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DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By
Cheng-Ta Li, M.S.

The Ohio State University
2000

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ABSTRACT

Dietary treatments involved supplemental Vitamin E at either: 1) Control. 15 IU; 2) 300 IU. 7 d prior to slaughter; 3) 300 IU. 21 d prior to slaughter. Peroxide value analyses demonstrated lower lipid oxidation in the fat from animals fed 300 IU (7 d) and 300 IU (21 d) of Vitamin E than in the fat from control animals (P<0.05). Results indicate that dietary intake of Vitamin E for either 7 or 21 d ante-mortem greatly reduced the rate of lipid oxidation of subcutaneous lamb.

A modified peroxide value (mPV) method and the TBA test were compared as methods of analyzing the lipid oxidation state in subcutaneous lamb fat obtained from lambs fed either 15 IU or 300 IU Vitamin E. The mPV and TBA analyses both demonstrated no significant difference (P>0.05) in the oxidation state of the fat from four different loin sections. These results demonstrate the efficacy of employing mPV to monitor the lipid-oxidation in animal fat.

Lipid oxidation in fresh lamb tissue and a corn oil sample were measured using two peroxide value assays: 1) AOCS peroxide method and 2) a new spectrophotometric peroxide method (SPM) in addition to the TBA method. Lowest CVs in SPM indicated that SPM is more rapid, effective, and reproducible than either AOCS or TBA methods to evaluate lipid oxidation.
Inferior quality of pale soft and exudative (PSE) meat and a surplus of mechanically deboned turkey meat (MDTM) have negative economical impacts to the meat industry. The objective of this research was to add the functional proteins from a salt extract of MDTM to PSE meat to create a value-added pork sausage product. Sausages manufactured from PSE using the MDTM extract exhibited a 30% increase in rigidity (P<0.05) compared to sausages made from PSE and brine alone. Sausages made from PSE and normal pork, added the MDTM extract, demonstrated an increase of 3.6 and 3.0% in WHC, and a reduction of 4.1 and 3.1% in cooking loss (P<0.05), compared to sausages made employing brine alone. Results indicate that proteins present in MDTM extract improve the physicochemical properties of fresh pork sausages.
Dedicated to my beloved family
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Meat Science: M. Wick "Improvement of the physicochemical properties of pale, soft, and exudative (PSE) meat products with an extract from mechanical deboned turkey meat (MDTM)."

Finally, I should thank my family and all my good friends. They are always there for me, and believe in me.
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Meat has been an important part of human diet throughout our history. Muscle food quality is often described in terms of palatability traits that related to physical and chemical changes of postmortem tissue. Processing operations, such as particle size reduction, cooking, and salting can speed up many chemical reactions and thereby alter the product quality (Decker and Xu, 1998). Of all chemical reactions, one of the major concerns in meat industry is lipid oxidation. Formation of undesirable off-flavors deters product quality and consumer acceptance. The oxidative stability of skeletal muscle is dependent of three major factors: Oxidative substrates (lipid and oxygen), catalysts (free radicals or metallic ions), and existence of antioxidant (Vitamin E). Inferior quality of pale soft and exudative (PSE) meat and a surplus of underutilized mechanically deboned turkey meat (MDTM) are two economical concerns in the processed meat industry. Some background information about these issues will be addressed in the following sections.
Vitamin E as an Antioxidant

The first dietary Vitamin E study was developed in the 1920's (Player, 1990). The function of Vitamin E was first associated with sterility and reproduction in rats (Parrish, 1980). Since the 1940's, there has been increased interest in the studies on the antioxidation activity of Vitamin E. \(\alpha\)-Tocopherol is the predominant form of vitamin E existing in muscle tissue (Erickson, 1998). Tocopherols prevent the unsaturated fatty acids in foods or tissues from oxidation by reacting with free radicals and blocking the chain reaction of lipid oxidation (Decker, 1998). Lipid is considered as a precursor of some undesirable volatile compounds (Suzuki, 1985). During cooking, flavor precursors from diets that accumulated in animal adipose tissue will be degraded, and some undesirable volatile compounds will be released (Suzuki, 1985). Tocopherols are very non-polar and exist mainly in the lipid phase of foods (Gregory, 1996). Some studies indicate that when tocopherol is supplied continuously in an animal diet, it accumulates in the muscle tissue (Erickson, 1998). Lipid oxidation can be blocked or inhibited under antemortem conditions. Thus, feeding an animal supplementary Vitamin E (tocopherol) could reduce the degree or rate of ante- and post-mortem lipid oxidation. More details in the effects of dietary Vitamin E on lipid oxidation of lamb tissue will be introduced in Chapter 2.

Lipid Oxidation in Muscle Foods

Oxidation of the highly unsaturated fatty acid constituents found in the membranes of muscle can be a very important contributor to the deterioration of meat (Foegeding and Lanier, 1996). In unsaturated fatty acids, the lipid oxidation rate
increases with additional unsaturation (Schaich. 1980). Free radicals, generated from direct energy deposition (light or heat) into some molecules, are capable of initiating the chain reaction of lipid oxidation. Unsaturated fatty acids, such as C\textsubscript{18:2} and C\textsubscript{18:3}, degrade into smaller molecules during the process of lipid oxidation. The mechanisms of lipid oxidation are summarized in Figure 1.1. In Figure 1.1, the initiation of lipid oxidation acquires the input of energy due to a higher energy of activation (35~65 kcal/mole) at this stage. However, only a few initiating free radicals (R*) are enough to build up the autoxidation chain through the formation of hydroperoxide (R•OOH) and the subsequent decomposition in the propagation stage (Schaich. 1980). Hydroperoxide might be stable without the presence of catalysts. However, the decomposition of hydroperoxide provides two radical initiators and the chain reaction starts almost instantaneously (Figure 1.1). With hundreds of decomposed short-chain molecules (secondary oxidative products) such as aldehydes, acids, and ketones, etc. released from lipids in muscle foods, some undesirable oxidative odors are also released.

Two lipid oxidation analyses, most commonly used as chemical measurements of lipid oxidation, are the 2-thiobarbaturic acid (TBA) test and American Oil Chemists' Society (AOCS) official peroxide value method. They are briefly described below.

(i) TBA test

TBA assay is based on the reaction of Malonaldehyde (MA) and TBA generating a TBA-MA complex with an absorbance maximum at 530 nm (Patton and Kurtz, 1951). Although widely employed, this method has certain limitations: the non-specificity of the TBA assay is due to interfering compounds that react with TBA (Nawar, 1996). For
instance, aldehydes are also the products of Strecker degradation of amino acids, which are not a part of lipid oxidation (Andreenkov, 1998). Another problem is that MA is not always extensively produced in all food systems. Some suggest the measurement of another wavelength at 450 nm to include other possible related substance (RS), such as alkanals, alkenals, and 2, 4-dienals (Nawar, 1996). However, TBA-RS is not a viable approach to solving the problem, because it is necessary to establish multiple standard curves to obtain a possible result while there were too many secondary oxidative products.

(ii) AOCS Official Peroxide Values

Formation of peroxides (primary oxidative products) has been recognized as the most important pathway for generating precursors of oxidative final products (Frankel, 1980). When peroxides in meat products are to be determined, it is necessary to extract the fat from meat samples to avoid the interference of other substance with peroxides (Mehlenbacher, 1960). However, this assay also has limitations. The traditional AOCS peroxide method is not reproducibly measuring low levels of oxidation due to the difficulty of titration endpoint determination (Fielder, 1974). Traditional titration methods have limitations due to the subjective error inherent in visual endpoint determinations. Some colormetric peroxide methods have been developed to address this problem, such as measurement of Cadmium iodide (Takagi et al. 1978) or triiodide ($I_3^-$) in the UV range (Lezerovich, 1985; Lovas, 1992). However, they have the disadvantages of using expensive and toxic compounds (CdI) or producing unstable or hazardous ions (some triiodide complex, such as NH$_4$I$_3$).
Another problem of AOCS official peroxide value method is the non-specificity of the reducing reagent. Sodium thiosulfate (Na$_2$S$_2$O$_3$). Sodium thiosulfate is a mild reducing agent, so it is able to react with more than one chemical compound in the peroxide value method. For instance, S$_2$O$_3$$^-$ can react with iodine (I$_2$) and form S$_4$O$_6$$^-$ and I$^-$ illustrated in the following equation.

$$2S_2O_3^- + I_2 \rightarrow S_4O_6^- + 2I^-$$

S$_2$O$_3$$^-$ can also react with the trace amount of triiodide (I$_3^-$; from I$_2$ + I$^-$ in the presence of oxygen) and form S$_4$O$_6$$^-$ and I$^-$ illustrated in the following equation.

$$2S_2O_3^- + I_3^- \rightarrow S_4O_6^- + 3I^-$$

There is no direct method to distinguish between I$_2$ and I$_3^-$ in aqueous solution. Sodium thiosulfate can also be reacted by the presence of oxidative secondary products (aldehydes, ketones and carboxylic acid) or other substances (peroxidants and metal ions, etc.). For instance, a reducing agent can reduce the following carbonyl compounds into alcohols:

(a) Aldehyde: R-CHO $\rightarrow$ RCH$_2$OH (primary alcohol)

(b) Ketone: R$_2$-C=O $\rightarrow$ R$_2$CH-OH (secondary alcohol)

(c) Carboxylic acid: R-COOH $\rightarrow$ R-CH$_2$OH (primary alcohol)
Therefore, the AOCS titration peroxide method is likely to report the result of non-specific reactions of sodium thiosulfate, rather than the reactions with iodine (I₂) formed by peroxides and KI. The titration method can generate higher peroxide values than the real values.

In conclusion, a simple, less expensive and more accurate way to measure lipid oxidation needs to be developed in order to eliminate the limitations of the TBA and AOCS peroxide values. Some innovative development in lipid oxidation analyses will be described in Chapter 3 and 4.
Initiation: \[ RH \rightarrow R^\bullet + H^\bullet \]

Propagation: \[ R^\bullet + O_2 \rightarrow ROO^\bullet \]
\[ ROO^\bullet + RH \rightarrow ROOH + R^\bullet \]

Termination: \[ R^\bullet + R^\bullet \rightarrow RR \]
\[ ROO^\bullet + R^\bullet \rightarrow ROOR \]
\[ ROO^\bullet + ROO^\bullet \rightarrow ROOR + O_2 \]

\( RH = \) Unsaturated fatty acid
\( ROOH = \) Hydroperoxide
\( R^\bullet = \) Free alkyl radical
\( ROO^\bullet = \) Free peroxy radical
\( RR \) and \( ROOR = \) Non-radical products

**Figure 1.1: The mechanism of lipid oxidation** (Nawar, 1996). The dot line indicates the formation of a chain-reaction loop.
Utilization of Mechanically Deboned Turkey Meat

The advantage of a mechanically deboned turkey meat (MDTM) process is to increase the utilization of turkey, and gain greater yields from the whole carcass (Kelleher and Hultin, 2000). If properly separated from bone, the meat fraction can be used as human food. At present, MDTM is treated as a by-product with little economic value (Schad, 1978). Similar to any whole muscle tissue, mechanically deboned turkey meat possesses excellent quality of functional myofibrillar proteins. There are three major problems about MDTM that prevents current meat processors from using this surplus by-product in formulation: 1) High fat and collagen content; 2) Susceptibility to lipid oxidation; 3) It is an excellent nutritive medium for supporting microbial growth (Schad, 1978). These three problems may be resolved by extracting myofibrillar proteins from MDTM. It has been reported that physical extraction of myofibrillar proteins by high-speed centrifugation produces the best results in high yield and protein functionality (Kelleher and Hultin, 2000). Removal of phospholipids or membrane lipids by centrifugation at temperature below 4°C should improve the quality and stability of the protein isolate. In addition, the meat industry can be benefited by increasing the use of MDTM. More details on MDTM extraction will be introduced in Chapter 5 and Appendix B.
PSE in the Meat Industry

(i) Genetic Selection and Occurrence of PSE

The Hampshire breed has been extensively used in crossbreeding programs to increase lean pork production (Monin and Sellier, 1985). As livestock producers become aware of the frequent occurrence of lower quality pork (lower ultimate pH, more pale color, and higher purge amount), researchers in U.S. also discovered in the early 1960s that Hampshires produce more acidic and pale meat than other breeds (Monin and Sellier, 1985). The Rendement Napole (RN) gene in Hampshires was first identified in France (Ellis and McKeith, 1997). The discovery in the Hampshire breed of a dominant major gene called Rendement Napole (RN), which increases the glycogen content in glycolytic muscles by about 70% (Estrade et al., 1993; Lundstrom et al., 1996). It is also known as the "acid meat gene", "RN gene", "Napole gene" or "Hampshire effect" (Miller, 1997). The Napole gene has two alleles, an unfavorable allele (RN') and a normal recessive allele (rn'). The dominant allele (RN') is responsible for the high glycolytic potential, which caused the characteristic increase of muscle acidity in post-mortem carcass (Estrade et al., 1993). The term of "glycolytic potential" was first introduced to predict whether an animal possessed excessive glycogen levels by Monin and Sellier (1985). Pigs with paired RN- alleles (RN' RN') or RN' rn' pairs have high glycogen levels, because the RN' gene is dominant (Ellis and McKeith, 1997). Glycolytic potential (GP) is expressed in μmole lactate equivalent per gram of fresh tissue (Monin and Sellier, 1985) as follows:

\[
GP = 2 ([\text{Glycogen}] + [\text{Glucose-6-phosphate}] + [\text{Glucose}] + [\text{Lactic acid}])
\]
GP is the sum of the process that glycogen is converted into lactic acid through glycogenolysis and glycolysis (Enfalt et al., 1997; McKeith et al., 1998). Animal with GP values greater than 180 m mole/g are generally classified as RN carrier (Enfalt et al., 1996; Lundstrom et al., 1996). Currently, GP is also one of the most common techniques to predict RN gene status in pigs. Carcasses from hogs with the RN gene were reported to have a lighter lean color, lower ultimate pH (< 5.4), more reduction in water holding capacity, more drip loss (> 21%) and more cooking loss (> 12%) than normal pork (Lundstrom et al., 1996). Other researchers have also reported that there is a significant decrease in the protein content of the RN" meat sample when compared with the rn" (Lundstrom et al., 1996). Despite the RN gene has many negative effects on meat quality (low water holding capacity and pale color), it still has potential, such as better growth performance (Ellis and McKeith, 1997). Recent researchers have developed DNA-based tests for the RN gene, e.g. gene markers & mapping (Milan et al., 1995), and these tests might allow the gene frequency to be controlled or manipulated in the future.

