecenoic acid (17:1) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat being stored up to ten days at 5-6°C

Meat type	Treat-ment ^c	State ^d			
		UNC	C0	C5	C10
White	1	0.0365d	0.0533bcd	0.0888ab	0.1036a
	2	0.0715abcd	0.1056a	0.0363d	0.0808abc
	3	0.0190d	0.1131a	0.1119a	0.0414bcd
Dark	1	0.0760ab	0.0722ab	0.1032a	0.0811ab
	2	0.0531b	0.0720ab	0.0756ab	0.1030a
	3	0.0620b	0.0732ab	0.0671ab	0.0758ab

^an=2; weight percentages of fatty acids.

^bFor any one meat, means followed by different letters differ (p<0.05).

^c1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^dUNC=uncooked; C0=cooked and stored zero day at 5-6°C; C5=cooked and stored five days at 5-6°C; and C10=cooked and stored ten days at 5-6°C.

Table 10-Mean concentrations^{ab} of linoleic acid (18:2) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat stored up to ten days at 5-6°C

Meat type	Treat-ment ^c	State ^d			
		UNC	C0	C5	C10
White	1	16.725ab	15.724d	14.251e	13.323f
	2	16.781a	16.511abc	16.091cd	16.172c
	3	16.089cd	16.433abc	16.233bc	16.160c
Dark	1	16.743a	15.579d	16.025bc	15.312d
	2	16.721a	16.319abc	16.465ab	16.340abc
	3	15.979cd	16.080abc	16.028c	16.200abc

^an=2; weight percentages of fatty acids.

^bFor any one meat, means followed by different letters differ (p<0.05).

^c1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^dUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; and C10=cooked meat stored ten days at 5-6°C.

Table 11-Mean concentrations^{ab} of 5,8,11,14,17-eicosapentenoic acid (20:5n3) in uncooked and cooked white and dark chicken meat treated in different ways with the cooked meat being stored up to ten days at 5-6°C

Meat type	Treat-ment ^c	State ^d			
		UNC	C0	C5	C10
White	1	0.0645e	0.0929bcd	0.0000e	0.1062bc
	2	0.0696e	0.0742de	0.1295a	0.1102b
	3	0.1023bc	0.0779de	0.0888cde	0.1085bc
Dark	1	0.0635c	0.0713bc	0.0627c	0.1747a
	2	0.0665c	0.0795bc	0.0757bc	0.1188b
	3	0.0919bc	0.0834bc	0.0843bc	0.0804bc

^an=2; weight percentages of fatty acids.

^bFor any one meat, means followed by different letters differ (p<0.05).

^c1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^dUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; and C10=cooked meat stored ten days at 5-6°C.

any of the acids, 17:0, 17:1, 18:2 and 20:5n3, across state. However, for any one of these acids in these meats, significant differences existed among the different treatments and states. The manner in which the concentration of each acid for each meat treated in different ways changed across state was different, however, resulting in the significant three way interaction. Therefore, the only PUFAs to use for proper assessment of oxidation in this study were 18:2 and 20:4 since they decreased steadily during the storage particularly in the salt and phosphate treated meat.

Cholesterol content

The cholesterol content, expressed mg/100 g on a wet basis, in chicken meat was affected by meat ($p < 0.05$), MEAT x TRT ($p < 0.10$), and MEAT x TRT x STATE ($p < 0.10$, Appendix A, Table A-4). The means of cholesterol content in each type of chicken meat from the different treatments and states are presented in Table 12. Overall, white chicken meat contained ($p < 0.05$) lower cholesterol content than dark chicken meat (43 versus 73 mg/100 g meat, wet basis). There was a wide range in cholesterol content of any one meat (23 to 63 mg/100 g meat for white chicken meat and 51 to 130 mg/100 g meat for dark chicken meat) with relatively few significant differences among the treatments and states of the meat. In the white meat, the uncooked meat treated with phosphate and antioxidant and the cooked meat treated with phosphate and stored for five days at 5-6°C had higher cholesterol levels ($p < 0.05$) only than cooked meat treated with phosphate and antioxidant and stored 0 days (Table 12). In

Table 12-Mean cholesterol levels^{ab} of white and dark chicken meats treated in different ways while uncooked and then cooked and stored up to ten days at 5-6°C

Meat type	Treat-ment ^c	State ^d			
		UNC	C0	C5	C10
White	1	34ab	60ab	39ab	43ab
	2	38ab	33ab	63a	26ab
	3	23b	62a	53ab	45ab
Dark	1	75bc	58bc	130a	57bc
	2	88abc	86abc	90ab	79bc
	3	59bc	51c	58bc	79bc

^an=2; mg/100 g meat, wet basis.

^bFor any one meat, means followed by different letters differ (p<0.05).

^c1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^dUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; and C10=cooked meat stored ten days at 5-6°C.

the dark meat, similar cholesterol levels were found for the following: salt treated, cooked meat stored for 5 days, phosphate treated uncooked meat, and phosphate treated, cooked meat stored 0 and 5 days (Table 12). However, the cholesterol levels in the latter three of these meat samples were significantly different only from cooked meat treated with phosphate and antioxidant and stored 0 days (Table 12).

The reported cholesterol content of meat, approximately 75 mg/100 g meat, wet basis, is below the level considered unhealthy for humans (300 mg/day, Fennema, 1985). In the present study, the average cholesterol content (43 mg/100 g meat) was below this level, and the average cholesterol content (73 mg/100 g meat) in the dark chicken meat was close to that value.

Cholesterol oxidation products

In the present study, the treatments and cooking and storage of the chicken meat at 5-6°C had no effect ($p < 0.05$) on concentrations of 7- α -hydroxycholesterol and 7- β -hydroxycholesterol, oxidation products of cholesterol (Appendix A, Tables A-5 and A-6, respectively). A significant difference was found between white and dark chicken meat in the level of 7- α -hydroxycholesterol, but no significant interactions among MEAT, TRT or STATE were found for either hydroxycholesterol.

White chicken meat contained a higher concentration of 7- α -hydroxycholesterol than did dark chicken meat (0.85 versus 0.27 $\mu\text{g}/100\text{ g}$ meat, wet basis). The concentrations of 7- β -hydroxycholesterol ranged from 0.01 $\mu\text{g}/100\text{ g}$ meat, wet basis, in salt treated uncooked chicken meat to as much as 10 $\mu\text{g}/100\text{ g}$

meat in phosphate + antioxidant treated, cooked meat stored for ten days at 5-6°C. The mean concentrations of 7- α -hydroxycholesterol and 7- β -hydroxycholesterol for each meat type, treatment and state are presented in Appendix B, Tables B-1 - B-3, respectively.

The levels of the cholesterol oxidation product, 7-ketocholesterol were different ($p < 0.05$) among meat types, treatments and states (Appendix A, Table A-7) and there was significant MEAT x TRT, TRT x STATE, and MEAT x TRT x STATE interactions ($p < 0.10$). Overall, white chicken meat had lower levels of 7-ketocholesterol than did dark chicken meat (3.4 versus 5.8 $\mu\text{g}/100\text{ g}$ meat, wet basis). The mean levels of 7-ketocholesterol for white and dark chicken meat from three treatments and four states are presented in Table 13.

Generally in the white meat, the level of 7-ketocholesterol increased significantly in salt treated, cooked chicken during 10 days storage at 5-6°C, but not in cooked chicken receiving phosphate or phosphate + antioxidant treatment. In dark meat, the concentration of 7-ketocholesterol was higher ($p < 0.05$) in salt treated, cooked chicken after 5 and 10 days storage at 5-6°C than in uncooked or freshly cooked, salt treated meat or in any other treated meat at any state with one exception (Table 13). This exception was the highest concentration of 7-ketocholesterol measured in dark meat was found in phosphate + antioxidant treated, cooked meat stored 10 days at 5-6°C.

Although, consistent decreases in cholesterol content across 10 day storage at 5-6°C were not found (Table 12), increases ($p < 0.05$) in the level of 7-ketocholesterol, an

Table 13-Mean levels^{ab} of 7-ketocholesterol in white and dark chicken meats treated in different ways while uncooked and then cooked and stored up to ten days at 5-6°C

Meat type	Treat-ment ^c	State ^d			
		UNC	C0	C5	C10
White	1	3.4bc	1.3c	7.4b	14.0a
	2	2.0c	1.3c	2.0c	2.4bc
	3	1.5c	1.2c	2.4bc	2.3bc
Dark	1	4.4bc	4.0bc	9.9ab	9.4ab
	2	6.6bc	4.1bc	4.9bc	3.9bc
	3	4.0bc	3.2bc	2.6c	12.9a

^an=2; µg/100 g meat, wet basis.

^bFor any one meat, means followed by different letters differ (p<0.05).