(ii) Inferior Quality and Economic Loss

PSE meat is defined as pale, soft, and exudative meat, caused by an abnormally rapid drop in pH while the carcass temperature is still high (Cloke et al. 1981). PSE pork has been researched extensively since the 1960s (Sayre et al., 1963). More than 10% of swine in the US exhibit a post-mortem PSE muscle condition (Kauffman and Meeker, 1996). With PSE discounting $0.34/head, the economic impact on the pork industry is nearly $32 million/yr (Kauffman and Meeker, 1996). The rapid accumulation of lactic
acid in combination with a high carcass temperature causes a denaturation of muscle proteins (Offer, 1991). The denaturation of muscle proteins and precipitation of sarcoplasmic proteins causes an increase in light scattering properties of the muscle tissue, and contribute to the pale color of PSE meat. A low postmortem muscle pH (5.2-5.4) results in a decrease in the electrostatic repulsion between myofibrillar proteins (net charge effect), which causes, in combination with the formation of cross-linkages between actomyosin at rigor onset, a shrinkage of the myofibrils. It has been reported that PSE muscle exhibits more postmortem disruption of cell components, and more myofibrillar breakdown with loss of material at the Z-line than normal muscle (Cloke et al. 1981). In previous studies, some researchers concluded that sarcoplasmic proteins were important for water holding capacity (Eikelenboom and Smulders, 1986; Lopez-Bote et al. 1989). Later the theory was proven to be incorrect, since sarcoplasmic proteins bind only 3% of total water in meat. So, it cannot be possibly responsible for the high drip losses in PSE pork (Wilson and van Laack, 1999). The exudative property and the low water holding capacity (WHC) of PSE meat have caused tremendous profit loss due to low product yield and poor consumer acceptance (Hedrick et al., 1994a). It has been recommended that PSE meat could be used with special formulations that contain ingredients or conditions to restore meat quality and protein functionality to reduce yield losses and improve texture (Owens et al., 2000). More details on improving PSE product quality will be introduced in Chapter 5.
Thermally Induced Gelation of Myofibrillar Proteins

Myosin, a major myofibrillar protein in muscle, has three important biological activities: 1) Myosin molecules spontaneously assemble into filaments in solutions of physiologic ionic strength and pH (Wick, 1999a); 2) Myosin is an enzyme (ATPase) (Stryer, 1988); 3) Myosin binds to the polymerized form of actin (Stryer, 1988). The capability of myosin to form thermally induced gel is essential to the water holding and binding capacities of the processed meat. When myosin molecules are heated, myosin monomers aggregate to form oligomers (Figure 1.2) (Yamamoto, 1990). Thermally induced protein-protein aggregation is considered to be the key element to transform the viscous protein extract (sol) to a three-dimensional matrix during meat processing (Xiong, 1992). Variations in gelling properties have also been observed in myofibrillar systems derived from different pH, ionic strength, and muscle types (Xiong, 1994). Yamamoto et al. (1988) also reported that longer myosin filaments form a finer network structure and higher gel rigidity than shorter ones (Figure 1.3). The sources of myofibrillar protein (myosin) have a significant influence on gel strength. It's been reported that the gel strength of the white myofibrillar protein is greater than gels formed from red myofibrillar protein at the same concentration (Morita et al., 1987; Xiong, 1994). However, recent research has revealed that the binding quality of either reconstituted or natural actomyosin is higher than that of myosin alone (Lin, 1996). Xiong and Brekke (1991) also reported that post-rigor muscle contains a higher amount of salt-extractable protein (mostly actomyosin) and produced a stronger gel than myofibrils from pre-rigor muscle (with the presence of myosin and actin, i.e., actomyosin cross-links have not extensively formed yet). The heat-induced gelation properties of salt
soluble myofibrillar proteins also improved the gelling properties in a mixed protein system (Smyth et al., 1998). Myosin or actomyosin from normal muscle might have the potential to lend functionality to other processed meat systems. From the standpoint of profit, the inclusion of a salt extract myosin or actomyosin could have an economic contribution to the meat industry due to the improvement of product quality and increase the utilization of surplus by-product, e.g., mechanically deboned turkey meat. More details in the new development of the utilization of myofibrillar protein extracts in meat systems will be described in Chapter 5 and Appendix B.
Figure 1.2: Thermally induced aggregation of myosin (Yamamoto, 1990).
Figure 1.3: Scanning electron micrographs of thermally induced gel of myosin filaments. A: short filaments (1.2 μm); B: long filaments (2.5 μm). Heating temperature was 65°C. 20 min. (Yamamoto et al. 1988)
CHAPTER 2

DIETARY SUPPLEMENTATION OF VITAMIN E AFFECTS PEROXIDE VALUES OF SUBCUTANEOUS LAMB FAT

2.1 ABSTRACT

Lipid oxidation is a major problem causing flavor deterioration in meat products. The objective of this research was to utilize an iodometric peroxide value method (PV) to analyze the effects of dietary Vitamin E on lipid oxidation of subcutaneous lamb fat. Lambs were fed an all-concentrate diet, offered ad libitum. Dietary treatments involved supplemental Vitamin E (based on daily diet DM) at either: 1) Control (C), 15 International Units (IU) for the entire feeding period; 2) Diet C until 7 d prior to slaughter, then 300 IU; 3) Diet C until 21 d prior to slaughter, then 300 IU. Peroxide value analyses demonstrated lower lipid oxidation in the fat from animals fed 300 IU (7 d) and 300 IU (21 d) of Vitamin E than in the fat from animals fed diets supplemented with 15 IU of Vitamin E (control) ($P < 0.05$). This trend was observed throughout the

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$^1$ Based on a paper presented during the 52nd Annual Reciprocal meat Conference. "Dietary intake of Vitamin E affects the peroxide value of subcutaneous fat in lamb" at Stillwater, Oklahoma, June 20-23, 1999.
entire storage time of 11 d. At 9 and 11 d of storage, PV analyses demonstrated a greater rate of increase ($P < 0.05$) in lipid oxidation in the subcutaneous fat from lambs fed control diets than in fat from animals fed either diet containing 300 IU supplemental Vitamin E. Results indicate that higher dietary intake of Vitamin E for either 7 or 21 d ante-mortem greatly reduced initial lipid oxidation, as well as the rate of lipid oxidation of subcutaneous lamb.
2.2 INTRODUCTION

Lipid oxidation is a major problem causing flavor deterioration and reduction in the shelf-life of meat and its by-products. Unsaturated fatty acids become oxidized and produce undesirable organoleptic characteristics. The initial step in lipid oxidation is the generation of highly transient hydroperoxides that further degrade into malonaldehyde (MA) and other secondary compounds. Evaluation of lipid oxidation is essential to predicting the shelf-life of muscle foods.

Many antemortem factors potentially contribute to lipid oxidation in animal fat. However, diet formulation could be one of the most effective ways of reducing lipid oxidation. Tocopherols are natural antioxidants, which have been utilized extensively in food processing to inhibit lipid oxidation (Bauernfeind, 1977). Tocopherols can quench free radicals by donating the phenolic H and an electron (Figure 2.1), while still maintaining their stable resonance forms (Figure 2.2). The tocopheryl radical intermediates are relatively stable due to resonance delocalization and the absence of positions suitable for attack by oxygen (Nawar, 1996). In addition, tocopherols can improve the oxidative stability of other compounds by scavenging singlet oxygen while being simultaneously degraded (Gregory, 1996). Previous research indicated that
supplementing dietary Vitamin E, a fat-soluble antioxidant, reduced lipid oxidation and increased the shelf life of lamb (Wulf et al., 1995).

Peroxide value (PV), an assay for determining the amount of peroxide present, is believed to provide the most direct measurement of lipid oxidation (Fiedler, 1974). The objective of this research was to employ PV to investigate the effects of different dietary Vitamin E regimen on the lipid oxidation of subcutaneous lamb fat.
Figure 2.1: α-Tocopherol as an antioxidant in a food system
Figure 2.2: The resonance forms of a phenolic antioxidant (Nawar, 1996). The shifting double bonds and the migrations of an electron on the phenolic ring indicate the delocalization (Kinsland, 1996).
2.3 MATERIALS AND METHODS

2.3.1. Sample Preparation

All reagents used were ACS grade (Fisher Scientific, Fair Lawn, NJ). This experiment was developed as a 3 (15 IU: 300 IU, 7 d: 300 IU, 21 d) by 4 (d 1, 7, 9 and 11 of storage) factorial design. Twenty-four fall-born Polypay lambs (initial wt 26.1 kg) regardless of sex were randomly divided into three groups of eight lambs. Feed consisted of an all-concentrate diet formulated to provide 16% crude protein, and the lambs were given *ad libitum* access to feed. Diets were fed for 82 d or until finishing wt of lambs was 47 kg. Dietary treatments involved supplemental Vitamin E (based on daily diet DM) at either: 1) Control (C) 15 International Units (IU) for the entire feeding period; 2) Diet C until 7 d prior to slaughter, then 300 IU daily; 3) Diet C until 21 d prior to slaughter, then 300 IU daily. One IU of Vitamin E is defined as 1 mg of dl-α-tocopheryl acetate (Parrish, 1980; NRC, 1985). Carcass fabrication was conducted at 1 d (for d 1 analysis) and 7 d (for d 7, 9 and 11 analyses) post-mortem in a cooler (2°C) respectively. Approximately 500 g of subcutaneous fat from the loin area was removed, placed in a meat tray, wrapped with Resinite® stretch meat film (OTR = 15 cm³/m²/24 h; Burdon Chemical, Griffin, GA) and stored at 4°C for 1 to 11 d prior to analysis. Lipid oxidation of the fat samples was analyzed by a PV method on d 1, 7, 9, and 11 post-mortem.
2.3.2 Peroxide Values (PV)

The AOCS method of determining peroxide values (AOCS, 1998) was modified as follows. Because of the difficulty in determining endpoints at extremely low PV in fresh lamb tissue, the modified method primarily combines the two steps of Na$_2$S$_2$O$_3$ titration in the AOCS method into one. Before Na$_2$S$_2$O$_3$ titration, a 5-min incubation period was employed. The reaction was allowed to proceed for 5 min rather than the 1 min required in the AOCS method due to one min incubation has been described as insufficient (Takagi et al., 1978; Lezerovich, 1985).

Five grams of unrendered ground lamb fat was placed into individual 250 mL volumetric flasks. Each sample was extracted with 30 mL of acetic acid-chloroform (3:2 vol/vol). The flasks were vigorously swirled. 0.5 mL of saturated potassium iodide was added and the solution mixed vigorously. Thirty milliliters of de-ionized water were added and the solution mixed again. Color of the upper aqueous layer ranged from pale to bright yellow, with the lower organic layer remaining white. Subsequently, 0.5 mL of 0.1% starch indicator in 0.3% chloroform was added and the color of the upper and lower layers observed. The mixture was allowed to stand for 5 min at room temperature, then titrated with 0.002 N Na$_2$S$_2$O$_3$ with constant shaking. Completion of the reaction was determined if the color of the upper aqueous layer ranged from light to dark purple (due to the formation of iodine-starch complex), while the lower organic layer remained white or gray. If the color of the lower organic layer remained yellow, the sample was vigorously swirled and allowed to stand for an additional 10 min. The end-point of
titration was established when the color of the upper aqueous layer became clear. The chemical reactions of PV method were summarized as follow as described in AOCS official method (AOCS, 1998).

\[
\begin{align*}
\text{KI} + \text{CH}_3\text{-C-OH} & \rightarrow 2\text{HI} + \text{CH}_3\text{-C-OK} \\
\text{R•OOH} + 2\text{HI} & \rightarrow \text{I}_2 + \text{H}_2\text{O} + \text{ROH} \\
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 & \rightarrow 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6
\end{align*}
\]

Where:

\(\text{R•OOH}\) is peroxide from food system.

PV was calculated employing the following equation:

\[
\text{PV} = (S - B) \times (N) \times (1000)/W
\]

Where:

\(\text{PV}\) = Peroxide Value (meq peroxide/kg fat)

\(S\) = mL \(\text{Na}_2\text{S}_3\text{O}_3\) used

\(B\) = mL \(\text{Na}_2\text{S}_2\text{O}_3\) used for blank

\(N\) = Normality of \(\text{Na}_2\text{S}_2\text{O}_3\)

\(W\) = g of fat
2.3.3. Rate of Lipid Oxidation

The rate of lipid oxidation was calculated using the following equation:

\[ m = \frac{PV_2 - PV_1}{d_2 - d_1} \]

Where:
- \( m \) = The rate of lipid oxidation, meq kg\(^{-1}\) d\(^{-1}\)
- \( PV_1 \) = meq/kg for first observation during storage
- \( PV_2 \) = meq/kg for second observation during storage
- \( d_2 - d_1 \) = Time period, d

2.3.4. Statistical Analysis

All analyses were performed in duplicate. Linear regression analysis between storage time and peroxide value was calculated. Statistical significance of the differences between means of peroxide values was determined by the t-test using SAS 7.0 for Windows (SAS, 1998).
2.4 RESULTS AND DISCUSSION

2.4.1. Peroxide Values (PV)

Oxidative rancidity of animal tissue starts to develop almost immediately after meat animal slaughter (Gray and Pearson, 1994). The results recorded in Table 2.1 demonstrate an increase in lipid oxidation in the fat from lambs fed all three supplemental Vitamin E treatments at d 9 and/or d 11 ($P < 0.05$). Lambs fed diet 1 (C) had greater lipid oxidation (3.94 meq/kg) on d 1 than lambs fed diets 2 and 3 (2.38 and 1.28 meq/kg). These results suggest that increased amounts of dietary Vitamin E supplement retarded lipid oxidation more than the control level of Vitamin E. The time required for the fat in lambs fed either diet containing 300 IU of supplemental Vitamin E to reach the same extent of lipid oxidation as that found in the fat from lambs fed the control diet on d 1 was at least 11 d (Figure 2.3). This observation is consistent with some previous researches, which indicated that increased supplemental dietary Vitamin E increased the shelf life of lamb meat and rendered pork (Wulf et al., 1995; Monahan et al., 1990).

Peroxide values were different ($P < 0.05$) in the fat obtained from lambs fed different diets on d 1 and d 7 (Table 2.1). Lamb fed control amounts of Vitamin E (15 IU) had greater initial peroxide value than those fed either of the diets supplemented with 300 IU of Vitamin E at the end of the feeding period (Figure 2.2). There was no difference in
the lipid oxidation in fat from lambs fed diets containing 300 IU of supplemental Vitamin E for either 7 d or 21 d ante-mortem at d 9 and d 11 ($P > 0.05$). These results suggest that a greater amount of dietary Vitamin E yielded lower initial PVs ($P < 0.05$), but diet 3 is not necessary to retard more lipid oxidation of subcutaneous lamb fat than diet 2 at longer storage time (d 9 and d 11) ($P > 0.05$).

As the plot of PV vs days of storage post-mortem in Figure 2.3 reflects, lipid oxidation in the fat from lambs fed all three Vitamin E supplements increased over time. The greatest increase occurred in the fat from lambs fed the control diet containing 15 IU of supplemental Vitamin E. All fat samples stored more than 7 d exhibited rapid increases in lipid oxidation ($P < 0.05$). However, fat from animals fed 300 IU, regardless of the feeding time, had equal or lower lipid oxidation after 7 d than the lipid oxidation in the fat from animals fed control amounts of Vitamin E.

Data in Table 2.1 also demonstrate that lambs fed in the control supplementary Vitamin E group had greater lipid oxidation than the groups fed 300 IU of Vitamin E at the end of each storage period ($P < 0.05$). Some researchers have reported that a longer period required to reach a certain PV indicates more effectiveness in inhibiting oxidation (Shahidi, 1994). Data reported here suggest that either feeding greater amounts of supplemental Vitamin E or increasing the supplemental feeding time increases the shelf-life of animal fat and reduces the rate of lipid oxidation.
# Table 2.1: Effects of three different dietary Vitamin E treatments on peroxide values (meq./kg) of lamb fat during storage

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>Vitamin E Regimen</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 IU</td>
<td>300 IU, 7 days</td>
<td>300 IU, 21 days</td>
</tr>
<tr>
<td>1</td>
<td>3.94± 1.46&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>2.38± 0.66&lt;sup&gt;B,a&lt;/sup&gt;</td>
<td>1.28± 0.51&lt;sup&gt;C,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>3.65± 0.98&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>2.18± 0.48&lt;sup&gt;B,a&lt;/sup&gt;</td>
<td>1.30± 0.50&lt;sup&gt;C,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>4.78± 1.24&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>2.23± 0.79&lt;sup&gt;B,a&lt;/sup&gt;</td>
<td>2.00± 0.33&lt;sup&gt;B,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>6.78± 1.44&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>3.88± 1.16&lt;sup&gt;B,b&lt;/sup&gt;</td>
<td>3.55± 0.46&lt;sup&gt;B,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means followed by same letters within same column are not significantly different (P > 0.05).

<sup>A,B,C</sup> Means followed by same letters within same row are not significantly different (P > 0.05).
Figure 2.3: Effects of dietary Vitamin E levels versus storage time on peroxide values of subcutaneous lamb fat
2.4.2. Rate of Lipid Oxidation

The plots in Figure 2.4 have been expanded in the range from d 9 through d 11 to compare the rate of increase in the lipid oxidation in the fat from lambs fed diets containing the three supplemental Vitamin E regimen. Linear regression analysis of the rate of increase in lipid oxidation from the fat from lambs fed the control diet. 300 IU for 7 d and 300 IU for 21 d produced slopes (m) of 1.0, 0.83, and 0.77 meq. kg⁻¹ d⁻¹ respectively. These analyses suggest that a diet containing 300 IU of Vitamin E for 7 d or 21 d ante-mortem had a significant effect on reducing the rate of lipid oxidation compared to the control diet (P < 0.05). The equations used to determine the rate of lipid oxidation during the period from d 9 to 11 of storage are as follows:

\[
\begin{align*}
Y_{15} &= 1.00X_{15} + 4.78 \\
Y_{300.7} &= 0.83X_{300.7} + 2.23 \\
Y_{300.21} &= 0.77X_{300.21} + 2.00
\end{align*}
\]

Where:

\(Y_{15}\) = PV of the group fed 15 IU

\(Y_{300.7}\) = PV of the group fed 300 IU for 7 d

\(Y_{300.21}\) = PV of the group fed 300 IU for 21 d

\(X\) = The number of storage d on different supplemental Vitamin E.

The rate of lipid oxidation was \(m_{300.21} > m_{300.7} > m_{15}\), which suggests the fat in lambs fed greater amounts and(or) longer periods of dietary Vitamin E had a greater resistance to lipid oxidation than the fat from lambs fed control supplementary amounts
of Vitamin E. However, the difference in rate and amount of lipid oxidation in the fat from lambs fed 300 IU of Vitamin E for 7 d in fat compared to those from lambs fed 300 IU of Vitamin E for 21 d was not great ($P > 0.05$). These data suggest that feeding lambs 300 IU of Vitamin E for only 7 d ante-mortem may be sufficient to retard both the initiation of and rate of lipid oxidation. In addition, the PV of the fat from lambs fed diets containing 300 IU of supplementary Vitamin E for even 7 d did not reach the same PV as the fat from animals fed the control diet even after 11 d of storage (Table 2.1). Data suggest that lambs fed diets with 300 IU of Vitamin E for even 7 d will have the same or lower rancidity after 11 d of storage as do animals at 24 h post-mortem.
Figure 2.4: Effects of dietary Vitamin E levels on rate of lipid oxidation of subcutaneous lamb fat
2.5 IMPLICATIONS

Peroxides are the primary products of lipid auto-oxidation. The results reported here indicate that Vitamin E is effective in inhibiting both the extent and rate of lipid oxidation in lambs. Peroxide values in the fat of lambs fed 300 IU for 7 or 21 d ante-mortem at d 9 and d 11 were not significant. However, the differences between those animals fed 15 IU and 300 IU regardless of the length of antemortem supplementation were significant ($P < 0.05$). Furthermore, these results indicate that the full antioxidant effect of dietary Vitamin E can be obtained with as little as 7 d of supplemental feeding prior to slaughter, which could result in significant economic savings for the meat industry. Further evaluation is necessary to determine the optimal time for feeding and amounts of antioxidants to increase the shelf life and palatability of fat from meat animals.
CHAPTER 3

EVALUATION OF LIPID OXIDATION IN LAMB TISSUE BY MODIFIED PEROXIDE VALUE (MPV) METHOD

3.1 ABSTRACT

Lipid oxidation is a major contributor to flavor deterioration in meat products. A modified peroxide value (mPV) method to the 2-thiobarbaturic acid (TBA) test were compared as methods of analyzing the lipid oxidation state in subcutaneous lamb fat obtained from lambs fed either 15 IU or 300 IU supplemental α-tocopherol (Vitamin E). mPV and TBA analyses both demonstrated no significant difference (p > .05) in the lipid-oxidation state of the fat from four different loin sections (n = 20). Both mPV and TBA methods demonstrated virtually identical differences (P < 0.05) in the lipid-oxidation state of the fat derived from lambs fed two different levels of Vitamin E. These results demonstrate the efficacy of employing mPV to monitor the lipid-oxidation in animal fat. The low temperatures inherent to the mPV method versus the TBA method along with mild extraction methods and speed of obtaining results reduces the potential of causing spurious autoxidation or generating substances that capable of interfering with the assay.
Both mPV and TBA methodologies indicate that the level of dietary intake of Vitamin E significantly affects the lipid oxidation-state of subcutaneous fat antemortem as well as 7 days post-mortem and thus, the shelf life of lamb fat.

\[1\] Based on research project "Evaluation of lipid oxidation by modified iodometric peroxide value". [Invention Patent Number: 001D02F. Office of Technology Transfer. The Ohio State University]
3.2 INTRODUCTION

Flavor is the trait responsible for consumer preference for meat and meat products. Water-soluble compounds in the lean portion of muscle impart meat taste while the lipids contribute the flavors characteristic of lamb species (Horstein and Crowe, 1963). Lipid oxidation during prolonged storage or short-term exposure to high temperatures is often associated with “off flavors”, “warmed over flavor”, “rancid”, and “stale” characteristics in mutton which result in product degradation and reduced case-life of an otherwise nutritious protein source.

One of the most important causes of meat food flavor deterioration is lipid oxidation, which affect fatty acids in general, and polyunsaturated fatty acids in particular (Gray, 1978; Allen and Allen, 1981). Postmortem factors can influence lipid oxidation and decrease the shelf life of meat products due to the initiation of peroxidation (Vercellotti et al., 1992). Oxidation of fatty acids in animal tissue starts to develop almost instantly after slaughter (Gray and Pearson, 1994).

Autoxidation of lipids is carried out by a free radical chain reaction (Gray, 1978; Allen and Hamilton, 1983; Rojarho and Sofos, 1993). The initial step in this reaction is the generation of transitory hydroperoxides, which degrade into malonaldehyde (MA) and several other reactive compounds (Figure 3.1). Due to their unstable state, peroxides
start their decomposition and form a series of secondary products, such as aldehydes, acids, and ketones, that produce undesirable rancid flavors (Shahidi. 1994).

The most common chemical measurement of lipid oxidation in muscle foods is the 2-thiobarbaturic acid (TBA) assay, developed by Patton and Kurtz (1951). It based on the reaction of MA and TBA generating a TBA-MA complex with an absorbance maximum at 530 nm (Figure 3.2). Although widely employed TBA reacts with other substances present in meat and the extracts of meat. The nonspecificity of the assay is due to interfering compounds that react with TBA such as sugars, ascorbic acid, and nonenzymatic browning products (Decker et al., 1998). In addition, the TBA assay is time-consuming and employ harsh conditions such as steam distillation or heating that are postulated to contribute to further lipid oxidation (Decker et al., 1998). Another potential drawback to the TBA method is that MA is often bound to proteins and the conditions for the optimal release of MA is often hard to determine. These forms vary from one sample to the next and require different hydrolytic conditions to release the MA. Also, it is difficult to release all of the MA from meat protein without employing strong acids and heat that adversely affect stability of the TBA-MA complex (Draper et al., 1986).

A comparison of a modified peroxide value (mPV) and TBA test as methods for analyzing the lipid oxidation state of the lipids in the subcutaneous fat layer obtained from lambs fed two different levels of the lipid soluble antioxidant α-tocopherol (Vitamin E) is reported here.
Figure 3.1: Mechanism of lipid oxidation in muscle foods (Frankel. 1985; Min and Lee. 1996).
Figure 3.2: TBA-malonaldehyde complex
3.3 MATERIALS AND METHODS

3.3.1. Comparison of TBA Test and mPV Method (Experiment I)

All reagents used were ACS grade (Fisher Scientific, Fair Lawn, NJ). Lamb was given *ad libitum* access to feed. Feed consisted of an all-concentrate diet formulated to provide 16% crude protein and 15 IU of supplemental Vitamin E per kg of diet dry matter. It should be noted that lambs are normally fed 15 IU supplemental Vitamin E per kg of diet dry matter (NRC, 1985). Diets were fed for 82 d or until finishing wt of lambs was 47 kg. Carcass fabrication was conducted at 2°C after 1 d post-slaughter. Two samples of subcutaneous fat (approximately 500 g) were removed from the right side loin and two samples from the left side loin of a lamb (Figure 3.3). Samples were placed in meat trays, wrapped with Resinite* stretch meat film. Lipid oxidation of the fat samples was analyzed by TBA and the modified PV method (described in Section 3.3.4) on d 1.
Figure 3.3: Sampling locations of subcutaneous fat from lamb loins. Right loin: A, B; Left loin: C, D.
3.3.2. Lipid Oxidation State in Lambs Fed Two Vitamin E Supplements (Experiment II)

A 2 (levels of Vitamin E) x 4 (days of storage) factorial comparison of the lipid oxidation state in the subcutaneous loin fat in lambs fed two different levels of supplemental Vitamin E was performed by employing both TBA and mPV. Twelve lambs were obtained from the Ohio Agricultural Research Development Center (OARDC), Wooster, Ohio where they had been fed an all-concentrate diet, offered *ad libitum* formulated to provide 16% crude protein with either the control 15 IU supplemental Vitamin E or 300 IU supplemental Vitamin E per kg of diet DM. 7 d prior to slaughter. Carcass fabrication was conducted 7 d post-slaughter. Samples were placed in meat trays, wrapped with Resinite® stretch meat film. Lipid oxidation of the fat samples was analyzed by a TBA and modified PV method on d 1.

3.3.3. The 2-Thiobarbituric Acid (TBA) Assay

The degree of lipid oxidation in this experiment was determined by the TBA (2-thio-barbituric acid) method described by Witte et al (1970), and modified by Pensel (1990). The major difference between the original method (Witte et al., 1970: AOAC, 1995) and the modified method is on sampling size, which may influence the k value but not the final result.

Briefly 5 g of unrendered lamb fat was placed into a coded polyethylene stomacher bag. An additional empty stomacher bag was prepared as a blank. 50-mL of ice cold 39.2 ± 3.6°F (4 ± 2°C) 20% of trichloroacetic acid (Fisher Scientific, Fair Lawn, New Jersey) in 1.6% of m-phosphoric acid (Fisher Scientific, Fair Lawn, New Jersey)
solution was immediately added to each stomacher bag. Samples were blended in a *Seward Laboratory blender* (Tekmar Co., Cincinnati, Ohio) for 2 min. 50-mL of cold (4 ±2°C) distilled water was added to each bag for a second blending for 30 s. The slurry was filtered through Whatman No. 1 filter paper (125 mm), and separately collected into a coded Pyrex (100± 0.16 ml) volumetric flask. If necessary, slightly pressing and squeezing of the filter paper was performed to assist with the aliquot from the residue in the final stages of filtration. Five mL of freshly prepared .02 M 4, 6-dihydroxypyrimidine-2-thiol (TBA) (Sigma Chemical Co., St. Louis. Miss.) was added to each tube and mixed for 4-5 s. Tubes were stored in the dark for 15 hours to develop the color. The color was measured by a spectrophotometer at a wavelength of 530 nm. By measuring the absorbance (OD) of the colored samples, the degree of the lipid oxidation could be calculated.

**Preparation of TEP Standard Curve**

To determine the concentration of red pigment, a standard curve was generated by reacting TBA with TEP (1.1.3.3 -tetra-ethoxypropane: Sigma Chemical Co., St. Louis. Mo). A TEP standard curve was required to calculate the constant K value, which was directly related to the TBA value. A solution of 10⁻³ M of 1.1.3.3.-tetra-ethoxypropane (TEP: Sigma Chemical Co., St. Louis. MO) stock solution was prepared for further dilution. A 0.220 g aliquot of the TEP reagent was weighed into a 5-mL graduate cylinder. One thousand mL of distilled water was used to rinse and transport the reagent into a 1.000 mL flask. This was mixed and brought to the total volume of 1.000 mL. Then 10 µL, 20 µL, 40 µL, 60 µL, and 80 µL of the stock solution were pipetted into
coded assay tubes. An additional assay tube, containing no stock solution, was prepared as a blank. A 5-mL aliquot of distilled water was added to each tube to dilute the sample and then 5 mL of freshly prepared 0.02 M TBA reagent was added to each tube for color formation. Each assay tube was mixed for 4-5 s. The tubes were then placed in the dark for 15 h to develop the color. After 15 h of color development, the coded samples from each treatment were measured with a spectrophotometer at a wavelength of 530 nm in a clean quartz cell. After 15 hours of color development, the color of each tube should change from the original light yellow color to red, and the absorbance of each tube would be proportionate to the concentration of the formed red pigments in the samples.