^c1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^dUNC=uncooked meat; C0=cooked meat stored zero day at 5-6°C; C5=cooked meat stored five days at 5-6°C; and C10=cooked meat stored ten days at 5-6°C.

oxidation product of cholesterol, occurred during the ten day refrigeration storage of salt treated meat (Table 13). This shows that the cholesterol in the cooked chicken samples did oxidize. However, the method for measuring cholesterol concentration in the present study apparently was not sensitive enough to measure cholesterol degradation under the present conditions. The lack of significant changes in cholesterol levels with increasing storage time in the present study is in disagreement with the findings of Kregel et al. (1986). They reported a decrease in cholesterol levels in heated ground beef during a 30 day storage. Pearson et al. (1983) also indicated that cholesterol may oxidize in cooked stored muscle. Kregel et al. (1986) suggested that conditions present during cooking of meat and subsequent storage influence the rate of cholesterol degradation. Pie et al. (1991) reported that cholesterol values for cooked beef and pork remained essentially the same before and after freezing of the products. However, with beef in which oxidation occurred during cooking, Pie et al. (1991) found that frozen (-20°C) storage resulted in a more intense breakdown of cholesterol.

The compound, 7-ketocholesterol, is the principal and most abundant cholesterol oxidation product (Pie et al., 1991). The pattern found in the present study of increasing amounts of 7-ketocholesterol in cooked meat during refrigeration storage also agrees with the data of Devore (1988) and Zubillaga and Maerker (1991), who found increasing levels of 7-ketocholesterol with increasing storage of cooked meats.

Fluorescent oxidation products

Three peaks, detected by the fluorescent detector at an excitation wavelength of 360nm and emission wavelength of 440nm during HPLC analysis, were consistently found in most of the chicken meat samples analyzed in the present experiment. These three peaks were designated as fluorescent oxidation product(FOP) I with a retention time of 2.9-3.0 min, FOP II with a retention time of 12.5-13.0 min and FOP III with a retention time of 19.0-20.0 min. These fluorescent products were quantitated as μg retinol equivalents/g total lipids in the chicken meat where data for the retinol standard curve was obtained under exactly the same conditions as the FOPs were analyzed. No MEAT, TRT or STATE effects ($p>0.05$) or interactions (MEAT x TRT, MEAT x STATE, TRT x STATE or MEAT x TRT x STATE) ($p>0.10$) were found for the levels of FOP I or FOP II (Appendix A, Tables A-9 and A-10). However, the concentration of FOP III was significantly affected by STATE and had MEAT x STATE, TRT x STATE, and MEAT x TRT x STATE interactions ($P<0.10$, Appendix A, Table A-10).

The levels for each FOP in each meat type and for each treatment are presented in Tables 14 and 15, respectively. The highest mean measured was FOP II and FOP III in white meat (Table 14). For FOP III, treated meat tended to have lower concentrations than did salt treated meat (Table 15).

The concentration of each FOP for each state of the meat is presented in Table 16. The only significant difference found in the concentration of any FOP was for FOP III. The cooked chicken stored for 10 days at 5-6°C had a higher concentration

Table 14-Mean concentrations^a of fluorescent oxidation products in white and dark chicken meat averaged across meat treatments and states

Product ^b	Meat type	
	White (n=24)	Dark (n=23)
FOP I	14.4	8.4
FOP II	128.7	70.9
FOP III	156.4	64.4

^aµg retinol equivalents/g total lipids.

^bRetention time in HPLC analysis for FOP I was 2.9 - 3.0 min; for FOP II was 12.6-13.0 min, and for FOP III was 19.0-20.0 min.

Table 15-Mean concentrations^a of fluorescent oxidation products (FOPs) in chicken meat receiving different treatments and averaged across meat types and states

Product ^c	Treatment ^b		
	1 (n=16)	2 (n=16)	3 (n=15)
FOP I	12.5	10.6	11.3
FOP II	112.9	112.3	74.6
FOP III	127.0	111.2	95.0

^an=µg retinol equivalents/g total lipids.

^b1=salt; 2=phosphate; and 3=phosphate + antioxidant.

^cRetention time in HPLC analyses for FOP I was 2.9-3.0 min; for FOP II was 12.6-13.0 min and for FOP III was 19.0-20.0 min.

Table 16-Mean concentrations^{ab} of fluorescent oxidation products in uncooked chicken meat and in cooked chicken meat stored at 5-6°C up to ten days as averaged across meat type and treatment

Product	State ^c			
	UNC (n=11)	C0 (n=12)	C5 (n=12)	C10 (n=12)
FOP I	11.4	10.5	11.2	12.8
FOP II	138.3a	105.1ab	69.6b	88 92.0ab
FOP III	120.2ab	76.6b	92.5b	157.0a

^aµg retinol equivalents/g fat.

^bMeans in a row followed by unlike letters are different (p<0.05).

^cUNC=uncooked meat, C0=cooked meat stored zero days at 5-6°C; C5-cooked meat stored five days at 5-6°C; C10 cooked meat stored ten days at 5-6°C.

of FOP III then chicken stored for 0 or 5 days at the same temperature (Table 16).

However, a significant three way interaction of MEAT x TRT x STATE (Appendix A, Table A-10) was found for FOP III which may make this latter observation dependent upon either type of meat (MEAT) and/or treatment (TRT). Means of FOP III concentration for each type of meat treated in different ways while uncooked and then cooked and stored 0, 5 and 10 days at 5-6°C are presented in Table 17. White chicken meat receiving salt treatment or phosphate treatment, then cooked and stored 10 days at 5-6°C had the highest levels of FOP III while lowest levels were found in white chicken meat receiving salt treatment, cooked and stored 0 days and in white meat treated with phosphate plus antioxidant, cooked and stored 10 days (Table 17). Overall dark meat for the most part appeared to have lower levels of FOP III than white meat, and only a few differences

Table 17-Mean concentrations^{ab} of FOP III in white and dark chicken meat treated differently while uncooked and then cooked and stored up to ten days at 5-6°C

Meat type	Treat-ment ^d	State ^c			
		UNC	C0	C5	C10
White	1	150bcd	68d	81cd	371a
	2	215bc	143bcd	96cd	265ab
	3	149bcd	119cd	168bcd	52d
Dark	1	92ab	80ab	68ab	105a
	2	46ab	17b	65ab	43ab
	3	15b	33ab	77ab	106a

^an=2; µg retinol equivalents/g fat.

^bFor any one type of meat, means followed by unlike letters are different (p<0.05).

^cUNC=uncooked meat, C0=cooked meat stored zero days at 5-6°C; C5-cooked meat stored five days at 5-6°C; C10 cooked meat stored ten days at 5-6°C.

^d1=salt; 2=phosphate; and 3=phosphate + antioxidant.

were found (p<0.05) for FOP III level in dark meat treated in different ways while uncooked and then cooked and stored up to 10 days at 5-6°C. Dark chicken meat salt treated, cooked and stored ten days and dark meat treated with phosphate plus antioxidant, cooked and stored 10 days at 5-6°C had higher amounts of FOP III than did phosphate treated, cooked meat stored 0 days or uncooked, phosphate plus antioxidant treated meat (Table 17).

Pikul et al. (1984a) reported that the total fluorescence of a lipid extract of cooked poultry meat increased after 4 days refrigeration storage and that these fluorescent products in the cooked chicken increased with increasing oxidation. Dillard and

Tappel (1973) reported an increase in fluorescent products but a decline in TBA values during increasing storage of fatty acid mixtures. They attributed this finding to the breakdown of malonaldehyde over time which formed fluorescent products.

Reasons for the lack of a consistent increase in concentration of FOP III with increasing state of oxidation (Table 17) is unknown at the present time. The identity and how these fluorescent oxidation products compare with those measured by Zheng (1995) in cooked turkey, pork and beef are also unknown. Zheng (1995) found the concentrations of three fluorescent oxidation products in cooked turkey, pork and beef to increase with increasing storage. However, Zheng (1995) analyzed the FOPs using reverse phase chromatography, and the FOPs in the present study were analyzed using normal phase chromatography. No other investigators have tried to separate and quantitate fluorescent oxidation products isolated from meat products by HPLC.

Thiobarbituric acid (TBA) values

The TBA values (mg malonaldehyde/kg meat) were affected ($p < 0.05$) by TRT and STATE and had a significant TRT x STATE interaction (Appendix A, Table A-12). The mean TBA values for each type of chicken meat receiving different treatments and at different states are given in Table 18. Averaged across meat type and state, meat receiving salt treatment had a higher ($p < 0.05$) TBA value than meat receiving the phosphate or phosphate plus antioxidant treatments (8.8 versus 1.3 and 0.8 mg malonaldehyde/kg meat, respectively). However, the TBA values

for any one state of meat was dependent upon the treatment.

As shown in Table 18, the TBA value of salt treated dark chicken meat increased ($p < 0.05$) from 0 up to 10 days storage at 5-6°C. The TBA-value of salt treated white meat increased ($p < 0.05$) during cooking and from 0 to 5 days storage of cooked meat before decreasing from 5 to 10 days storage (Table 18). TBA values of white or dark chicken receiving phosphate or phosphate plus antioxidant treatment failed to increase significantly during cooking and subsequent storage at 5-6°C.