**Calculation of K values**

The TBA value was defined as the mg of an oxidative intermediate, malonaldehyde (MA), per 1.000 g of sample (Ockerman, 1985). It can be calculated by multiplying the absorbance by a constant K, and the K value was obtained from the following formula:

\[
K = \frac{\text{Conc. of MA in moles in 5 ml of filtrate}}{\text{M. W.} \times 10^7 \times \text{absorbance (OD)} \times \frac{\text{sample wt.}}{\text{sample wt.}} \times \% \text{ of recovery}}
\]

Where:

M.W. of malonaldehyde = 72.03

Sample weight = 5 g

Percentage of recovery = 91%, described by Pensel (1990).
Conc. (moles) of malonaldehyde in 5 ml of filtrate = Slope from the standard curve. A value of $5.837786825 \times 10^{-8}$ was obtained.

\[
K = \frac{10^7}{5} \times \frac{100}{72.03 \times \frac{5}{91}} = 9.242
\]

Calculation of TBA value

A K value of 9.242 was obtained ($n = 20$, $R^2 = 0.996$). The TBA of each sample was determined employing the following formula:

The TBA value = $K \times O.D_{530nm} = 9.242 \times OD$ of each sample.

3.3.4. Modified Peroxide Value (mPV)

The PV method of analysis was modified from the AOAC (1995) procedure. Briefly, a 50 g sample of unrendered subcutaneous loin fat each was ground in a Waring lab blender (Waring Products Division, Dynamic Cooperation of America, New Hartford, CN) for 20-30 s and extracted with 30 mL ice cold (3:2 v/v) acetic acid:chloroform. The extraction was vigorously swirled to distribute the sample and reagents. After the samples were dissolved in the acetic acid:chloroform mixture 0.5 mL of saturated potassium iodine (KI) ($83.2$ g solid KI / 40 mL H$_2$O) was added and mixed vigorously. Subsequently, 30 mL deionized water was added and the solution mixed thoroughly. Color of the upper aqueous layer ranged from pale yellow to bright yellow, with the
lower organic layer remaining white. The mixture was allowed to stand for 5-10 min. at room temperature then titrated with 0.02 M Na$_2$S$_2$O$_3$ (Sigma Chemical, Fair Lawn, NJ) gradually with vigorous shaking. During the titration 0.5 mL of starch indicator 1% (in 0.3% chloroform) (Lab Chem. Inc., Pittsburgh, PA) was added. Color of the upper aqueous layer ranged from light purple to dark purple, and the lower organic layer remained white to gray. If the color of the lower organic layer remained yellow, the sample was vigorously swirled and allowed to stand for an additional 10 min. The end-point of titration was established when the color of the upper aqueous layer disappears. mPV was calculated employing the following formula:

$$mPV = \frac{(S)(N)(1000)}{W}$$

Where:

mPV = Modified Peroxide Value (meq oxygen/ kg fat)
S = mL Na$_2$S$_2$O$_3$
N = Normality of Na$_2$S$_2$O$_3$ (0.02 N)
W = g of fat

3.3.5. Statistical Analysis

All analyses were performed in five replications. Linear regression analysis for TEP standard curve was calculated. Statistical significance of the differences between means was determined by t-test using SAS 7.0 for Windows (SAS, 1998).
3.4 RESULTS AND DISCUSSION

3.4.1. Comparison of TBA Test and mPV Method (Experiment I)

The results reported in Table 3.1 indicate that both TBA and mPV methods detected no differences in the lipid oxidation state at four locations in the subcutaneous loin fat (p > 0.05). In addition, results also demonstrate the consistency and repeatability of both TBA and mPV. In addition these data also indicate that lipids in the subcutaneous fat surrounding the loin from lambs fabricated within 24 h of slaughter exhibit a very low oxidation state.

The comparative advantages of mPV in contrast to TBA as a method of analyzing the lipid oxidation states of lamb fat are summarized in Table 3.2. The ability of mPV to detect the oxidation of mono unsaturated fatty acids suggests that this method is more accurate in determining the lipid oxidation states of all the fatty acids in the sample in contrast to the conventional TBA methodology. The potential for further autoxidation of the sample is minimized in the mPV method due to the use of non acidic and low temperature condition during sample preparation and extraction in. In addition, the potential to compare the oxidation state in fat derived from different muscles is more appropriate with mPV than traditional TBA due to the fact that mPV detects only the degree of peroxide formation in the unconjugated fatty acid fraction from animal fat
(Decker et al., 1998). Final color development for mPV is almost instantaneous while color development for the traditional TBA method requires at least 15 h. This greatly shortens the "turn around" time for analysis of the lipid oxidation state of lamb fat by mPV versus the traditional TBA method.
<table>
<thead>
<tr>
<th>Locations</th>
<th>mPV</th>
<th>TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N= 5/location</td>
<td>meq./kg</td>
<td>μg/g</td>
</tr>
<tr>
<td>A</td>
<td>2.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.150&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>2.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.133&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.138&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>1.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.143&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means followed by same letters within same column are not different (P > 0.05).

**Table 3.1: Lipid oxidation at different locations of lamb fat by TBA and mPV methods.** Right loin: A. B; Left loin: C. D.
<table>
<thead>
<tr>
<th></th>
<th>mPV</th>
<th>TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temp. of sample preparation (°C)</strong></td>
<td>4 ± 2</td>
<td>25 (cold extraction)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 (steam distillation)</td>
</tr>
<tr>
<td><strong>Sample autoxidation potential</strong></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td><strong>Analysis time</strong></td>
<td>1 h</td>
<td>15 h</td>
</tr>
<tr>
<td><strong>Specificity for peroxides</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Detection of oxidized mono and unsaturated fatty acids</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Potential to compare oxidation between muscles with different fatty acid composition</strong></td>
<td>Appropriate</td>
<td>Inappropriate</td>
</tr>
</tbody>
</table>

Table 3.2: Comparison between TBA and mPV
3.4.2. Lipid Oxidation State in Lambs Fed Two Vitamin E Supplements (Experiment II)

Both mPV and TBA methods demonstrate an increase in the lipid oxidation state in loin area subcutaneous fat obtained from lambs fed on both the control (15 IU) and 300 IU supplemental dietary Vitamin E (P < 0.05). Both methodologies demonstrate similar lipid oxidation states of the fat from animals fed on both regimen until 11 d post slaughter. The greatest increase of lipid oxidation in 11 d was observed in the fat from lambs fed 15 IU (control animals) by both methods. There was no difference in the mPV or TBA of fat obtained from animals fed 300 IU Vitamin E on days 1, 7 or 9, suggesting that high dietary Vitamin E treatment retards the rate of lipid oxidation to a greater extent than control levels of dietary Vitamin E treatment. In addition, lambs fed 15 IU Vitamin E had higher initial mPV and TBA values than those fed 300 IU Vitamin E (Table 3.3). Furthermore, mPVs significantly increased at d 9 and 11 (P < 0.05), indicating that increased dietary Vitamin E treatment reduces the rate of lipid oxidation more than control dietary Vitamin E intake, thus increasing the usable shelf life of lamb fat.
Table 3.3: Effects of two different levels of dietary Vitamin E on peroxide and TBA values of lamb fat during storage

<table>
<thead>
<tr>
<th>Post slaughter</th>
<th>MPV meq./kg</th>
<th>TBA µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>300 IU</td>
</tr>
<tr>
<td>1</td>
<td>1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>2.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>2.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>3.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means followed by same letters within columns are not different (P > 0.05). Total observation, N = 96
3.5 IMPLICATIONS

Previous research indicated that supplemental dietary Vitamin E reduces the lipid oxidation state of rendered fat from lamb (Wulf et al., 1995). The rate of lipid oxidation is controlled by various antemortem factors. Diet composition is one of the most effective ways of inhibiting lipid oxidation in animal fat. In this study, lambs fed 300 IU of Vitamin E apparently had lower mPVs than those fed 15 IU. Results suggested that higher levels of dietary Vitamin E contribute to lower mPVs and less oxidation of subcutaneous lamb fat. In addition increased levels and longer duration of dietary Vitamin E contributed to a greater inhibition of lipid oxidation in the subcutaneous loin fat in lambs.

The mPV method reported here reproducibly analyzes the lipid oxidation states of fatty acids derived from animal tissue. The reaction time for the entire analysis is less than 1 h. In contrast the TBA method reported here is time consuming and incapable of detecting oxidized mono-unsaturated fatty acids.

TBA values have been commonly considered as an index of lipid rancidity. However, the TBA method is primarily based on determining the concentration of a TBA-MA complex, a secondary oxidative product of fatty acid oxidation, but MA is not always present in all oxidized systems. In addition, the TBA method is not sensitive to the oxidation state of mono-or di-unsaturated fatty acid derivatives (Nawar, 1996). Gray
(1978) also reported no color development by TBA method for linoleate, a mono unsaturated fatty acid, in spite of the fact that the peroxide value had already reached to 2.000 (Gray, 1978). Interaction of MA with available amino groups in meat components have been reported to affect the results of TBA analysis (Shahidi, 1994).

Lipid oxidation determinations are normally performed on animal fat samples more than 7 days post slaughter. This is the first report of the lipid oxidation state determination on fresh unrendered (< 24 h) fabricated subcutaneous lamb fat. Both mPV and TBA yielded similar results however. mPV is more rapid, simpler, more reproducible, costs less, and uses unrendered fat. These results demonstrate that mPV is a rapid, low cost, simple and reproducible method to monitor the lipid oxidation state in animal fat. These aspects of the mPV method could have a potentially great economic contribution for the meat industry.
CHAPTER 4

SPECTROPHOTOMETRIC DETERMINATION OF LAMB TISSUE PEROXIDE VALUES

4.1 ABSTRACT

Flavor is the predominant characteristic of meat responsible for consumer preferences. One of the most important causes of flavor deterioration in meat is lipid oxidation that begins almost instantly after slaughter. Lipid oxidation in fresh lamb tissue (n = 6) and a corn oil control (n = 6) were measured using two peroxide value assays: 1) AOCS peroxide method and 2) a new spectrophotometric peroxide method (SPM) in addition to the 2-thiobarbituric acid (TBA) method. PVs determined by the AOCS and SPM methods were 1.4 and 1.5 meq./kg (P > 0.05), respectively. Coefficients of variation (CVs) for AOCS and SPM methods were 33.7% and 10.7%, respectively. The TBA mean of fresh lamb tissue was 0.10 µg/g with a CV of 14.5%. The PVs of corn oil determined by the AOCS and SPM methods were 52.2 and 51.7 meq./kg (P < 0.05).

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1 Based on a paper presented during the 53rd Annual Reciprocal Meat Conference, "Spectrophotometric determination of lamb tissue peroxide value" at Columbus, Ohio. June 18-21, 2000.
respectively. The CVs of AOCS and SPM methods were 0.36% and 0.21%, respectively. The TBA mean was 0.39 μg/g with a CV of 4.6%. Low CVs in SPM indicated that it has better reproducibility than either the TBA or AOCS method in measuring lipid oxidation in both lamb tissue and corn oil. In addition, SPM yields results in less than 30 min. These results demonstrate that SPM is more rapid and reproducible than either AOCS or TBA methods and could be an effectively method to evaluate lipid oxidation in animal tissue.
4.2 INTRODUCTION

Flavor is the predominant characteristic of meat responsible for consumer preferences. One of the most important causes of flavor deterioration in meat is lipid oxidation that begins almost instantly after slaughter (Gray and Pearson, 1994). Autoxidation of lipids is carried out by a free radical chain reaction. The initial step in this reaction is the formation of peroxide that further degrades into several reactive compounds including malonaldehyde (MA) (Ranken, 1989; Raharjo and Sofos, 1993). These secondary products are responsible for undesirable or “rancid” flavors (Shahidi, 1994).

Due to its non-homogeneous nature there are no good methods to evaluate the lipid oxidation of meat. Currently, one of the most common analytical methods for determining lipid oxidation in meat is the 2-thiobarbituric acid (TBA) assay, which measures a TBA-MA complex with an absorbance maximum at 530 nm (Nawar, 1996). The TBA method has certain limitations in food systems. TBA non-specifically reacts with compounds such as sugars, ascorbic acid, and non-enzymatic browning products often present in food (Decker et al., 1998). Another potential drawback to the TBA method is that MA is not always present in all food systems, which could produce biased results (Gray, 1978).
Another common analytical method of determining lipid oxidation is the peroxide value. The assay for peroxides is the most direct measurement of lipid oxidation (Fielder, 1974). The AOCS method requires a multi-step titration and a 1 min incubation that has been described as insufficient (Lezerovich, 1985; Takagi et al., 1978). The AOCS method also is subject to inherent experimental error due to the subjectivity in visual endpoint determination (Fielder, 1974). Some colorimetric peroxide methods have been developed to minimize the subjectivity in visual endpoint determination, including the measurement of Cadmium iodide (Takagi et al., 1978) or triiodide in the UV range (Lezerovich, 1985; Lovaas, 1992). However, these methods have the disadvantages of requiring the use of expensive or toxic compounds (Takagi et al., 1978) and result in the generation of unstable or hazardous ions (Lezerovich, 1985; Lovaas, 1992).

The purpose of this research was to develop a rapid, sensitive, and reproducible spectrometric method of determining peroxide values, incorporating the advantages and eliminating many disadvantages of the TBA and AOCS methods.
4.3 MATERIALS AND METHODS

4.3.1. Sample Preparation

Lamb tissue

Fresh subcutaneous ovine fat from the loin (one-week post-mortem) was obtained from The Ohio State University Meat Laboratory, Columbus, Ohio. The fat was placed in meat trays, wrapped with Resinite™ stretch meat film (Burdon Chemical, Griffin, GA) and stored at 4°C prior to analysis. Immediately prior to analyses, 50 g of fat was coarsely ground in a Waring™ lab blender (Model 1120, Dynamic Corp of America, New Hartford, CN) for 30 s. No rendering or homogenization procedures were employed in order to prevent heat-induced oxidation. All procedures were conducted at 4°C.

Corn Oil

A four-year-old, aged Mazola® brand 100% pure corn oil (with 57.1% polyunsaturated, 14.3% saturated, and 28.6% mono-unsaturated fatty acids), which was in a laboratory under light and room temperature, was used for PV and TBA analyses. The mean and CV of SPM for corn oil were used to compare those of TBA and AOCS methods.
4.3.2. The 2-Thiobarbituric Acid (TBA) Assay

All chemicals used were ACS reagent grade (Fisher Scientific, Fair Lawn, NJ). The lipid oxidation of each sample was determined and repeated six times by the TBA method described by Witte et al. (1970) as modified by Pensel (1990). The details of the TBA methodology are shown in Figure 4.1. Five grams of either coarsely ground unrendered lamb fat or corn oil was placed in a polyethylene bag. An additional empty polyethylene bag was prepared as a blank. Fifty mL of a cold (4°C) 20% trichloroacetic acid and 1.6% m-phosphoric acid mixture was added to each polyethylene bag and blended in a Seward™ Laboratory blender (Tekmar Co., Cincinnati, OH) for 2 min. Fifty mL of cold (4°C) distilled water was added to each bag and blended for an additional 30 s. The slurry was filtered through Whatman No. 1 filter paper. Five mL of the filtered slurry was added to 5.0 mL of freshly prepared 0.02 M 2-thiobarbituric acid and mixed for 5 s. The samples were subsequently stored in the dark for 15 h to develop color. The absorbance of the chemical reactions was measured at 530 nm using a Gilford™ Response UV-VIS Spectrophotometer (Ciba Corning Diagnostic Co., Oberlin, Ohio).

The calculation of the TBA value was same as described in Section 3.3.3 in Chapter 3. It can be calculated by the following formula:
The TBA value = \( K \times O.D_{530nm} = 9.242 \times OD \) of each sample.

### 4.3.3. AOCS for Peroxide Value

The American Oil Chemists’ Society (AOCS) official method of determining peroxide values (AOCS, 1998) was used, and the procedures were shown in Figure 4.2. The chemical reactions of the AOCS official peroxide value were shown in following equations (§4.1 ~ §4.4):

\[
\begin{align*}
R^* + O_2 + H^* & \rightarrow R^{OO*H} \quad (§4.1) \\
2 \text{KI} + 2 \text{CH}_3\text{COOH} & \rightarrow 2 \text{HI} + 2 \text{CH}_3\text{COO}'K^- \quad (§4.2) \\
R^{OO*H} + 2 \text{HI} & \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{I}_2 \quad (§4.3) \\
\text{I}_2 + 2 \text{Na}_2\text{S}_2\text{O}_3 & \rightarrow \text{Na}_2\text{S}_4\text{O}_6 + 2 \text{NaI} \quad (§4.4)
\end{align*}
\]

AOCS official Peroxide value was based on the amount of \( \text{Na}_2\text{S}_2\text{O}_3 \) used for titration, which was established, when the purple color of the upper aqueous layer disappears (in §4.4). PV was calculated employing the following formula:
$PV = (S)(N)(1000)/W$

Where:

$PV =$ Peroxide Value (meq/kg fat)

$S =$ mL Na$_2$S$_2$O$_3$

$N =$ Normality of Na$_2$S$_2$O$_3$ (0.02 N or 0.002N)

$W =$ g of fat
5.0 g sample fat

Cold extraction, 2 min
50mL 20% TCA (with 1.6% -HPO₄)

Mix with 50 mL dH₂O, 30 s

Filtration

Collect 5 mL aliquot

Add 5 mL 0.02 M TBA, mix 4~5 s

Store in a dark room, 15 h

Measure Abs. at 530 nm for TBA-MA complex

Figure 4.1: Flow diagram depicting TBA methodology
5.0 g sample fat

Extract with CH$_3$COOH : CHCl$_3$
3:2 (v/v), 30 mL

AOCS

Add 0.5 mL saturated KI
Incubate 1 min, RT
Add 30 mL dH$_2$O
Titrate with 0.02 N Na$_2$S$_2$O$_3$
(Until yellow color disappears)
Add 0.5 mL starch indicator, 1%
Titrate with 0.002 N Na$_2$S$_2$O$_3$
(Until purple color disappears)

SPM

Incubate 5 min, RT
Collect 10 mL up. aqueous layer
Add 0.5 mL starch indicator, 1%
Measure Abs. at 563nm

Figure 4.2: Flow chart comparing AOCS and SPM methods of determining peroxide values
4.3.4. Spectrophotometric Method (SPM) for Peroxide Value

Survey Scanning

The absorption maximum of an iodine solution alone and an iodine-starch complex were determined by scanning a 0.001 N iodine solution and a 10 mL 0.001 N iodine with 0.5 mL 1% starch indicator solution from 400 nm to 900 nm (Figure 4.3). A 10-mL deionized water along with 0.5 mL 1% starch indicator solution was also prepared for scanning.

Linear Regression for the Standard Curve: Iodine Equation

An iodine standard curve was developed in order to establish the iodine equation to determine the peroxide value. A 0.1 N iodine solution was freshly prepared as a stock solution for dilution. Four dilutions of 0.0005 N, 0.001 N, 0.002 N, and 0.004 N iodine were prepared from the stock solution in de-ionized water. Ten mL of each iodine solution was dispensed into assay tubes and 0.5 mL of the 1% starch indicator was added to each tube. The absorption of a blank and samples were immediately measured at 563 nm, the absorption maximum for the starch-iodine complex. Absorption was plotted against concentration and linear regression analyses performed (Figure 4.4). Iodine equation from the standard curve (with $R^2 = 0.99$) is obtained as follows:

$$ Y = (m)(X) + b $$

$$ OD\ value = 34^{\pm}.3 \times (\text{meq of iodine}) - 0.0131 $$
Measurements

The flow diagram of the SPM analysis for peroxide value is shown in Figure 4.2. Five grams of fat were placed into a 250 mL flask. 30 mL of acetic acid-chloroform (3:2, v/v) reagent was added, and the flask was shaken vigorously for 1 min. One half mL of saturated potassium iodide was added to a flask, mixed for 1 min. and 30-mL of de-ionized water added and mixed again for 1 minute. After incubating the flask for 5 min., 10 mL of the upper aqueous solution from each flask was dispensed into a 16 mm x 120 mm borosilicate glass assay tube. If preliminary analyses indicated that the sample was highly oxidized the sample was diluted 10 fold with de-ionized water. The procedure continued by the addition of 0.5-mL of 1% starch indicator (with 0.3% chloroform) and mixing for 5 s. The absorption of samples was immediately measured at 563 nm.

The Calculation of the Spectrophotometric Peroxide Value

The determination of spectrophotometric peroxide values was based on the chemical reactions, shown in following equations (§4.1' - §4.3').

\[
\begin{align*}
R^* + O_2 + H^* & \rightarrow R^*O^*H \quad (§4.1') \\
2 \text{Kl} - 2 \text{CH}_3\text{COOH} & \rightarrow 2 \text{HI} + 2 \text{CH}_3\text{COOK}^- \quad (§4.2') \\
R^*O^*H + 2 \text{HI} & \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{I}_2 \quad (§4.3') \\
\text{I}_2 + \text{Starch} (\text{indicator}) & \rightarrow \text{Starch-Iodine complex} \\
\text{Purple} & \rightarrow \text{Blue}
\end{align*}
\]
SPM was based on the iodine \( (I_2) \) concentration formed in §4.3', not on the amount of \( Na_2S_2O_3 \) used. By measuring the absorbance (OD) of the blue pigment, the amount of \( I_2 \) and the degree of the lipid oxidation can be calculated. Peroxide value can be originally calculated from the required amount of \( Na_2S_2O_3 \) mL in §4.4. However, no \( Na_2S_2O_3 \) was used in the spectrometric peroxide value method (SPM). According to the chemical reaction in §4.4, the ratio of \( I_2/Na_2S_2O_3 \) is 1/2. By knowing the concentration (meq.) of starch-iodine complex in 10 mL of solution from the absorbance and the iodine equation from the standard curve (with \( R^2 = 0.99 \)), the original meq \( Na_2S_2O_3 \) is equal to twice of meq of \( I_2 \).

\[
Spectrometric \ peroxide \ value = \left[ \frac{OD \ value + 0.0131}{3477.3} \right] \times 2 \times \left( \frac{48}{10} \right)
\]

Where:

Iodine equation is \( OD \ value = -0.0131 + 3477.3 \times (\text{meq of iodine}) \)

The ratio of \( I_2/Na_2S_2O_3 \) is 1/2. Amount of \( Na_2S_2O_3 \) would be twice of the \( I_2 \)

The total volume of acetic acid-H\(_2\)O (upper aqueous layer) is:

\[30 \text{mL} \times 60\% \text{ of acetic acid} + 30 \text{mL of } H_2O = 48 \text{mL}\]

10 mL of solution was sampled

For pure oil sample (e.g., corn oil), an adjustment on the specific density (\( \sigma \)) is required (meq / kg of \( H_2O \)/ \( \sigma \) of oil = meq/ kg of oil). The \( \sigma \) of the corn oil is 0.90 g/mL and adjusted equation is shown below.

\[
Spectrometric \ peroxide \ value = \left[ \frac{OD \ value + 0.0131}{3477.3} \right] \times 2 \times \left( \frac{48}{10} \right) / \sigma
\]
4.3.5. Statistical Analyses

All analyses were performed in six replications. The coefficient of variation (CV) of the AOCS, SPM, and TBA for lamb tissue and corn oil were calculated by the following equation:

\[
\text{Coefficient of Variation (C.V.)} = \left( \frac{S}{\overline{X}} \right) \times 100\%
\]

Where:

\[
S = \text{standard deviation}
\]

\[
\overline{X} = \text{mean}
\]

Statistical significance of the differences between means of PV and TBA were determined by the Tukey's multiple comparison test at 95% confidence level using the SAS 7.0 for Windows (SAS, 1998).
Figure 4.3: Spectrophotometric analysis of $10^{-3}$ N iodine (dotted line) and iodine-starch complex (solid line) from 400 nm to 900 nm. Absorption spectrum indicates the absorption maximum of iodine-starch complex is 563 nm.
Figure 4.4: Iodine standard curve. Curve derived by plotting starch - iodine concentration versus absorbance at 563 nm. Linear regression analyses (straight line) used to produce the iodine equation employed to calculate the peroxide value.
4.4 RESULTS AND DISCUSSION

4.4.1. Absorption Maximum of an Iodine–Starch Complex

Figure 4.3 indicates that the absorption maximum of a 0.001 N iodine solution was essentially 0 between 560 nm and 900 nm, and the absorption maximum of the iodine-starch complex solution was 563 nm. The absorption of a 0.5 mL 1% starch indicator was 416 nm, which does not contribute to absorption maximum of the iodine-starch complex (563 nm). The absorbance at 563 nm of each sample is proportional to the concentration of the blue pigment as a result of the iodine-starch complex formed in samples.

4.4.2. Iodine-Starch Equation

The amount of iodine produced was determined by comparing the absorbance of the sample to a freshly generated iodine standard curve (Figure 4.4) and the peroxide value calculated from the linear regression equation as follows:

\[ Y = (m)(X) + b \]
\[ Y = (1906.29)(X) + 0.27 \]
Where:
Y = Absorption at 563 nm
X = Normality of iodine solution
b = 0.27
m = 1906.29

4.4.3. Analytical Values in Lamb Tissue and Corn Oil

The analytical values of lamb tissue and corn oil by TBA, AOCS, and SPM are summarized in Table 4.1, 4.2, and 4.3.

Lamb Tissue

Lamb tissue analyzed by AOCS and SPM had mean peroxide values of 1.43 and 1.52 meq./kg., respectively (P > 0.05). The CV for AOCS and SPM were 33.7 and 10.7%, respectively (Table 4.1). The TBA of the lamb tissue was 0.1 μg/g with a CV of 14.5%. The greater CVs obtained for fresh lamb tissue by AOCS, SPM and TBA were postulated to be due to the non-uniform variation of lamb tissue. The CV of 10.7% for SPM in contrast with 14.5% for TBA and 33.7% for AOCS PV indicates that SPM is a more reproducible method for evaluating the lipid oxidation of lamb tissue.

Corn Oil

In order to test the hypothesis, the PV and TBA of corn oil were determined by AOCS, SPM and TBA. The means of peroxide value analyses by AOCS and SPM were 52.3 and 51.7 meq/kg., respectively (P < 0.05). In general, PV > 8 is considered as rancid
(Ockerman, 1985). Results indicated that the corn oil sample was extremely stale. The TBA value was 0.39 μg/g. The CVs for AOCS, SPM and TBA were 0.36, 0.21, and 4.62% respectively.
## TABLE 4.1: Coefficients of variations for the analyses of 2-thiobarbituric acid (TBA), AOAC peroxide value, and spectrometric peroxide value method (SPM) in lamb tissue

<table>
<thead>
<tr>
<th>Analyses</th>
<th>TBA µg/g</th>
<th>AOCS PV meq./kg</th>
<th>SPM Meq./kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.120</td>
<td>1.24</td>
<td>1.31</td>
</tr>
<tr>
<td>2</td>
<td>0.074</td>
<td>1.24</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>0.102</td>
<td>1.08</td>
<td>1.30</td>
</tr>
<tr>
<td>4</td>
<td>0.111</td>
<td>2.08</td>
<td>1.68</td>
</tr>
<tr>
<td>5</td>
<td>0.102</td>
<td>2.08</td>
<td>1.53</td>
</tr>
<tr>
<td>6</td>
<td>0.092</td>
<td>0.84</td>
<td>1.71</td>
</tr>
<tr>
<td>Mean</td>
<td>0.100</td>
<td>1.43</td>
<td>1.52</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.01453</td>
<td>0.4809</td>
<td>0.1630</td>
</tr>
<tr>
<td>C.V. %</td>
<td>14.53%</td>
<td>33.71%</td>
<td>10.73%</td>
</tr>
</tbody>
</table>
### TABLE 4.2: Coefficients of variations for the analyses of TBA, AOAC Peroxide value and spectrometric peroxide value method (SPM) in corn oil sample

<table>
<thead>
<tr>
<th>Analyses</th>
<th>TBA (μg/g)</th>
<th>AOCS PV (meq./kg)</th>
<th>SPM (Meq./kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.416</td>
<td>52.00</td>
<td>51.69</td>
</tr>
<tr>
<td>2</td>
<td>0.388</td>
<td>52.40</td>
<td>51.62</td>
</tr>
<tr>
<td>3</td>
<td>0.388</td>
<td>52.40</td>
<td>51.62</td>
</tr>
<tr>
<td>4</td>
<td>0.407</td>
<td>52.40</td>
<td>51.91</td>
</tr>
<tr>
<td>5</td>
<td>0.407</td>
<td>52.40</td>
<td>51.76</td>
</tr>
<tr>
<td>6</td>
<td>0.360</td>
<td>52.00</td>
<td>51.62</td>
</tr>
<tr>
<td>Mean</td>
<td>0.394</td>
<td>52.27</td>
<td>51.70</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.01823</td>
<td>0.1886</td>
<td>0.1065</td>
</tr>
<tr>
<td>C.V. %</td>
<td>4.62%</td>
<td>0.36%</td>
<td>0.21%</td>
</tr>
</tbody>
</table>
The corn oil with a high PV of 51 meq./kg gave TBA of only 0.4 μg/kg. This apparent discrepancy can be explained by the fact that the TBA method is primarily based on determining the concentration of a TBA-malonaldehyde (MA) complex. MA is a secondary oxidative product of linolenic acid not always present in oxidized systems. The interaction of MA with available amino groups in meat components has been reported to adversely affect the results of TBA analyses (Nawar, 1996). Furthermore, the TBA method is not sensitive to the oxidation of oleic and linoleic acids (Nawar, 1996).

The low temperature conditions employed during the preparation and extraction steps reduce the potential for further autoxidation in samples being analyzed by the SPM method. The comparisons of CVs for AOCS and SPM indicate that SPM is the more reproducible of the two methods in analyzing PV for lamb tissue and corn oil.