The lack of significant differences in the TBA values between white chicken meat and dark chicken meat, which has a higher lipid content than white meat, is contrary to the results reported by Igene and Pearson (1979) who found that meat systems containing the greatest lipid content exhibited the highest TBA values. Pikul et al. (1984a) also reported a higher TBA number in leg meat of chicken than the breast meat because the leg contained more than twice as much total fat as the breast meat. In addition, several investigators have found slightly higher TBA values in dark chicken meat than in white meat (Igene et al., 1985; Wilson et al., 1976). Higher TBA values in dark meat compared to white meat may be due to larger amounts of myoglobin in dark meat which serves as a source of Fe(II) that is broken down during cooking and is available then to catalyze lipid oxidation.

The low TBA values found in uncooked chicken meat (Table 18) is in agreement with other investigators who studied the TBA values of uncooked versus cooked meats. Lyon et al. (1988) found lowest TBA values for raw chicken patties (compared with

Table 18-Mean 2-thiobarbituric acid (TBA) values^{ab} of white and dark chicken meat treated differently while uncooked and then cooked and stored for up to ten days at 5-6°C

Meat type	Treat-ment ^d	State ^c			
		UNC	CO	C5	C10
White	1	0.5d	8.1bc	14.5a	8.6b
	2	0.5d	1.3d	1.8d	3.0cd
	3	0.6d	0.7d	1.2d	1.7d
Dark	1	0.4c	7.2bc	11.6b	23.3a
	2	0.4c	1.4c	0.4c	1.2c
	3	0.4c	0.4c	0.4c	0.6c

^an=2; mg malonaldehyde/kg meat.

^bFor any one type of meat, means followed by unlike letters are different ($p < 0.05$).

^cUNC=uncooked meat, CO=cooked meat stored zero days at 5-6°C; C5-cooked meat stored five days at 5-6°C; C10 cooked meat stored ten days at 5-6°C.

^d1=salt; 2=phosphate; and 3=phosphate + antioxidant.

cooked chicken patties). The TBA value increased more than 10-fold in salt treated white and dark chicken meat during cooking. This large increase is consistent with other investigators who studied the TBA value of raw and cooked poultry meat (Lyon et al., 1988; Pikul et al., 1984a). This increase is to be expected since during the cooking process, levels of free Fe(II) increase greatly and accelerate lipid oxidation in cooked muscle (Igene et al., 1979).

The increase in TBA value with increasing storage time (with the exception of the salt treated white meat from 5 to 10 days storage) for the salt treated meat is in agreement with published data (Ang and Lyon, 1990; Brewer et al., 1992a; Lyon et al., 1988; Miller et al., 1994; Poste et al., 1986; Su et al., 1991). This increase in TBA value corresponds to overall decreases in the levels of the polyunsaturated fatty acids (PUFA), 20:4 and 18:2 (Tables 8 and 10, respectively). According to Miller et al. (1994) during the early storage of cooked meat there is a disruption of the porphyrin ring resulting in oxidation of PUFA (Igene et al., 1979; Pearson et al., 1986). The increase in TBA value and decrease in selected PUFA is also in agreement with Poste et al. (1986) who found an increase of TBA values after eight days storage of meat with an associated decrease in PUFA levels during the same period.

The decrease in TBA values of salt treated white meat from 5 to 10 days storage at 5-6°C (Table 18) is indicative of the reactive nature of malonaldehyde. Other investigators have found decreases in the TBA values in uncooked and cooked chicken that was stored frozen (Igene et al., 1979). Malonaldehyde is

only a secondary product of lipid oxidation and not the final product (Bailey et al., 1980). Melton (1983) stated that malonaldehyde does not necessarily increase with increasing storage time of meats. Malonaldehyde is very reactive with other food components such as proteins, amino acids and phospholipids, and this could be the reason for the observed decrease in TBA values in the present study.

The lack of increase in the TBA value during the storage of cooked chicken treated with phosphate is in agreement with Liu et al. (1992) who reported lower TBA values in pork steaks from treatments containing sodium tripolyphosphate and rosemary oleoresin than in the controls without treatment. The lack of significant differences in the TBA values between white and dark chicken, however, is in disagreement with the published data of Ajuyah et al. (1993) who found that cooked dark chicken meat had a higher TBA value than white meat throughout a five day storage period.

Phosphate treatment greatly reduced the formation of malonaldehyde; the addition of rosemary oleoresin did not significantly increase this reduction (Table 18). The antioxidant effect of phosphate is thought to be due to its sequestering of heavy metals, especially iron, which is a major prooxidant metal in meats (Igene et al., 1979). The lack of an additional reduction in TBA value in chicken meat when rosemary was added is in agreement with Liu et al. (1992) who found no additional benefits in preventing lipid oxidation when rosemary was added with phosphate.

Relationship of TBA values with other measurements of oxidation

Correlation coefficients between TBA values and concentration of each fatty acid analyzed, 7- β -hydroxy-cholesterol, 7- α -hydroxycholesterol, 7-ketocholesterol, and each of the three fluorescent oxidation products were determined. TBA values were correlated ($p < 0.05$) with percentages of 14:0 ($r = -0.37$), 18:2 ($r = -0.54$), 18:3 ($r = -0.35$), 20:4 ($r = -0.34$), and 22:4n3 ($r = 0.58$) across meat type, treatment and storage ($n = 48$). A negative correlation implies that as the TBA value increased with increasing oxidation, the concentration of the fatty acid decreased. For 18:2, 18:3 and 20:4, it is reasonable to suspect that their concentrations (expressed in weight percentages of fatty acids) decreased during cooking and storage of the meat because they were oxidized with increasing oxidation in the meat. The negative correlation of 14:0 with TBA value and the positive correlation of 22:4n3 ($r = 0.58$) are hard to explain. The possibility exists that the 22:4n3 was misidentified and really is tetraeicosanoic acid (24:0) which is very stable to oxidation. These latter two fatty acids have similar retention times under the GC conditions the fatty acids methyl esters were analyzed (Melton, 1995). As the percentage concentrations of the unsaturated fatty acids decrease because they are more subject to oxidation, the percentages of more stable fatty acids increase. However, 14:0 is also stable to oxidation, and it is not likely that this fatty acid was misidentified. Therefore, it is unknown why it has a negative correlation with increasing oxidation as measured by the TBA value. Averaged across meat type and treatment, the percentages of 14:0 in cooked meat

stored 0, 5 and 10 days were ($p < 0.05$) lower than in uncooked chicken meat, but the level of 14:0 did not decrease significantly during refrigerated storage of the cooked meat (Table 5).

The only significant correlation of TBA value with the other measurements of lipid oxidation in the present study was with the concentration of 7-ketocholesterol (0.47; $n = 46$; $p < 0.05$). This positive correlation indicates that as the TBA value increased with increasing oxidation in the chicken meat, 7-ketocholesterol also increased in concentration. In fact, the level of 7-ketocholesterol increased steadily in cooked, white chicken meat from 0 to 10 days refrigerated storage (Table 13), but the TBA value increased only up to 5 days storage before decreasing significantly at 10 days storage (Table 18). However, in dark chicken meat, the TBA value increased steadily across 10 days refrigerated storage (Table 18), but the level of 7-ketocholesterol did not. The control cooked, dark chicken meat stored for 5 and 10 days at 5-6°C did have ($p < 0.05$) higher levels of 7-ketocholesterol than did control of uncooked or cooked, dark chicken meat stored 0 day (Table 13). Zheng (1995) concluded that multiple tests, each measuring a different component of lipid oxidation, are necessary to follow oxidation in stored cooked muscle foods. She found that measurement of the TBA value and 7-ketocholesterol were two such tests. However, Zheng (1995) also found that measurement of fluorescent oxidation products extended the time during which the lipid oxidation in cooked muscle foods could be followed. Results of the present study for fluorescent oxidation products did not

confirm this latter observation in cooked chicken meats. Results, however, do support that, in combination with TBA value, measurement of 7-ketocholesterol in stored, cooked chicken meat may be an additional indicator of lipid oxidation.

Chapter V

Summary

In the present investigation, white (boneless, skinless, breast meat) and dark (boneless, skinless, thigh meat) chicken meats were treated with (1) salt, (2) phosphate or (3) phosphate + antioxidant (rosemary oleoresin) while raw, and then were cooked. Enough of each type of chicken meat was obtained at one time and prepared to complete a planned experiment with two replications in which uncooked meat samples were treated and most of them cooked. A replication consisted of the 2 types of meat, 3 treatments and 4 states (uncooked, cooked and stored 0 day, cooked and stored 5 days, and cooked and stored 10 days at 5-6°C). Forty-eight meat samples were used. The meat treated with salt, phosphate and antioxidant was similar to the frozen, cooked chicken entrees that may be purchased in local retail markets.

The moisture and total lipid concentrations, the fatty acid composition of the total lipids, the cholesterol content, the levels of 7- α -hydroxycholesterol, 7- β -hydroxycholesterol and 7-ketocholesterol, the amounts of three fluorescent products (FOPs), and the 2-thiobarbituric acid or TBA value (mg malonaldehyde/kg meat) were determined on each uncooked and cooked, stored chicken meat sample. These dependent variables were analyzed statistically as a function of meat type, treatment, oxidation state and their appropriate interactions. Effects for the independent variables were determined to be significant at $p < 0.05$, and interactions significant at $p < 0.10$.

Correlation coefficients of the TBA value with the concentration of each fatty acid, each cholesterol oxide (COP), and each FOP were also determined.

The moisture level was lower in dark chicken meat than white meat ($p < 0.05$), and the total lipid concentration in the dark meat was more than twice that present in the white meat (5.87%). Phosphate treated white chicken meat generally had higher moisture levels than the meat from other treatments, and cooked meat had significantly lower moisture levels than uncooked meat.

Compared with dark chicken meat, white meat had lower levels of the 18 carbon fatty acids: stearic (18:0) and oleic (18:1), and the monounsaturated fatty acid, palmitoleic (16:1), but higher percentage concentrations of myristic (14:0) and palmitic (16:0) acids and the polyunsaturated fatty acids: eicosatrienoic (20:3), and arachidonic (20:4). Treatment did not affect the concentrations of the fatty acids in the chicken meat to any great extent. However, storage at 5-6°C decreased concentrations of the polyunsaturated fatty acids, 18:2 and 20:4, particularly in meat receiving salt treatment or phosphate treatment.

Dark chicken meat had higher levels of cholesterol than white chicken meat, but no consistent trend for treatment or storage was found for cholesterol content. White chicken meat contained a higher concentration of 7- α -hydroxycholesterol than dark chicken meat (0.85 versus 0.27 $\mu\text{g}/100\text{ g meat}$). The levels of 7- β -hydroxycholesterol ranged from 0.01 to 10 $\mu\text{g}/100\text{ g meat}$. The three lipid soluble, polar, FOPs extracted from the chicken

were detected at an excitation wavelength of 360 nm and an emission wavelength of 440 nm and had retention times of 2.9-3.0 min, 12.5-13.0 min and 19.0-20.0 min in a gradient elution normal phase HPLC analysis.

In general, levels of 7- α -hydroxycholesterol and 7- β -hydroxycholesterol and two of the FOPs were unaffected ($p>0.05$) by treatment or cooking and 5-6°C storage of the chicken meats. Generally, 7-ketocholesterol, one FOP (retention time of 19.0-20.0 min) and the TBA value (mg malonaldehyde/kg meat) increased during the 10 day storage at 5-6°C of the white and dark chicken meats receiving only salt treatment. The one exception to this was the significant decrease in TBA-value from 14.5 to 8.5 mg malonaldehyde/kg meat in dark meat from five to ten days storage at 5-6°C. The TBA value, which is a well accepted measurement of lipid oxidation in meat, was negatively correlated ($p<0.05$) with percentage concentration of 18:2 ($r=-0.54$), positively correlated with 7-ketocholesterol level ($r=0.47$) and not significantly correlated with any other COP or FOP. The level of 7-ketocholesterol may be an additional indicator of lipid oxidation in stored, cooked muscle foods in combination with the TBA value.

List of References

List of References

- Ahn, D.U., Ajuyah, A., Wolfe, F.H., and Sim, J.S. 1993. Oxygen availability affects prooxidant catalyzed lipid oxidation of cooked turkey patties. *J. Food Sci.* 58(2):278-282,291.
- Ajuyah, A.O., Fenton, T.W., Hardin, R.T., and Sim, J.S. 1993. Measuring lipid oxidation volatiles in meats. *J. Food Sci.* 58(3):270-273,277.
- Allen, C.E. and Foegeding, E.A. 1981. Some lipid characteristics and interactions in muscle foods--a review. *Food Techn.* 35(5):253-255.
- Ang, C.Y.W. 1988. Comparison of broiler tissues for oxidative changes after cooking and refrigerated storage. *J. Food Sci.* 53(4):1072-1075.
- Ang, C.Y.W. and Lyon, B.G. 1990. Evaluations of warmed-over flavor during chill storage of cooked broiler breast, thigh and skin by chemical, instrumental and sensory methods. *J. Food Sci.* 55(3):644-648,673.
- AOAC. 1980. Official Methods of Analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC.
- AOCS. 1975. Official and Tentative Methods of Analysis, 4th ed. American Oil Chemists' Society, Champaign, IL.
- Asghar, A., Gray, J.I., Buckley, D.J., Pearson, A.M., and Booren, A.M. 1988. Perspectives on warmed-over flavor. *Food Techn.* 42(6):102-108.
- Bailey, M.E., Dupuy, H.P., and Legendre, M.G. 1980. Lipid oxidation in meats. In: The Analysis and Control of Less Desirable Flavors in Foods and Beverages, p. 31-52. G. Charalambous (Ed.), Academic Press, New York.
- Bailey, M.E., Rourke, T.J., Gutheil, R.A., and Wang, C.Y.-J. 1992. Rancidity in meats. In Off-flavors in Foods and Beverages, G.Charalambous (Ed.), p.127-159. Elsevier Science Publishers. Amsterdam.
- Bailey, M.E., Shin-Lee, S.Y., Dupuy, H.P., St. Angelo, A.J., and Vercellotti, J.R. 1987. Inhibition of warmed-over flavor by Maillard reaction products. In Warmed-Over Flavor of Meat, A.J. St. Angelo and M.E. Bailey (Ed.), p. 237-266. Academic Press, Inc., Orlando.
- Block, G. and Langseth, L. 1994. Antioxidant vitamins and disease prevention. *Food Techn.* 48(7):80-84.

- Brewer, M.S., Ikins, W.G., and Harbers, C.A.Z. 1992a. TBA values, sensory characteristics, and volatiles in ground pork during long-term frozen storage: effects of packaging. *J. Food Sci.* 57(2):558-563,580.
- Brewer, M.S., McKeith, F.K., and Britt, K. 1992b. Fat, soy and carrageenan effects on sensory and physical characteristics of ground beef patties. *J. Food Sci.* 57(4):1051-1052,1055.
- Brown, H.G. 1995. Personal communication, Tyson's Foods, Springdale, AR.
- Chastain, M.F., Huffman, D.L., Hsieh, W.H., and Cordary, J.C. 1982. Antioxidants in restructured beef/pork steaks. *J. Food Sci.* 47:1779-1782.
- Chio, K.S. and Tappel, A.L. 1969. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. *Biochem.* 8:2827-2831.
- Csallany, A.S., Kindom, S.E., Addis, P.B., and Lee, J-H. 1989. HPLC method for quantitation of cholesterol and four of its major oxidation products in muscle and liver tissue. *Lipids.* 24(7):645-651.
- Decker, E.A. and Hultin, H.O. 1990. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *J. Food Sci.* 55:947-950.
- Decker, E.A. and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* 38:674-677.
- Decker, E.A., Crum, A.D., Shantha, N.C., and Morrissey, P.A. 1993. Catalysis of lipid oxidation by iron from an insoluble fraction of beef diaphragm muscle. *J. Food Sci.* 58(2):233-236,258.
- deMan, J.M. 1980. Principles of Food Chemistry. The Avi Publishing Co., Inc., Westport, Conn.
- DeVore, V.R. 1988. TBA values and 7-ketocholesterol in refrigerated raw and cooked ground beef. *J. Food Sci.* 53(4):1058-1061.
- Dillard, C.J. and Tappel, A.L. 1971. Fluorescent products of lipid peroxidation of mitochondria and microsomes. *Lipids.* 6(10):715-721.
- Dillard, C.J. and Tappel, A.L. 1973. Fluorescent products from reaction of peroxidizing polyunsaturated fatty acid with phosphatidyl ethanolamine and phenylamine. *Lipids.* 8(4):183-189.
- Dorko, C. 1994. Antioxidants used in foods. *Food Techn.* 48(4):33.

- Empson, K.L., Labuza, T.P., and Graf, E. 1991. Phytic acid as an antioxidant. *J. Food Sci.* 56(3):560-563.
- Ensminger, M.E. (Ed.). 1980. Poultry Science, 2nd ed. The Interstate Printers & Publishers, Inc., Danville, Illinois.
- Fennema, O.R. (Ed.). 1985. Food Chemistry, Marcel Dekker, New York.
- Finocchiaro, E.T. and Richardson, T. 1983. Sterol oxides in foodstuffs: a review. *J. Food Prot.* 46(10):917-925.
- Goll, S.J., Kastner, C.L., and Kropf, D.H. 1992. Glucose and internal cooking temperature effects on low fat, pre- and post-rigor, restructured beef roasts. *J. Food Sci.* 57:834-840.
- Gray, J.I. 1978. Measurement of lipid oxidation: a review. *J. Am. Oil Chem. Soc.* 55:539.
- Gray, G.I. and Pearson, A.M. 1987. Oxidative measurements in meats. In Advances in Meat Research, A.M. Pearson and Dutson, T.R. (Eds.), p. 221-269. Van Nostrand Reinhold Co., New York.
- Ho, C.P., Huffman, D.L., Bradford, D.D., Egbert, W.R., Mikel, W.B., and Jones, W.R. 1995. Storage stability of vacuum packaged frozen pork sausage containing soy protein concentrate, carrageenan, or antioxidants. *J. Food Sci.* 60:257-261.
- Igene, J.O. and Pearson, A.M. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat systems. *J. Food Sci.* 44(5):1285-1290.
- Igene, J.O., Pearson, A.M., Merkel, R.A., and Coleman, T.H. 1979. Effect of frozen storage time, cooking and holding temperature upon extractable lipids and TBA values of beef and chicken. *J. Anim. Sci.* 49:701-707.
- Igene, J.O., Pearson, A.M., Dugan, L.R., and Price, J.F. 1980. Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chem.* 5:263-276.
- Igene, J.O., Yamanchi, K., Pearson, A.M., Gray, J.I., and Aust, S.D. 1985. Evaluation of 2-thiobarbituric acid reactive substances (TBRS) in relations to warmed-over flavor (WOF) development in cooked chickens. *J. Agric. Food Chem.* 33:364-367.
- Jantawat, P. and Dawson, L.E. 1980. Composition of lipids from mechanically deboned poultry meats and their composite tissues. *Poultry Sci.* 59:1043-1052.