It was reported that the boiling procedure in TBA test used to speed up color development had negative impact on result due to the further breakdown of MA (Decker et al., 1998). TBA method does not detect the oxidation of mono- and di-unsaturated fatty acids (Decker et al., 1998), while there are very small portions of fatty acids in beef, pork, mutton and poultry (Foegeding and Lanier, 1996). Since most muscle foods consist of mono- and di-unsaturated fatty acids, peroxide value may report more accurately the lipid oxidation of foods than TBA. The color developments for AOCS and SPM for PV are instantaneous while color development for the TBA cold extraction method requires at least 15 h. Therefore, peroxide value determination greatly shortens the time to analyze lipid oxidation.
Titration methods for determining peroxide values have long been criticized for their lack of reproducibility because of the difficulty in determining the titration endpoint, which can be influenced by light and subjectivity (Fielder, 1974). The SPM method provides a rapid, yet more objective and more accurate method for measuring the lipid oxidation in animal tissue.
TABLE 4.3: Measurements of lipid oxidation of lamb tissue and corn oil by 2-thiobarbituric acid (TBA) test, AOCS peroxide value and spectrophotometric peroxide value method (SPM)

<table>
<thead>
<tr>
<th>Analyses&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TBA (µg/g)</th>
<th>PV (meq./kg)</th>
<th>SPM (meq./kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb Fat</td>
<td>Mean 0.10 ± .01</td>
<td>1.43 ± .48</td>
<td>1.52 ± .16</td>
</tr>
<tr>
<td></td>
<td>C.V. 14.53%</td>
<td>33.71%</td>
<td>10.73%</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>Mean&lt;sup&gt;b&lt;/sup&gt; 0.39 ± .02</td>
<td>52.27 ± .19</td>
<td>51.70 ± .11</td>
</tr>
<tr>
<td></td>
<td>C.V. 4.62%</td>
<td>0.36%</td>
<td>0.21%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Six replications in each sample fat
<sup>b</sup>Statistical analyses (for two peroxide value methods): $P < 0.05$
4.5 CONCLUSION

Lipid oxidation determinations are normally performed on animal fat samples more than 7 days post slaughter. This is the first report of the lipid oxidation state determination on fresh fabricated lamb tissue. SPM is rapid, sample, sensitive and reproducible compared to AOCS method. This new spectrophotometric PV method could make a potentially great analytical contribution to the meat and other food industries.
4.6 IMPLICATIONS

The advantages of SPM over TBA and AOCS PV methods were summarized as follows:

Reproducible

The low CVs of SPM in contrast to the relatively high CVs for TBA and AOCS methods in both high and low oxidation states indicates that SPM is the more reproducible method for evaluating the lipid oxidation. Titration methods for AOCS peroxide values have long been criticized for their lack of reproducibility because of the inherent subjectivity of determining visual endpoints. The SPM method provides a more objective and accurate method for measuring the lipid oxidation.

Accurate

TBA method does not detect the oxidation of mono- and di-unsaturated fatty acids. Since many foods consist of mono-, di- and poly-unsaturated fatty acids, peroxide values may report more accurately the lipid oxidation of foods than TBA.
Rapid

The color development for SPM is instantaneous while color development for the TBA method requires at least 15 h. Therefore, peroxide value determination greatly shortens the time to analyze lipid oxidation.
CHAPTER 5

IMPROVEMENT OF THE PHYSICOCHEMICAL PROPERTIES OF PALE SOFT AND EXUDATIVE (PSE) MEAT PRODUCTS WITH AN EXTRACT FROM MECHANICAL DEBONED TURKEY MEAT (MDTM)¹

5.1 ABSTRACT

The physicochemical states of the myofibrillar proteins confer functionality to meat systems and thereby have a direct role in determining the quality and value of processed meat. Inferior quality and loss of protein functionality of pale soft and exudative (PSE) meat and a surplus of underutilized mechanically deboned turkey meat (MDTM) are negative economical concerns in the processed meat industry. The objective of this research was to add the functional proteins present in a salt extract of MDTM to PSE meat during processing to create a value-added cooked pork sausage product. Myofibrillar and sarcoplasmic proteins were extracted from fresh MDTM with brine (1.4 M NaCl, 0.1 M Na₃PO₄, and 0.05 M sucrose) and the extracted proteins

¹ Based on a paper presented during the Annual IFT 2000 Muscle Foods Division Graduate Paper Competition. "Improvement of the physicochemical properties of pale soft and exudative (PSE) meat products with an extract from mechanical deboned turkey meat (MDTM)" at Dallas, TX., June 10-14, 2000. The first runner-up winning paper.
recovered in the supernatant following centrifugation. Fresh pork sausages were made from either PSE or normal pork trimmings by preblending with either brine alone or MDTM extract. The water holding capacity (WHC) of precooked sausages was evaluated by the Carver Press filter paper method. Cooking loss and rheological analysis were performed on cooked sausages. Statistical analyses were determined by SAS. Sausages manufactured from PSE using the MDTM extract exhibited a 30% increase in rigidity (P < 0.05) compared to those manufactured from PSE using brine alone. Sausages made from PSE and normal pork in which the MDTM extract was part of the preblend demonstrated an increase of 3.6 and 3.0% in WHC, and a reduction of 4.1 and 3.1% in cooking loss (P < 0.05), respectively, compared to sausages made employing brine alone. Results indicate that proteins present in a salt extract of MDTM have the potential to "lend" functionality to processed meat systems, and improve the physicochemical properties of fresh pork sausages. Introducing functional proteins derived from MDTM to processed meat made with PSE pork has the potential to enhance the economic value to both of these low value raw materials. Thus, inclusion of a salt extract of MDTM could have a great economic contribution to the meat industry.
5.2 INTRODUCTION

Improving the quality and value of muscle foods is a major objective of the meat industry. The physicochemical properties of comminuted meat system are essential to final product quality and consumer acceptability (Smith, 1988; Barbut and Mittal, 1989). The physicochemical state of the myofibrillar proteins, myosin and actomyosin, confer functionality to meat systems and thereby have a direct role in determining the quality and value of processed meat (Yasui et al., 1982; Camou and Sebranek, 1991).

Among the problems affecting the economic value of muscle foods are the inferior quality of pale, soft and exudative (PSE) meat and the surplus of mechanically deboned turkey meat (MDTM). Ten percent of swine in the US exhibit a post-mortem muscle condition resulting in PSE (Kauffman and Meeker, 1996). With PSE discounting $0.34/head, the economic impact on the pork industry is nearly $32 million/yr due to the shrinkage of meat and the loss of weight during storage (Hedrick et al., 1994b). Research indicated that the loss of protein functionality in PSE pork is responsible for the inferior quality of PSE meat (Boles et al., 1992). MDTM is a low cost, underutilized by-product of fresh meat processing with high nutritive value. MDTM is approved for use in commercially processed meat products. However, the high proportion of fat and collagen in MDTM and subsequent high rate of lipid oxidation make it a low value raw material to
meat processors (Field, 1981; Yang and Froning, 1992; Lee et al. 1975). It has been recommended that PSE meat could be used with special formulations that contain ingredients or conditions to restore meat quality and protein functionality thereby reducing yield losses and improving texture (Owens et al., 2000).

Thermally induced meat protein gelation is the key element required to bind meat pieces together with optimal strength in processed meat products (Hedrick et al. 1994c). Heat-induced gelation of the meat proteins, myosin and actomyosin, plays an essential role in the development of binding properties of comminuted meat products such as sausages (Samejima et al., 1969; Ishioroshi et al., 1983). Samejima et al. (1969, 1981, 1982) reported that myosin and actomyosin are the most important factors for the development of the binding properties of thermally induced meat protein gels. Not only do myosin and actomyosin contribute to the rigidity of thermally induced meat gels, they are also critical to the water holding capacity (WHC) and high cooking yields of comminuted meat products such as sausages (Hamann, 1988; Smith, 1988). Heating transforms the meat sol to a gel resulting in the formation of a three-dimensional network (Samejima et al., 1969; Acton et al., 1981; Yamamoto et al., 1988). The protein network physically and chemically enhances the water retention by their capillarity and non-covalent bonding (Acton and Dick, 1984). Water retention in processed meat product is the major contributor to the sensation of juiciness (Hedrick et al., 1994c). Cooking loss directly impacts both economic and palatable value of processed meats. The greater the amount of moisture retained during processing the greater the value of the product. The loss of weight of processed meat products during processing is a function of the water holding capacity. However, PSE meat has tremendously reduced palatability of meat due
to higher moisture losses during cooking. The objective of this research was to employ myosin/actomyosin extracted from MDTM as an ingredient to improve physicochemical properties of processed meat products.
5.3 MATERIALS AND METHODS

A 2 (PSE and normal pork trimmings) x 2 (standard brine and MDTM extract) factorial comparison of the physiochemical properties in four comminuted sausage products was performed. Experimental design is shown in Figure 5.1.

5.3.1. Raw Material

Fresh mechanically deboned turkey meat (MDTM) was obtained from a local processing facility (Cooper Farms. St. Henry. OH). The MDTM was immediately extracted to minimize any further lipid oxidation during processing. Normal and PSE pork trimmings were obtained from The Ohio State University Meat Lab. Columbus. OH. The evaluation of PSE was determined by visual appearance and exudative condition of pork. Normal and PSE pork were ground with 1/4" (6.4 mm) plate openings and stored at 4 ± 2°C prior to the sausage manufacture respectively.
5.3.2. Standard Brine (STB)

The standard brine formula was modified from the Ohio State Meat Lab. Columbus, OH, which includes: 1.4 M sodium chloride (Fisher Scientific, Fair Lawn, NJ), 0.1 M sodium tripolyphosphate (Food grade; Kemira Kemi AB, Helsingborg, Sweden) and 0.05 M sucrose (Domino* granulated sugar; Tate & Lyle North American Sugars Inc., New York, NY).

5.3.3. Mechanically Deboned Turkey Meat (MDTM) Extract

An ionic strength of 0.6 M or higher is required to achieve a better result of myosin or actomyosin extraction and solubility (Whiting, 1988; Stanley et al., 1994). Salt soluble proteins were extracted with a meat: standard brine ratio of 1:3 (w/w). The meat and brine were mixed for 1 h at 4°C. The salt-soluble proteins were recovered in the supernatant following at 10,000 x g, 4°C, 15 min centrifugation by a Sorvall* RC-5B Refrigerated Superspeed Centrifuge (Sorvall Instrument, Newton, CT). Fat, collagen, and meat residue pellets were separated from the salt soluble protein supernatant. Supernatant was then sieved through a 1/16-inch (1.6 mm) mesh filter to remove any solidified fats and meat residues. The procedures of MDTM extraction were summarized in Figure 5.2. The recovery rate of the MDTM extract was measured in a 4-liter cylinder (Figure 5.3)
5.3.4. Sausage Manufacturing

Pork sausages were made from PSE or normal pork trimmings by preblending with either STB alone or MDTM extract in a ratio of 100: 35 (w/w) at 4°C for 1 h. Sausage materials were stuffed into artificial casings (length/diameter = 15 cm: 2 cm) using an electric food grinder (The Rival Company, Kansas City, MO). Sausage links were refrigerated and measured within 24 h.
Figure 5.1: Experimental design
Figure 5.2: The procedures of MDTM extraction

1. Fresh MDTM
2. Mix MDTM:STB 1:3 (w/w), 1 h at 4°C
3. Centrifuge 10,000x G, 15 min
4. Sieve, 1/16-inch mesh
5. Collect supernatant
6. Store at 4°C or immediate use
Figure 5.3: Physical separation of MDTM extract. The recovery rate of MDTM extract is 75–78% (free oil: 2–3%; sediment: 19–23%).
5.3.5. Determination of the MDTM Protein Concentration

Protein determination of MDTM extract was performed using the Kjeldahl procedure in triplicate. The Kjeldahl method was used to determine the organic and non-protein nitrogen content of the sample (AOAC, 1995). By the known nitrogen amount from the digested samples, the protein content can be estimated by calculation. The determination of protein is based on titration and calculation to find the protein content in the MDTM samples. The procedures are described below.

Digestion

All reagents used were ACS grade (Fisher Scientific, Fair Lawn, NJ). Approximately 4 g of MDTM extract was weighed in a clean and dry 300 mL Kjeldahl flask, along with 1 g of catalyst tablets (K_2SO_4: CuSO_4 = 1:1; w/w). An additional clean Kjeldahl flask was prepared as a blank. Twenty-five mL of concentrated (99.9%) sulfuric acid was added to each flask for sample digestion. All flasks were placed on a digestion rack, and the ventilation system and burners turned on. All samples were digested until the solution had a clear bluish green color, then an additional 30 min of digestion heating was continued. This procedure took approximately 70 min.

Distillation

After digestion, the flasks were allowed to cool, and then 20 mL of distilled water was carefully added to the flasks. Kjeldahl flasks were prepared for the absorption of distilled ammonium. The Kjeldahl flasks were placed in a Kjeltec Auto Sampler system 1035 Analyzer (Foss Tecator, Foss North America, Inc., Eden Prairie, MN) for
distillation. Before starting the reaction, the condenser cooling water was turned on. After 75 mL of saturated NaOH was added into each Kjeldahl flask, the solution was well-mixed and the distillation began. Ammonium salts (NH$_4^+$) were broken down into ammonia (NH$_3$), chilled in the condenser and finally absorbed by the 4% boric acid (containing an indicator of Bromo Cresol Green). Due to weak base environment (ammonium salts), the solution with indicator turned from purplish red (acid) to bluish green (base). When ammonia collection was finished, the titration procedure was proceeded by the Kjeltec Analyzer.

**Titration**

The principle of titration is based on neutralization between a strong acid (HCl) and a weak base (ammonium salt). Each flask containing ammonium-boric acid mixture was titrated with 0.100 N HCl. At titration endpoint, the color of solution turn from bluish green to colorless. The amount of HCl, which had been used for titration in each flask including the blank, was recorded respectively.

**Calculation**

The crude protein content was calculated by the following formula:

\[
\text{Protein, \%} = \frac{[(\text{mL of HCl for the sample}) - (\text{mL of HCl for the blank})] \times 1.4 \times 6.25 \times N \text{ of HCl}}{\text{Original moist sample weight, g}}
\]
Normality of HCl used in this research was 0.100 N

5.3.6. Determination of the pH of the MDTM Extract

The pH measurement of the MDTM extract was determined with a basic pH meter (Denver Instrument Company, Arvada, CO).