- Kregel, K.K., Prusa, K.J., and Hughes, K.V. 1986. Cholesterol content and sensory analysis of ground beef as influenced by fat level, heating, and storage. *J. Food Sci.* 51:1162-1165,1190.
- Kumar, N., and Singhal, O.P. 1991. Cholesterol oxides and atherosclerosis: a review. *J. Sci. Food Agric.* 55:497-510.
- Lee, B.J. and Hendricks, D.G. 1995. Phytic acid protective against beef round muscle lipid peroxidation. *J. Food Sci.* 60:241-244.
- Liu, H.F., Booren, A.M., Gray, J.I., and Crackel, R.L. 1992. Antioxidant efficacy of oleoresin rosemary and sodium tripolyphosphate in restructured pork steaks. *J. Food Sci.* 57:803-806.
- Lyon, B.G., Lyon, C.E., Ang, C.Y.W., and Young, L.L. 1988. Sensory analysis and thiobarbituric acid values of precooked chicken patties stored up to three days and reheated by two methods. *Poultry Sci.* 67:736-742.
- Maerker, G. 1987. Cholesterol autooxidation-current status. *J. Am. Chem. Soc.* 64:388-392.
- Maerker, G., Nungesser, E.H., and Zulak, I.M. 1988. HPLC separation and quantitation of cholesterol oxidation products with flame ionization detection. *J. Agric. Food Chem.* 36:61-63.
- Marion, J.E. and Woodroof, J.G. 1965. Lipid fractions of chicken broiler tissues and their fatty acid composition. *J. Food Sci.* 30:38-43.
- Melton, S.L., Moyers, R.E., and Playford, C.G. 1979. Lipids extracted from soy products by different procedures. *J. Am. Oil Chem. Soc.* 56:489-493.
- Melton, S.L. 1983. Methodology for following lipid oxidation in muscle foods. *Food Techn.* 37(7):105-111,116.
- Melton, S.L. 1985. Methods of Assessing Oxidation. In Proceedings of 1985 Meat Industry Research Conference, Chicago, IL., October 18-19, 1985.
- Melton, S.L. 1995. Personal communication, The University of Tennessee, Knoxville.
- Miller, D.K., Smith, V.L., Kanner, J., Miller, D.D., and Lawless, H.T. 1994. Lipid oxidation and warmed-over aroma in cooked ground pork from swine fed increasing levels of iron. *J. Food Sci.* 59:751-756.
- Mount, J.R. 1993. Personal communication. The University of Tennessee, Knoxville.

- O'Brein, J.M. and Labuza, T.P. 1994. Symposium provides new insights into nonenzymatic browning reactions. *Food Techn.* 48(7):56-58.
- Park, S.W. and Addis, P.B. 1987. Cholesterol oxidation products in some muscle foods. *J. Food Sci.* 52(6):1500-1503.
- Pearson, A.M., Love, J.D., and Shorland, F.B. 1977. Warmed-over flavor in meat, poultry and fish. *Adv. Food Res.* 23:1-68.
- Pearson, A.M. and Gray, J.I. 1983. Mechanism responsible for warmed-over flavor in cooked meat. Ch. 13, In The Maillard Reaction in Foods and Nutrition, G.R. Waller and M.S. Feather (Ed.), p. 287-300. Am. Chem. Soc. Symp. Ser. 215, Washington, DC.
- Pearson, A.M., Gray, J.I., Wolzak, A.M., and Horenstein, N.A. 1983. Safety implications of oxidized lipids in muscle foods. *Food Techn.* 37(7):121-129.
- Pie, J., Spahis, K., Seillan, C. 1991. Cholesterol oxidation in meat products during cooking and frozen storage. *J. Agric. Food Chem.* 39(2): 250-254.
- Pikul, J., Leszczynski, D.E., and Kummerow, F. 1983. Elimination of sample autoxidation by butylated hydroxytoluene additives before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. *J. Agric. Food Chem.* 31:1338-1342.
- Pikul, J., Leszczynski, D.E., Niewiarowicz, A., and Kummerow, F.A. 1984a. Lipid oxidation in chicken breast and leg meat after sequential treatments of frozen storage, cooking, refrigerated storage and reheating. *J. Food Techn.* 19:575-584.
- Pikul, J., Leszczynski, D.E., Betchel, P.J., and Kummerow, F.A. 1984b. Effects of frozen storage and cooking on lipid oxidation in chicken meat. *J. Food Sci.* 49:838-842.
- Poste, L.M., Willemot, C., Butler, G., and Patterson, C. 1986. Sensory aroma scores and TBA values as indices of warmed-over flavor in pork. *J. Food Sci.* 51:886-888.
- Rhee, K.S. 1978. Minimization of further lipid peroxidation in the distillation 2-thiobarbituric acid test of fish and meat. *J. Food Sci.* 43(6):1776-1778,1781.
- Rhee, K.S., Ziprin, Y.A., and Rhee, K.H. 1981. Antioxidant activity of methanolic extracts of various oilseed protein ingredients. *J. Food Sci.* 46:37-40.
- Rhee, K.S. 1988. Enzymic and nonenzymic catalysis of lipid oxidation in muscle foods. *J. Food Techn.* 42(3):127-130.

- Sander, B.D., Smith, D.E., Addis, P.B., and Park, S.W. 1989. Effects of prolonged and adverse storage conditions on levels of cholesterol oxidation products in dairy products. *J. Food Sci.* 54:874-879.
- Sato, K., Hegarty, G.R., and Herring, H.K. 1973. The inhibition of warmed-over flavor in cooked meats. *J. Food Sci.* 38(2):398-402.
- SAS Institute, Inc. 1989. SAS User's Guide: Basic, Version 5 Ed. Caryville, NC, SAS Institute Inc.
- Shahidi, F., Rubin, L.J., and D'Souza, L.A. 1986. Meat flavor volatiles: a review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.* 24:141-150.
- Shahidi, F., Rubin, L.J., and Wood, D.F. 1987. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrihemochrome. *J. Food Sci.* 52(3):564-567.
- Simic, M.G., Jovanovic, S.V., and Niki, E. 1992. Mechanisms of lipid oxidative processes and their inhibition. In Lipid Oxidation in Food, ACS Symposium Series 500, A.J. St. Angelo (Ed.), p. 14. ACS Books, Inc., Washington, DC.
- Sinnhuber, R.O. and Yu, T.C. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. *Food Techn.* 12(1):9-12.
- Siu, G.M. and Draper, H.H. 1978. A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.* 43:1147-1149.
- Smith, J.S. and Alfawaz, M. 1995. Antioxidative activity of maillard reaction products in cooked ground beef, sensory and TBA values. *J. Food Sci.* 60:234-236,240.
- Steinberg, D., Parthasarathy, S., Carew, T.E., and Witztum, J.L. 1989. Beyond cholesterol: modifications of low-density lipoprotein that increases its atherogenicity. *New England J. Med.* 320:915-924.
- Su, Y., Ang, C.Y.W., and Lillard, D.A. 1991. Precooking method affects warmed-over flavor of broiler breast patties. *J. Food Sci.* 56(5):881-883,898.
- Tarladgis, B.G., Watts, B.M., Younathan, M.T., and Dugan, L.A. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* 37:44-48.
- Tims, M.J. and Watts, B.M. 1958. Protection of cooked meats with phosphates. *Food Techn.* 12:240-243.

- Wheeler, T.L., Seideman, S.C., Davis, G.W., and Rolan, T.L. 1990. Effect of chloride salts and antioxidants on sensory and storage traits of restructured beef steaks. *J. Food Sci.* 55(6):1276-1279.
- Wilson, B.R., Pearson, A.M., 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.* 24:7-11.
- Wu, T.W., Lee, M.H., Ho, C.T., and Chang, S.S. 1982. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. *J. Am. Oil Chem. Soc.* 59:339-345.
- Wu, S.Y. and Brewer, M.S. 1994. Soy protein isolate antioxidant effect on lipid peroxidation of ground beef and microsomal lipids. *J. Food Sci.* 59(5):702-706.
- Zheng, Y. 1995. Fatty acids and cholesterol in and oxidation measurement of different cooked ground meats during 5-6°C storage. Ph.D. Dissertation, University of Tennessee, Knoxville.
- Zubillaga, M.P. and Maerker, G. 1991. Quantification of three cholesterol oxidation products in raw meat and chicken. *J. Food Sci.* 56(6):1194-1196,1202.

Appendices

Appendix A

Table A-1-Analyses of variance for total moisture of uncooked dark and white chicken meat and cooked dark and white chicken meat receiving different treatments and stored for up to ten days at 5-6°C

General Linear Models Procedure				
Dependent Variable: TM				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	100.70580378	5147.05	0.0089
REP	1	0.97895113		
MEAT*REP (Error a)	1	0.01956573	0.08	0.7744
TRT	2	3.86060803	8.31	0.0021
STATE	3	443.34905030	636.08	0.0001
MEAT*TRT	2	6.37129401	13.71	0.0001
MEAT*STATE	3	0.79086592	1.13	0.3568
TRT*STATE	6	6.67498427	4.79	0.0029
MEAT*TRT*STATE	6	4.62722578	3.32	0.0176
Error B	22	5.11131187		
Corrected Total	47	572.48966081		

Table A-2-Analyses of variance for percentage of fat in uncooked dark and white chicken meat and cooked dark and white chicken meat receiving different treatments and stored at 5-6°C for up to ten days.

General Linear Models Procedure				
Dependent Variable: WTFAT				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	824.59552566	800.64	0.0225
REP	1	0.40861216		
MEAT*REP (Error a)	1	1.02992432	0.16	0.6948
TRT	2	0.97334416	0.07	0.9283
STATE	3	13.06954609	0.67	0.5803
MEAT*TRT	2	4.31879245	0.33	0.7214
MEAT*STATE	3	7.11815303	0.36	0.7795
TRT*STATE	6	12.47654944	0.32	0.9200
MEAT*TRT*STATE	6	21.65126298	0.55	0.7619
Error B	22	143.35498666		
Corrected Total	47	1028.99669694		

Table A-3-Analyses of variance for percentages of fatty acids in total lipids of uncooked dark and white chicken meat receiving different treatments and then stored at 5-6°C for up to ten days

Fatty acid = Lauric acid (12:0)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0000136	0.0000136	4.64	0.2766
REP	1	0.0000012	0.0000012		
MEAT*REP(Error a)	1	0.0000029	0.0000029	2.67	0.1178
TRT	2	0.0000032	0.0000016	1.43	0.2620
STATE	3	0.0000151	0.0000050	4.59	0.0133
TRT*MEAT	2	0.0000075	0.0000038	3.42	0.0529
MEAT*STATE	3	0.0000002	0.0000001	0.07	0.9734
TRT*STATE	6	0.0000163	0.0000027	2.47	0.0599
TRT*MEAT*STATE	6	0.0000076	0.0000013	1.15	0.3691
Error B	20	0.0000220	0.0000011		
Corrected Total	45	0.0000818			

Fatty acid = Myristic acid (14:0)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0920905	0.0920905	165.43	0.0494
REP	1	0.0001240	0.0001240		
MEAT*REP(Error a)	1	0.0005567	0.0005567	0.30	0.5895
TRT	2	0.0064527	0.0032264	1.74	0.1992
STATE	3	0.0368901	0.0122967	6.62	0.0023
TRT*MEAT	2	0.0056599	0.0028300	1.52	0.2399
MEAT*STATE	3	0.0025828	0.0008609	0.46	0.7105
TRT*STATE	6	0.0033502	0.0005584	0.30	0.9298
TRT*MEAT*STATE	6	0.0114680	0.0019113	1.03	0.4328
Error B	22	0.0408447	0.0018566		
Corrected Total	47	0.2000196			

continued

Table A-3 (continued)

Fatty acid = Myristoleic acid (14:1)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0073021	0.0073021	36.65	0.1042
REP	1	0.0001258	0.0001258		
MEAT*REP (Error a)	1	0.0001992	0.0001992	0.57	0.4563
TRT	2	0.0009884	0.0004942	1.43	0.2616
STATE	3	0.0001074	0.0000358	0.10	0.9572
TRT*MEAT	2	0.0013253	0.0006627	1.91	0.1715
MEAT*STATE	3	0.0013260	0.0004420	1.28	0.3073
TRT*STATE	6	0.0019795	0.0003299	0.95	0.4792
TRT*MEAT*STATE	6	0.0016358	0.0002726	0.79	0.5896
Error B	22	0.0076225	0.0003465		
Corrected Total	47	0.0226120			

Fatty acid = Palmitic (16:0)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	10.227897	10.227897	461.52	0.0296
REP	1	0.000540	0.000540		
MEAT*REP(Error a)	1	0.022161	0.022161	0.10	0.7519
TRT	2	1.287680	0.643840	2.98	0.0717
STATE	3	1.637834	0.545945	2.53	0.0839
TRT*MEAT	2	3.888799	1.944400	8.99	0.0014
MEAT*STATE	3	0.270376	0.090125	0.42	0.7426
TRT*STATE	6	2.513754	0.418959	1.94	0.1193
TRT*MEAT*STATE	6	1.175144	0.195857	0.91	0.5085
Error B	22	4.755862	0.216176		
Corrected Total	47	25.780047			

continued

Table A-3-(continued)

Fatty acid = Palmitoleic (16:1)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	5.4455772	5.4455772	2594.71	0.0125
REP	1	0.0000012	0.0000012		
MEAT*REP(Error a)	1	0.0020987	0.0020987	0.04	0.8500
TRT	2	0.0278228	0.0139114	0.24	0.7865
STATE	3	0.2660493	0.0886831	1.55	0.2303
TRT*MEAT	2	0.3224411	0.1612205	2.81	0.0816
MEAT*STATE	3	0.3439424	0.1146475	2.00	0.1433
TRT*STATE	6	0.3787080	0.0631180	1.10	0.3927
TRT*MEAT*STATE	6	0.5568924	0.0928154	1.62	0.1888
Error B	22	1.2605069	0.0572958		
Corrected Total	47	8.6040399			

Fatty acid=Margaric (17:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0006009	0.0006009	46.44	0.0928
REP	1	0.0000308	0.0000308		
MEAT*REP(Error a)	1	0.0000129	0.0000129	0.26	0.6170
TRT	2	0.0004273	0.0002136	4.31	0.0333
STATE	3	0.0007625	0.0002542	5.12	0.0123
TRT*MEAT	2	0.0001898	0.0000949	1.91	0.1820
MEAT*STATE	3	0.0001118	0.0000373	0.75	0.5383
TRT*STATE	6	0.0005418	0.0000903	1.82	0.1622
TRT*MEAT*STATE	4	0.0006217	0.0001554	3.13	0.0464
Error B	15	0.0007440	0.0000496		
Corrected Total	38	0.0043587			

continued

Table A-3-(continued)

Fatty acid=9-heptadecenoic (17:1)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0002341	0.0002341	5.68	0.2529
REP	1	0.0005896	0.0005896		
MEAT*REP(Error a)	1	0.0000412	0.0000412	0.10	0.7518
TRT	2	0.0003405	0.0001703	0.42	0.6598
STATE	3	0.0063750	0.0021250	5.29	0.0071
TRT*MEAT	2	0.0003776	0.0001888	0.47	0.6312
MEAT*STATE	3	0.0024963	0.0008321	2.07	0.1344
TRT*STATE	6	0.0094259	0.0015710	3.91	0.0088
TRT*MEAT*STATE	6	0.0093464	0.0015577	3.88	0.0092
Error B	21	0.0084298	0.0004014		
Corrected Total	46	0.0355929			

Fatty acid=stearic acid (18:0)

General Linear Models Procedure					
Dependent variable: PERCENT		Percent of total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0758017	0.0758017	1849.00	0.0148
REP	1	0.0003690	0.0003690		
MEAT*REP (Error a)	1	0.0000410	0.0000410	0.00	0.9516
TRT	2	0.1289120	0.0644560	5.93	0.0087
STATE	3	0.0247411	0.0082470	0.76	0.5291
MEAT*TRT	2	0.0260940	0.0123047	1.20	0.3199
MEAT*STATE	3	0.0408526	0.0136175	1.25	0.3147
TRT*STATE	6	0.0684224	0.0114037	1.05	0.4213
MEAT*TRT*STATE	6	0.0652247	0.0108708	1.00	0.4498
Error B	22	0.2390479	0.0108658		
Corrected Total	47	0.6695062			

continued

Table A-3- (continued)

Fatty acid=Oleic (18:1)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	4.8721078	4.8721078	1849.00	0.0148
REP	1	0.0237150	0.0237150		
MEAT*REP (Error a)	1	0.0026350	0.0026350	0.00	0.9516
TRT	2	8.2857450	4.1428725	5.93	0.0087
STATE	3	1.5902201	0.5300734	0.76	0.5291
MEAT*TRT	2	1.6771750	0.8385875	1.20	0.3199
MEAT*STATE	3	2.6257736	0.8752579	1.25	0.3147
TRT*STATE	6	4.3978084	0.7329681	1.05	0.4213
MEAT*TRT*STATE	6	4.1922797	0.6987133	1.00	0.4498
Error	22	15.3646619	0.6983937		
Corrected Total	47	43.0321202			