5.3.7. Determination of the presence of actomyosin in MDTM Extract

The SDS-PAGE is a very sensitive method that commonly used to detect molecular changes in postmortem muscle (Koohmaraie et al., 1984). The presence of actomyosin was determined by using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) described by Weber and Osborn (1969). A high salt/low salt extract was prepared by mixing 1 vol. of MDTM with 3 volumes of formulated brine (9% sodium chloride, 5% dextrose, 2.75% sodium tripolyphosphate, 83.25% water) at 4°C for 1h. After centrifugation at 10,000 x g, 4°C, 15 min. the supernatant was recovered and diluted with 7 volumes of ice cold water. The pellet was recovered after centrifugation at 10,000 x g, 4°C, 15 min. The precipitated actomyosin was resuspended in formulated brine and the concentration of sodium chloride, dextrose and sodium tripolyphosphate returned to 9.5 & 2.75 % respectively by the addition of solid reagents.
5.3.8. Cooking Loss

All sausage links were cooked in an automatic time/temperature controlled smokehouse (DEC International, Inc., Lodi, WI) at 60°C (140°F), RH 100% for 1 h to achieve the maximal meat binding ability (Ishioroshi et al., 1979 and 1981; Samejima et al., 1985). The cooking loss (%) of each treatment was calculated by dividing the final product weight by the stuffed weight. Each sausage casing was recorded before cooking. Final weight of each linkage was recorded and the cooking loss was calculated as follows:

\[
\text{Cooking loss} \% = \frac{\text{Stuffed weight} - \text{Final weight}}{\text{Stuffed weight}}
\]

5.3.9. Texture Analysis

Texture analyses were conducted within 48 h and all sausage links were stored at 10°C for the best result of rigidity (Foegeding, 1988). The shear force required to rupture the samples was used to evaluate the rigidity of the sausage. The rigidity of 2 cm x 2 cm x 2-cm cubes of sausages was determined using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). All data were automatically imported into a spreadsheet of a computer ready for data analysis. Rupture force was expressed as g/cm².

5.3.10. Water Holding Capacity (WHC)

The determination of the WHC of the sausages was modified from the Carver Press filter paper method (Barge et al., 1991), and used on the uncooked raw meat samples. Approximately 500 mg of well-minced meat sample was placed between two Whatman® #1 filter papers (diameter = 150 mm). Filter papers were then placed between
two Teflon® plates and placed between the plates of a Carver Laboratory Press™ (Fred S. Carver Inc., Summit, NJ), individually. A mechanical force of 500 psi was applied to the plates for exact 1 min. After compression, the filter papers were separated from the plates, and the images of two circles (trace inner and outer circles for the areas) were scanned by on a BioRad® Model 700 Imaging Densitometer (BioRad Inc., Hercules, CA) using computer software Imagine-Pro® Plus 3.0 for Window™ 95 (Media Cybernetics Inc., Sliver Spring, MD).

Moisture Determination

The % moisture of the meat sample was determined by the oven-drying method (100°C, 18 h) described by Ockerman (1985). Moisture determination is based on drying the material at a specified temperature and time and observing the loss of weight, which is determined as the moisture content. All procedures were conducted in triplicate. Prior to moisture determination, aluminum weighing pans (VWR Scientific Inc., West Chester, PA) were coded for each treatment (3 replications x 4 treatments), and placed in a drying oven (Boekel Inc., 115 volt, 2000 Watts) for 2 h at 100°C. After drying, the aluminum pans were transferred to a desiccator for chilling to room temperature for 1 h. After recording dried pan weight, approximate 4-5 g of samples from treatment were weighed and recorded. The aluminum pans along with samples were then placed in the drying oven at 100°C. After 18 h, the pans with dried samples were transferred to a desiccator for chilling to the room temperature for 1 h. Pans and samples were removed from the desiccator, the weight recorded, the weight loss calculated. The moisture content (%) for each sample was calculated by the following equation:
Where: Weight loss = [Sample net wt.] - [(Pan with dried meat sample) - dry pan weight].

The area between the two traced circles on the filter paper was defined as amount of free water in meat (Figure 5.4). The same compression procedure was also employed to determine the area of 20 mg of water as a reference for free water (n = 5). The result from each sample was recorded, and WHC was calculated as follows.

\[ \text{WHC. \%} = \left( \frac{\text{free water, mg}}{\text{original meat weight, mg}} \right) \times 100\% \]
Figure 5.4: The distribution of free and bound water in a sausage sample prior to cooking. The large light circle surrounding the dark central region represents the free water expressed from the uncooked sausage material at 500 psi, 1 min. The central dark region represents the bound water remaining in the sausage material and the sausage material remaining after application of 500 psi, 1 min.
5.3.11. Statistical Analyses

Data were analyzed by SAS statistical analysis system (SAS, 1998). Statistical significance ($P < 0.05$) of the differences between means was determined by Tukey's multiple comparison test.
5.4 RESULTS AND DISCUSSION

5.4.1. Determination of the MDTM Protein Concentration

The crude protein concentration (in triplicate), calculated from (the percent of free nitrogen content) x (6.25), was 2.97%.

5.4.2. Determination of the pH of the MDTM Extract

The pH of MDTM was 6.41 (in triplicate).

5.4.3. Determination of the Presence of Actomyosin in MDTM Extract

The presence of actomyosin in the extract was determined by SDS-PAGE (Figure 5.5). Proteins were identified based on the SDS-PAGE reports of Whipple and coworkers (Whipple et al., 1994). Figure 5.5 also demonstrates differences in the protein profiles between high salt extracts (HS) of fresh MDTM and the high salt/low salt extract (HS/LS) from the same material. The major constituents of the HS extract are myosin heavy chain (206 kDa), myosin light chains (17–21 kDa), actin (45 kDa), most likely from extracted actomyosin, and myoglobin (18 kDa). Notice when the HS extract is returned to low salt conditions only myosin and actin are precipitated (Figure 5.5, lane HS/LS).
The results of adding STB or MDTM extract on the physicochemical properties (rigidity, cooking loss, and WHC) of cooked fresh pork sausages were summarized in Table 5.1 and the details described in the sections 5.4.4, 5.4.5 and 5.4.6.
Figure 5.5: SDS-PAGE of myofibrillar protein from high salt (HS) and high salt/low salt (HS/LS) extracts (Adapted from Wick, 1999b). Lane identification:

**Stds:** Standard. MHC (myosin heavy chain), 200 kDa; A (actin), 45 kDa; Mb (myoglobin), 18 kDa.

**HS:** High salt (HS) extract contains many proteins including those with similar mobilities to myosin, actin, and myoglobin.

**HS/LS:** Further myofibrillar purification by dilution in water to reduce the ionic strength < 0.2 M results in significant increases in concentration of myofibrillar proteins, myosin and actin and significant reduction in proteins with molecular weights < 40 kDa, including myoglobin.
<table>
<thead>
<tr>
<th>Pork</th>
<th>Brine</th>
<th>Rupture Force (g)</th>
<th>Cooking Loss (%)</th>
<th>WHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSE</td>
<td>STB</td>
<td>1584 ± 107&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.8 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PSE</td>
<td>MDTM</td>
<td>2041 ± 264&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.9 ± 0.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>63.0 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>STB</td>
<td>2995 ± 283&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.8 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.6 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>MDTM</td>
<td>3081 ± 116&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.4 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.5 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means followed by identical letters within the same column are not different (P > 0.05); n = 6

Table 5.1: Effect of added STB and MDTM extract on rigidity, cooking loss, and WHC of comminuted pork sausages
5.4.4. Cooking loss (%)

Cooking loss analyses demonstrated that when a 1:3 (MDTM: STB, w/w) blend was added as an ingredient during the manufacture of cooked pork sausages, the cooking loss of sausages prepared from PSE meat as well as sausages manufactured from normal pork trimmings was slightly reduced (P < 0.05) (Table 5.1 and Figure 5.6). There were no differences in the cooking loss among the three groups of sausages made from: 1) normal pork trimmings and MDTM, 2) normal pork trimmings and STB or, 3) sausages made from PSE pork and MDTM extract (P > 0.05). The reduction in the cooking loss exhibited in the sausages made from PSE and the MDTM extract is most likely due to the presence of the functional myofibrillar proteins, myosin and actomyosin, present in the MDTM extract. The absence of a difference in the cooking loss (P > 0.05) between sausages made from PSE with STB and sausages made from PSE and MDTM extract (Table 5.1 and Figure 5.6), is likely due to a low concentration of myofibrillar proteins present in the MDTM extract (< 1.5%). Experiments are underway to study the effect of adding higher concentrations of myofibrillar proteins derived from MDTM on processed meat products made from PSE meat.
Figure 5.6: Cooking loss (%) of comminuted pork sausages
5.4.5. Texture Analyses

The rigidity of sausages made from PSE was lower than sausages made from normal pork (P < 0.05) (Figure 5.7). In contrast, sausages manufactured from PSE including a MDTM extract exhibited approximately a 30% increase in rigidity compared to sausages made employing STB alone (P < 0.05) (Table 5.1 and Figure 5.7). The plausible cause is due to the contribution of the myofibrillar proteins from MDTM extract to PSE pork. However, the difference in the rigidity between sausages made from normal pork trimmings with either MDTM or STB was not significant (P > 0.05). This observation could be due to high concentrations of functional myofibrillar proteins already present in the normal pork muscle. Therefore, the contribution of the MDTM extract to the functionality of the meat system would be minimal.

5.4.6. Water Holding Capacity (WHC)

Results of WHC analysis demonstrate no difference in WHC among all four groups of sausages (P > 0.05) (Table 5.1 and Figure 5.8). The difference in the ability of myofibrillar proteins derived from normal or PSE meat to bind water was only observed as differences in cooking loss in the sausages made from the two sources, but not WHC. Prior to thermal gelation, myosin and actomyosin from PSE or normal muscle have the same capacity for binding water. However, the capacity for binding water changes upon thermal denaturation through an unknown mechanism. Therefore, these observations may be a reflection of different mechanisms of water binding in native and thermally denatured myofibrillar proteins.
Figure 5.7: Rupture force (g) of comminuted pork sausages
Figure 5.8: Water holding capacity (WHC) of comminuted pork sausages
5.5 CONCLUSIONS

Functional properties of processed meat products are mainly due to the existence of myofibrillar proteins (Samejima et al., 1992). Myofibrillar proteins are critical to the development of a three dimensional matrix exhibiting the rigidity and WHC necessary to the manufacture of high quality comminuted meat products (Xiong, 1992; Samejima et al., 1981). In our study, an extract of MDTM included in the preblend produced a value-added cooked meat product by employing extracted myofibrillar proteins to increase the physicochemical properties of cooked meat products. These results demonstrate significant positive effects on the rheological and cooking loss properties of cooked pork sausages when a salt extract of MDTM was added as an ingredient during preblending. These data further support our hypothesis that myosin/actomyosin has the potential to lend functionality to processed meat. Researchers have been demonstrating this for years. Our hypothesis is that myosin and actomyosin derived from mechanically deboned meat can be used to increase the functionality of processed meats systems containing PSE. Thus, the inclusion of a salt extract of MDTM could have an important economic contribution to the meat industry.
5.6 IMPLICATIONS

The thermal gelation of myofibrillar proteins is the primary factor for forming the physicochemical properties associated with high quality processed meat products. Results of this study demonstrate that functional meat proteins derived from an underutilized, low economic value by product can be used as a source of functional myofibrillar proteins for use in the manufacture of a processed meat product and partially compensate the loss of functionality in the PSE meat. In addition, with the removal of phospholipid or membrane lipid by centrifugation, the quality of protein isolate should be more stable. High quality of functional meat proteins can also be extracted from meat sources from many animal species contributing a positive economic impact for the meat industry.
APPENDICES
APPENDIX A

THE EVALUATION OF A MECHANICAL LIPID EXTRACTION OF OVINE TISSUE - CHEMICAL ANALYSES

A.1 INTRODUCTION

Meat provides excellent quality protein and caloric value in our daily diets (Hedrick et al. 1994e). The amount of lipid in meat depends on amount of untrimmed fat within and between muscles and amount of external fat remaining after trimming. Lipid oxidation is a major problem, which causes flavor deterioration in meat and meat products. In this project, a mechanical extraction method used to generate extracted lipid sample was developed. The objectives of this study were: 1) To systematically develop an efficient and practical method for the separation of lipids from meat without using chemicals or heat; 2) To study the effects of mechanical lipid extraction on the changes of chemical composition in mutton loin samples; 3) To observe the effects of physical compression on the chemical compositions of mutton sample.

The official methods of chemical analyses for meat and meat products (AOAC, 1995) are commonly used to provide some basic nutritional information in meat. In this
project. the proximate analyses were conducted to compare the difference in the changes of chemical components in mutton samples and the TBA method were used to measure lipid oxidation between different compression treatments. These analytical results might provide some information to the meat industry for their further studies.
A.2 MATERIALS AND METHODS

The experiment was a 1 x 4 (4 compression times: 0, 1, 2, and 3 times) factorial design. The loin from a fresh 5-year-old ewe was obtained from the Ohio State University Meat Lab, Columbus, OH. All visible fat and connective tissue were carefully removed from the meat surface. A similar loin sample was prepared for proximate and TBA analyses.

The procedure for physical lipid extraction involved a hydraulic force by a Carver Laboratory Press™ (Fred S. Carver Inc., Summit, NJ), which was applied to the samples until the pressure reached 5,000 psi (Figure A.1). The fluid portion was absorbed by filter paper. Approximately 2 g of cubed mutton samples was placed between two Teflon™ plastic plates (6 x 6 inches sq.) and pressed. Exudate (fluid portion) was removed by filter paper and a syringe, and the pressed matter was then collected and placed between the pressing plates again for second compression. Moisture, Keljadel protein, mineral and TBA analyses were performed on d 1. Crude fat and nitrogen analyses were conducted on d 2 after the moisture analysis was conducted.