Fatty acid=Linoleic (18:2)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.9078681	0.9078681	4.88	0.2705
REP	1	0.0038946	0.0038946		
MEAT*REP(Error a)	1	0.1859226	0.1859226	2.57	0.1230
TRT	2	7.9093131	3.9546565	54.72	0.0001
STATE	3	5.5572882	1.8524294	25.63	0.0001
TRT*MEAT	2	2.5188766	1.2594383	17.43	0.0001
MEAT*STATE	3	2.1273444	0.7091148	9.81	0.0003
TRT*STATE	6	7.4513372	1.2418895	17.19	0.0001
TRT*MEAT*STATE	6	1.9627119	0.3271186	4.53	0.0039
Error B	22	1.589827	0.072265		
Corrected Total	47	30.214384			

continued

Table A-3. Continued

Fatty acid=Arachidic (20:0)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0000097	0.0000097	3.17	0.3259
REP	1	0.0001226	0.0001226	0.04	0.8688
MEAT*REP(Error a)	1	0.0000031	0.0000031	0.00	0.9789
TRT	2	0.0060612	0.0030306	1.08	0.5624
STATE	3	0.0115841	0.0038614	1.38	0.5433
TRT*MEAT	0	0.0000000	.	.	.
MEAT*STATE	1	0.0000266	0.0000266	0.01	0.9382
TRT*STATE	1	0.0034153	0.0034153	1.22	0.4687
TRT*MEAT*STATE	0	0.0000000	.	.	.
Error B	1	0.0028043	0.0028043		
Corrected Total	15	0.0380969			

Fatty acid=Linolenic (18:3)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0116261	0.0116261	0.23	0.7149
REP	1	0.0012100	0.0012100		
MEAT*REP(Error a)	1	0.0503640	0.0503640	2.02	0.1695
TRT	2	0.2030367	0.1015184	4.07	0.0314
STATE	3	0.0909500	0.0303167	1.21	0.3278
TRT*MEAT	2	0.0162857	0.0081429	0.33	0.7251
MEAT*STATE	3	0.0188661	0.0062887	0.25	0.8591
TRT*STATE	6	0.2582877	0.0430480	1.72	0.1623
TRT*MEAT*STATE	6	0.0313486	0.0052248	0.21	0.9700
ErrorB	22	0.5490814	0.0249582		
Corrected Total	47	1.2310565			

continued

Table A-3. (continued)

Fatty acid=11-eicosenoic (20:1)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0310040	0.0310040	2.18	0.3791
REP	1	0.0027152	0.0027152		
MEAT*REP(Error a)	1	0.0142334	0.0142334	1.16	0.2944
TRT	2	0.0122234	0.0061117	0.50	0.6152
STATE	3	0.0159227	0.0053076	0.43	0.7321
TRT*MEAT	2	0.0310536	0.0155268	1.26	0.3039
MEAT*STATE	3	0.0262885	0.0087628	0.71	0.5552
TRT*STATE	6	0.0900892	0.0150149	1.22	0.3358
TRT*MEAT*STATE	6	0.0322515	0.0053752	0.44	0.8448
Error B	20	0.2455270	0.0122763		
Corrected Total	45	0.4989246			

Fatty acid=8,11,14-eicostrienoic(20:3)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.1005789	0.1005789	279.29	0.0380
REP	1	0.0006985	0.0006985		
MEAT*REP(Error a)	1	0.0003601	0.0003601	0.56	0.4629
TRT	2	0.0118079	0.0059040	9.15	0.0013
STATE	3	0.0050606	0.0016869	2.61	0.0767
TRT*MEAT	2	0.0038706	0.0019353	3.00	0.0705
MEAT*STATE	3	0.0013854	0.0004618	0.72	0.5531
TRT*STATE	6	0.0096252	0.0016042	2.49	0.0546
TRT*MEAT*STATE	6	0.0055576	0.0009263	1.44	0.2460
Error B	22	0.0141945	0.0006452		
Corrected Total	47	0.1531394			

continued

Table A-3-(continued)

Fatty acid=Arachidonic (20:4)

General Linear Models Procedure
 Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	1.2642868	1.2642868	865993.09	0.0022
REP	1	0.0003185	0.0003185		
MEAT*REP(Error a)	1	0.0000146	0.0000146	0.01	0.9370
TRT	2	0.1833291	0.0916646	40.08	0.0001
STATE	3	0.0192587	0.0064196	2.81	0.0633
TRT*MEAT	2	0.0257860	0.0128930	5.64	0.0106
MEAT*STATE	3	0.0148799	0.0049600	2.17	0.1205
TRT*STATE	6	0.1226309	0.0204385	8.94	0.0001
TRT*MEAT*STATE	6	0.0126194	0.0021032	0.92	0.4997
Error B	22	0.0503115	0.0022869		
Corrected Total	47	1.6934354			

Fatty acid=5,8,11,14,17-eicosapentaenoic (20:5n3)

General Linear Models Procedure
 Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0001770	0.0001770	0.41	0.6387
REP	1	0.0000042	0.0000042		
MEAT*REP(Error a)	1	0.0004354	0.0004354	1.40	0.2524
TRT	2	0.0000151	0.0000076	0.02	0.9759
STATE	3	0.0092265	0.0030755	9.92	0.0005
TRT*MEAT	2	0.0004786	0.0002393	0.77	0.4778
MEAT*STATE	3	0.0015082	0.0005027	1.62	0.2217
TRT*STATE	6	0.0056524	0.0009421	3.04	0.0331
TRT*MEAT*STATE	5	0.0043157	0.0008631	2.78	0.0516
Error B	17	0.0052726	0.0003102		
Corrected Total	41	0.0273297			

continued

Table A-3- (continued)

Fatty acid=10,13,16,19-docosatetraenoic(22:4n3)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0538940	0.0538940	25.51	0.1244
REP	1	0.0148555	0.0148555		
MEAT*REP(Error a)	1	0.0021125	0.0021125	0.36	0.5575
TRT	2	0.3150769	0.1575385	26.50	0.0001
STATE	3	0.1323987	0.0441329	7.42	0.0014
TRT*MEAT	2	0.0471025	0.0235513	3.96	0.0347
MEAT*STATE	3	0.0227237	0.0075746	1.27	0.3089
TRT*STATE	6	0.2603359	0.0433893	7.30	0.0003
TRT*MEAT*STATE	6	0.0329114	0.0054852	0.92	0.4986
Error B	21	0.1248275	0.0059442		
Corrected Total	46	1.0350087			

Fatty acid=7,10,13,16-docosatetraenoic(22:4n6)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.1728053	0.1728053	53.61	0.0864
REP	1	0.0010021	0.0010021		
MEAT*REP(Error a)	1	0.0032235	0.0032235	2.41	0.1350
TRT	2	0.0134309	0.0067155	5.02	0.0160
STATE	3	0.0029687	0.0009896	0.74	0.5399
TRT*MEAT	2	0.0001005	0.0000503	0.04	0.9632
MEAT*STATE	3	0.0075073	0.0025024	1.87	0.1642
TRT*STATE	6	0.0382755	0.0063792	4.77	0.0030
TRT*MEAT*STATE	6	0.0159036	0.0026506	1.98	0.1122
Error B	22	0.0294467	0.0013385		
Corrected Total	47	0.2846640			

continued

Table A-3-(continued)

Fatty acid=7,10,13,16,19-docosapentaenoic (22:5)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0378693	0.0378693	22.64	0.1319
REP	1	0.0112199	0.0112199		
MEAT*REP(Error a)	1	0.0016723	0.0016723	0.75	0.3968
TRT	2	0.0286609	0.0143304	6.40	0.0064
STATE	3	0.0166165	0.0055388	2.47	0.0883
TRT*MEAT	2	0.0108307	0.0054154	2.42	0.1123
MEAT*STATE	3	0.0081334	0.0027111	1.21	0.3291
TRT*STATE	6	0.0127871	0.0021312	0.95	0.4793
TRT*MEAT*STATE	6	0.0209130	0.0034855	1.56	0.2068
Error B	22	0.0492539	0.0022388		
Corrected Total	47	0.1979571			

Fatty acid=4,7,10,13,16,19-docosaheptaenoic(22:6)

General Linear Models Procedure

Dependent Variable: PERCENT Percent of Total Frequency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
MEAT	1	0.0473479	0.0473479	5.26	0.2618
REP	1	0.0216921	0.0216921	2.59	
MEAT*REP(Error a)	1	0.0090022	0.0090022	1.07	0.3120
TRT	2	0.0761146	0.0380573	4.54	0.0230
STATE	3	0.0637556	0.0212519	2.53	0.0845
TRT*MEAT	2	0.0193046	0.0096523	1.15	0.3356
MEAT*STATE	3	0.0155248	0.0051749	0.62	0.6117
TRT*STATE	6	0.0823973	0.0137329	1.64	0.1864
TRT*MEAT*STATE	6	0.0547794	0.0091299	1.09	0.4012
Error B	21	0.1761582	0.0083885		
Corrected Total	46	0.5637218			

Table A-4-Analysis of variance of cholesterol content of uncooked white and dark chicken meat treated differently, and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: CHOL				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	11882.8811546	428.35	0.0307
REP	1	40.7696970		
MEAT*REP (Error a)	1	27.7411255	0.10	0.7532
TRT	2	759.7649120	1.39	0.2718
STATE	3	2440.4280009	2.98	0.0559
MEAT*TRT	2	1843.1494467	3.38	0.0545
MEAT*STATE	3	1746.1655764	2.13	0.1281
TRT*STATE	6	1975.2796634	1.21	0.3437
MEAT*TRT*STATE	6	4254.9752318	2.60	0.0501
Error b	20	5459.8386364		
Corrected Total	45	27498.9610870		

Table A-5-Analysis of variance for concentration of 7- α -hydroxycholesterol in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: TC7A0H				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	3.44745718	163.16	0.0497
REP	1	0.07044962		
MEAT*REP	1	0.02112995	0.00	0.9460
TRT	2	10.65632382	1.19	0.3255
STATE	3	3.22667011	0.24	0.8676
MEAT*TRT	2	17.05804666	1.90	0.1754
MEAT*STATE	3	10.41533672	0.77	0.5221
TRT*STATE	6	17.85614958	0.66	0.6798
MEAT*TRT*STATE	6	9.44028190	0.35	0.9011
Error	20	89.70997222		
Corrected Total	45	172.45433212		

Table A-6-Analysis of variance for concentration of 7- β -hydroxycholesterol in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: TC7B0H				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	34.09956366	0.50	0.6093
REP	1	45.34703277		
MEAT*REP	1	68.70519890	3.00	0.0985
TRT	2	48.25858758	1.05	0.3669
STATE	3	123.95665760	1.81	0.1785
MEAT*TRT	2	51.98533875	1.14	0.3409
MEAT*STATE	3	34.12298492	0.50	0.6884
TRT*STATE	6	227.23512590	1.66	0.1840
MEAT*TRT*STATE	6	273.22467974	1.99	0.1151
Error	20	457.55874097		
Corrected Total	45	1397.92184108		

Table A-7-Analysis of variance for concentration of 7-ketocholesterol in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: TC7KET0				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	52.45219777	54.02	0.0790
REP	1	1.85610506		
MEAT*REP	1	0.97095333	0.16	0.6949
TRT	2	106.37901230	8.67	0.0019
STATE	3	148.50455594	8.07	0.0010
MEAT*TRT	2	39.12939961	3.19	0.0627
MEAT*STATE	3	4.04031937	0.22	0.8816
TRT*STATE	6	113.47838549	3.08	0.0265
MEAT*TRT*STATE	6	98.04354994	2.66	0.0021
Error	20	457.55874097		
Corrected Total	45	1397.92184108		

Table A-8-Analysis of variance for concentration of fluorescent oxidation product I (FOP I) in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: FOP I				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	430.81578635	1.17	0.4749
REP	1	381.24986538		
MEAT*REP	1	367.84009736	7.32	0.0132
TRT	2	32.01149393	0.32	0.7306
STATE	3	35.74744370	0.24	0.8694
MEAT*TRT	2	71.59760174	0.71	0.5019
MEAT*STATE	3	272.35370974	1.81	0.1768
TRT*STATE	6	124.94621830	0.41	0.8610
MEAT*TRT*STATE	6	357.31852058	1.19	0.3517
Error	21	1055.01472899		
Corrected Total	46	3193.94843078		

Table A-9-Analysis of variance for concentration of fluorescent oxidation product II (FOP II) in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: FOP II				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	39532.7189716	46.41	0.0928
REP	1	16455.1661976		
MEAT*REP	1	851.8852251	0.26	0.6167
TRT	2	15333.4957396	2.32	0.1226
STATE	3	21651.7559713	2.19	0.1196
MEAT*TRT	2	9047.0610967	1.37	0.2757
MEAT*STATE	3	1963.4489144	0.20	0.8963
TRT*STATE	6	34409.9597915	1.74	0.1614
MEAT*TRT*STATE	6	16771.0429435	0.85	0.5483
Error	21	69289.867093		
Corrected Total	46	230498.530621		

Table A-10-Analysis of variance for concentration of fluorescent oxidation product III (FOP III) in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: FOP III				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	101182.436617	13.60	0.1686
REP	1	5591.251102		
MEAT*REP	1	7439.655472	3.03	0.0964
TRT	2	10168.634656	2.07	0.1512
STATE	3	43528.753142	5.91	0.0044
MEAT*TRT	2	11196.540398	2.28	0.1271
MEAT*STATE	3	18630.681248	2.53	0.0849
TRT*STATE	6	50144.568145	3.40	0.0167
MEAT*TRT*STATE	6	67583.492900	4.59	0.0040
Error	21	51576.713119		
Corrected Total	46	366200.037436		

Table A-11-Analysis of variance for 2-thiobarbituric acid (TBA) value in uncooked white and dark chicken meat treated differently and then cooked and stored at 5-6°C for up to ten days

General Linear Models Procedure				
Dependent Variable: TBA value				
Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	1.66109648	0.05	0.7750
REP	1	2.45974959		
MEAT*REP	1	31.35254356	2.58	0.1223
TRT	2	756.81444988	31.17	0.0001
STATE	3	229.74812650	6.31	0.0030
MEAT*TRT	2	35.28431672	1.45	0.2554
MEAT*STATE	3	53.74195304	1.48	0.2486
TRT*STATE	6	317.95394767	4.36	0.0048
MEAT*TRT*STATE	6	141.44069632	1.94	0.1091
Error	22	267.09322446		
Corrected Total	47	1825.99529970		

Table A-12-Analyses of variance for TBA values of uncooked dark and white chicken meat and cooked dark and white chicken meat receiving different treatments and stored at 5-6°C for up to 10 days

General Linear Models Procedure

Dependent Variable: TBA

Source	DF	Sum of Squares	F Value	Pr > F
MEAT	1	1.66109648	0.14	0.7150
REP	1	2.45974959	0.20	0.6570
MEAT*REP(Error a)	1	31.35254356	2.58	0.1223
TRT	2	756.81444988	31.17	0.0001
STATE	3	229.74812650	6.31	0.0030
MEAT*TRT	2	35.28431672	1.45	0.2554
MEAT*STATE	3	53.74195304	1.48	0.2486
TRT*STATE	6	317.95394737	4.36	0.0048
MEAT*TRT*STATE	6	141.44059532	1.94	0.1186
Error B	22	267.09322446		
Corrected Total	47	1825.99529970		

Appendix B

Table B-1-Mean^{ab} concentrations of 7- α -hydroxycholesterol, 7- β -hydroxycholesterol, and total lipids for each type of chicken meat as averaged across treatment and state

Variable	Type of meat	
	White	Dark
7- α -hydroxy-cholesterol (μ g/100 g meat)	0.848a	0.268b
7- β -hydroxy-cholesterol (μ g/100 g meat)	1.809a	3.718a
Total lipids (g/100 g meat)	5.87b	13.76a

^an=24

^bMeans in a row followed by unlike letters are different ($p < 0.05$).

Table B-2-Mean^{ab} concentrations of 7- α -hydroxycholesterol, 7- β -hydroxycholesterol, and total lipids for each treatment as averaged across type of meat and state

Variable	Treatment		
	Salt	Phosphate	Phosphate + antioxidant
7- α -hydroxy-cholesterol (μ g/100 g meat)	1.413a	0.348a	0.020a
7- β -hydroxy-cholesterol (μ g/100 g meat)	1.483a	3.874a	2.773a
Total lipids (g/100 g meat)	9.84a	9.53a	9.57a

^an=16.

^bMeans in a row followed by unlike letters are different ($p < 0.05$).

Table B-3-Mean^{ab} concentrations of 7- α -hydroxycholesterol and 7- β -hydroxycholesterol, and total lipids for each state of meat as averaged across type of meat and treatment

Variable	State ^c			
	UNC	C0	C5	C10
7- α -hydroxy- cholesterol (μ g/100 g meat)	0.193a	0.744a	0.937a	0.358a
7- β -hydroxy- cholesterol (μ g/100 g meat)	2.810a	1.827a	1.007a	5.268a
Total lipids (g/100 g meat)	9.10a	9.24a	10.41a	9.84a

^an=12.

^bMeans in a row followed by unlike letters are different ($p < 0.05$).

^cUNC=uncooked, C0=cooked and stored zero day at 5-6°C, C5=cooked and stored five days at 5-6°C, and C10=cooked and stored ten days at 5-6°C.

VITA

Cynthia Edith Adcox was born in East Detroit, Michigan, on February 11, 1970. She attended schools in the public systems of East Detroit, Michigan, Westland, Michigan and Overton County, Tennessee, where she graduated from Livingston Academy High School in May, 1988. She entered Tennessee Technological University, Cookeville, Tennessee, during August, 1988, where in May, 1993, she received the Bachelor of Science degree in Chemistry. She then entered the Master of Science program in Food Science and Technology at The University of Tennessee, Knoxville, in August, 1993, officially receiving the M.S. degree in May, 1996. During her work at The University of Tennessee she was a Graduate Teaching Assistant for Food Chemistry.

She is presently working toward a Doctoral degree in Food Science at North Carolina State University Food Science and Technology program where she is a Graduate Teaching Assistant in the Biological Sciences.

28
MJ