In this project, chemical analyses (moisture, total solid, crude fat, crude protein, and mineral) and a TBA method were performed to investigate the compressed samples for their chemical components and lipid oxidation state. The apparatus and chemical
reagents used in all analyses are summarized in Table A.1. Moisture, crude fat, crude protein (Keljadel protein and nitrogen analyzer methods), and mineral analyses were determined by the AOAC procedure (1995) and outlined in the following sections A.2.1–A.2.6.
Figure A.1: Apparatus used for the physical extraction of animal fat
<table>
<thead>
<tr>
<th>Analysis method &amp; Total Solid (TS), %</th>
<th>Apparatus and Instrument</th>
<th>Chemicals &amp; Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture &amp; Total Solid (TS), %</td>
<td>Oven (100°C, 18 h)</td>
<td>Petroleum ether (B.P.: 35 - 60°C)</td>
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<tr>
<td></td>
<td>Thermometer</td>
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<td></td>
<td>Desiccator</td>
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<tr>
<td></td>
<td>Aluminum weighing pans</td>
<td></td>
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<tr>
<td>Crude Fat</td>
<td>Soxhlet (pulp) thimbles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soxhlet extraction unit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glass beads</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hot plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Graduated cylinder</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Kjeldahl flasks</td>
<td>Sulfuric acid.</td>
</tr>
<tr>
<td></td>
<td>Digestion racket</td>
<td>Catalyst Tablets</td>
</tr>
<tr>
<td></td>
<td>Tecator Keljtec distillation unit</td>
<td>(Potassium sulphate: 89.7%; Cupric sulphate: 10.30%).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40% Saturated NaOH.</td>
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<tr>
<td></td>
<td></td>
<td>Boric acid (with Methyl Red and Bromo Cresol Green as the indicator).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.100 N HCl solution.</td>
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<tr>
<td></td>
<td>Nitrogen analyzer</td>
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<td></td>
<td>Tin capsules</td>
<td></td>
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<tr>
<td>Mineral</td>
<td>Sybron Thermolyne Furnace 6000 (525°C, 18 hours)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Porcelain evaporating dishes</td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>Graduated cylinder</td>
<td>20% Trichloroacetic acid (TCA. in 1.6% of phosphoric acid).</td>
</tr>
<tr>
<td></td>
<td>Polyethylene stomacher bags</td>
<td>Deionized water</td>
</tr>
<tr>
<td></td>
<td>Seward Laboratory blender</td>
<td>0.02 M 2-thiobarbituric acid (TBA = C₄H₄N₂O₂S; or 4.6-dihydroxypyrimidine-2-thiol).</td>
</tr>
<tr>
<td></td>
<td>Funnels</td>
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<td></td>
<td>Whatman No. 1 filter paper (125 mm)</td>
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<td></td>
<td>Pyrex volumetric flask (100 mL)</td>
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<td></td>
<td>Gilford Spectrophotometer</td>
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</table>

Table A.1: The apparatus and chemical reagents for chemical analysis
A.2.1. Moisture

The moisture analysis was same as described in Chapter 5. Section 5.3.9 (Moisture Determination). The moisture content (%) for each sample was calculated by the following formula (1):

\[
\text{Moisture content, \%} = \frac{\text{Weight loss, g}}{\text{Sample net weight, g}} \times 100\% \quad (1)
\]

Where:

Weight loss = [Sample net wt.] - [(Pan with dried meat sample) - dry pan weight].

After recording the dried sample weight, samples were retained in a desiccator for crude fat analysis.

A.2.2. Crude Fat

Lipids are associated with other molecules through 1) van der Waals interaction (e.g., lipid-protein interaction). 2) Electrostatic and hydrogen bonding (mainly between lipids and proteins). 3) Covalent bonding between lipids, carbohydrates, and proteins (Shahidi and Wanasundara. 1998). Therefore, it is important to separate and isolate lipids from the complex cellular matrix for further quantification and analyses. The insolubility of lipids in water makes possible their separation from proteins, carbohydrates, and water in the tissue. Solubility of lipids in organic solvents is mainly determined by the length and proportion of the non-polar hydrocarbon chain of the fatty
acids or other aliphatic moieties (Shahidi and Wanasundara, 1998). Since most fats contain no distinguishable polar groups, the most common method of analyzing meat for fat is using an organic non-polar solvent extraction. Petroleum ether (non-polar) extraction of fat and fat-soluble substances was applied in this procedure. The principle of this solvent extraction is based on the affinity between the hydrophobicity of the non-polar organic solvent (anhydrous ether) to the hydrophobicity of another non-polar substance (fats).

The procedure for crude fat extraction was performed after the moisture determination. Prior to crude fat determination, Soxhlet (pulp) thimbles, along with Whatman No. 1 filter paper (for wrapping the sample) were dried in a 100°C drying oven for 2 h. After drying, the thimbles and filter paper were placed in a desiccator to chill to the room temperature for 1 h. Each thimble, along with a piece of filter paper, was weighed and recorded. With the aid of petroleum ether and a spatula, dried samples (from moisture determination) were then transferred from the aluminum pans to filter paper individually. Samples were wrapped in the filter paper and then placed into the thimbles. Thimbles along with the samples were placed inside a Soxhlet extractor. The upper end of the extractor was connected to a condenser and the lower end was connected to a 400 mL extraction flask. Each extraction flask was filled with at least 300 mL of petroleum ether (Ligroine, 35°-60°C; Mallinckrodt Baker Inc., Paris, KY). Three glass beads were placed into the extraction flask to stir and in order to ensure a uniform heating process. Running water was passed through each condenser for chilling. Then the hot plate of the Sohlext units (GLAS-COL apparatus Co., Terre Haute, Indiana) was turned on to 65~70°C. After checking water flow, connected parts and warming of the hot plate
(approximately 15 min), extraction was started. After 8 h of extraction, thimbles were then removed from extractors and placed on absorbing paper towels and left in a ventilating hood for ether evaporation for 2 h. After evaporation, thimbles along with samples were placed into a 100 °C drying oven for 2 h. After drying, samples were removed from the drying oven, and placed into a desiccator to chill to the room temperature for 1 h, and then weighed. By calculating the weight loss of the sample before and after either extraction, the crude fat content (%) of each sample was obtained by the following formula (2):

\[
\text{Crude fat. \%} = \frac{\text{Weight loss of the dried tissue after extraction}}{\text{Original sample weight before moisture determination}} \times 100\% \quad (2)
\]

A.2.3. Kjeldahl Protein

Protein determination of MDTM extract was performed using the Kjeldahl procedure in triplicate. The Kjeldahl protein method was used to determine the organic and non-protein nitrogen content of the sample (AOAC. 1995) as described in Chapter 5, Section 5.3.5. The crude protein content can be calculated by the following formula (3):

\[
\text{Crude Protein. \%} = \frac{[(\text{mL of HCl for the sample}) - (\text{mL of HCl for the blank})] \times 1.4 \times 6.25 \times N \text{ of HCl}}{\text{Original moist sample weight, g}}
\]

\quad (3)

Where Normality of HCl used in this research was 0.100 N
A.2.4. Nitrogen Analysis (Protein Content)

Nitrogen analysis was another method to determine the protein content. The principle is based sample combustion and ionization. The disintegrated molecules were flushed out by the carrier gas CO₂ and detected by a nitrogen analyzer. Prior to nitrogen analysis, moisture and fat were removed from the samples, as describe in session A.2.1 and A.2.2. Approximately 0.05 g (0.04-0.06 g) of sample was weighed in a tin caplet on balance. Both weights of tin caplet and meat sample were recorded. The top of the tin caplets were folded $\frac{1}{4}''$ to close the opening. Caplets were then rolled into a small ball and placed into the analyzer. A caplet containing EDTA was used as a standard prior to sample measurement. Data were imported and into a computer for recording.

A.2.5. Ash (Minerals)

Ash content in meat is most commonly used to represent the total mineral content, since high temperature can incinerate most substance in foods except minerals. The procedure described in AOAC (1995) and summarized as follows. All measurements were conducted in triplicate. Prior to mineral determination, twelve porcelain-evaporating dishes were prepared for the removal of any possible organic residue on dishes by the following procedures:

The coded porcelain evaporating dishes were placed into a Sybron™ Thermolyne Furnatrol (Model FA-1730. 240 volts. 5800 Watts: Thermolyne Corp., Dubuque, IA), at a
temperature of 525°C (977°F) for 8 h. Dishes were retained in the oven for chilling for 1 h. All porcelain dishes were removed from the oven and placed into a desiccator at room temperature for at least 1 h prior to mineral determination.

Mineral Determination

Each coded dish was weighed and recorded before an approximately 2~3 g sample was added. After all dish weights and sample net weights were recorded, samples were placed into a 100°C drying oven to remove moisture as described in A.2.1. During drying procedure, samples were charred in the drying oven (with a hood) until the sample stopped smoking. Samples were then placed into a Furnatrol oven. Before the ash furnace was turned on, the ventilation system was activated. When the temperature reached 525°C (977°C) in the ash oven, timing was started and the samples were kept in the oven for 18 h. After proceeding, the oven was turned off and the samples were held in the oven to cool. After porcelain dishes cooled, the samples were removed from the ash oven and transported to a desiccator for 1 h for chilling (the color of ash was white to gray). All samples were then weighed in a closed and isolated environment to prevent air currents from disturbing the samples during weighing and recording. The mineral content can be calculated by formula (4).

\[
\text{Ash} \% = \frac{(\text{Wt. porcelain dish} + \text{Wt. ash residue}) - (\text{Wt. porcelain dish})}{\text{Net sample weight}} \times 100\% \quad \text{(4)}
\]
A.2.6. The 2-thiobarbituric Acid (TBA) Test

The degree of lipid oxidation in this experiment was determined by the TBA (2-thio-barbituric acid), which was described in Chapter 3, Session 3.3.3. The color of TBA-MA complex was measured by a spectrophotometer at a wavelength of 530 nm. By measuring the absorbance (OD) of the colored samples, the degree of the lipid oxidation could be calculated.

A.2.7. Statistical Analysis

All analyses were performed in triplicate. Statistical significance of the differences between means value was determined by t-test using SAS 7.0 for Windows (SAS, 1998).
A.3 RESULTS AND DISCUSSION

Results of all chemical analyses are summarized in Table A.2. For moisture content analysis, the treatment without compression had a significantly higher percentage of moisture content (67.13%) than those with compression treatments. The exudation from the lamb meat samples was simply caused by the physical force, which will also alter the proportions of other components (protein, fat, and minerals) in meat samples. In addition, results indicate that compression times (once, twice and three times) have no significant influence on the moisture content (Figure A.2). The same trends are shown for crude fat (Figure A.3), mineral (Figure A.4) and two crude protein analyses (Figure A.5 and A.6). TBA values have been commonly used as an index of lipid rancidity. Table A.2 and Figure A.7 indicate that the TBA values decreased, when the time of compression increased. Ockerman (1985) reported that a TBA number of 0.7-1.0 in fresh meat product is considered as an unacceptable level for rancidity by some processors. None of the data exceeded this level, but all mutton (ewe) samples exhibited a noticeable unpleasant odor. The result may suggest that TBA is not an appropriate indicator of mutton freshness. Either a lower freshness threshold for mutton may be set, or a better (more accurate) lipid oxidation test needs to be developed.
### Table A.2: The effects of different compression time on chemical composition of meat

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Compression Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Moisture. %</td>
<td>67.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude Fat. %</td>
<td>11.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude Protein&lt;sup&gt;1&lt;/sup&gt;. %</td>
<td>19.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude Protein&lt;sup&gt;2&lt;/sup&gt;. %</td>
<td>17.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mineral. %</td>
<td>0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBA. µg/g</td>
<td>0.4639&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means followed by identical letters within the same row are not different (P > 0.05).

<sup>1</sup> indicates the protein contents were done by Kjeldahl method. Kjeldahl factor: 6.25.

<sup>2</sup> indicates the protein contents were done by Nitrogen analyzer. Kjeldahl factor: 6.25.
Figure A.2: Moisture content vs. Time(s) of compression

Figure A.3: Crude fat content vs. Time(s) of compression
Figure A.4: Mineral content vs. Time(s) of compression

Figure A.5: Crude protein (Kjeldahl Method) vs. Time(s) of compression
Figure A.6: Crude Protein (Nitrogen analyzer method) vs. Time(s) of compression

Figure A.7: TBA values vs. Time(s) of compression
A.4 SUMMARY

Mutton samples lost approximately half of their weight during compression. Compression is also the major factor that influenced the chemical analysis (moisture, total solid, crude fat, crude protein, and mineral).

Multiple compression (once, twice, and three times) did not significantly change moisture, crude fat, and mineral contents of mutton.

Compression reduces:
- 59–63% of moisture
- 41–47% of fat
- 18%–24% of protein
- 35%–42% of mineral

The TBA values were decreased by compression. (32–38%)

TBA value reported here may also more closely represent the true oxidation state in this experiment prior to the onset of the lipid oxidation. It can also be speculated that storage time will contribute a significant effect on the freshness. Some supplementary research of the sensory evaluation on rancidity may be conducted in the future as further support.
In this experiment, the results of the chemical analyses provided the basic nutritional information required by meat industry that may take an interest of this study. With the appropriate machinery design, mechanical lipid extraction shall have its potential. In addition, the difference between compression times (once, twice, and three times) did not seem to have much influence on most analyses; therefore, there is no need for additional compression that would save both time and labor.
A.5 IMPLICATIONS

The by-product from compressed mutton has lower moisture, lower fat, but is still high in protein contents. On the other hand, the by-product of meat could serve as a good sustenance for human consumption, because it also would have the advantages of light weight, low fat and high protein. It implies that the product from compressed mutton could be an excellent dietary substance. On product development standpoint, it is possible. TBA values indicated a lower degree of rancidity in the compressed sample due to the exudates was removed from the rancid mutton samples. However, some off-the-shelf meat blocks (those which caused a flavor concern, not microbial problems) could be "brought back to life" with additional compression treatment, and converted into "another kind of product", which is still suitable for consumption. This technique may also be applied to the muscle tissue from other animal species. If this approach is successful, the meat industry could expect lower costs in analyses and the increased profits from new product development.
APPENDIX B

REDUCTION OF COOKING LOSS OF TURKEY LOAF PRODUCTS WITH A PURIFIED MYOSIN/ACTOMYOSIN EXTRACT FROM MECHANICAL DEBONED TURKEY MEAT (MDTM) - A PRELIMINARY REPORT

B.1 INTRODUCTION

Development of value-added muscle foods is one of the fastest growing areas in the turkey industry (McKee and Sams, 1998). Sectioned and formed products have become increasingly popular due to the advantages of easier and more accurate portion control and the simulation of boneless high quality cuts (Siegel et al., 1978). It was reported that myosin and actomyosin confer functionality to meat systems and thereby have a direct role in determining the quality and value of processed meat (Yasui et al., 1982; Camou and Sebranek, 1991). As previously mentioned in the Chapter 5, thermally induced protein gelation is the key element required to bind meat pieces together with optimal strength in processed meat products. Binding properties in meat products are produced by extracting protein from the meat to serve as a binder between the meat
pieces. (Siegel and Schmidt, 1979) (Figure B.1). Samejima et al. (1969, 1981, and 1982) also reported that myosin and actomyosin are the most important factors for the development of the binding properties of thermally induced meat protein gels.

Cooking loss directly impacts the economic value of processed meats. The greater the amount of moisture retained during processing, the greater the value of the product to the producer. Therefore, introducing functional proteins extracted from MDTM to turkey loaves in order to increase the total concentration of myofibrillar proteins present during meat processing may have the potential to reduce cooking loss, increase binding and thereby enhance economic value of restructured meat products. The objective of this research was to employ purified myosin/actomyosin extracted from MDTM as an ingredient to improve the cooking yield of turkey loaf products.
Figure B.1: Binding in restructured meat product
B.2 MATERIALS AND METHODS

B.2.1. Raw Materials

Fresh mechanically deboned turkey meat and turkey breasts (whole muscle) were obtained from a local processing facility (Cooper Farms, St. Henry, OH). The MDTM and turkey breasts were stored at 4°C for immediate use.

B.2.2. Brines

Two brine solutions (A & B) were prepared for myosin/actomyosin extraction and purification. The formula of Brine A was obtained from the Ohio State Meat Lab, Columbus, OH, which included: 1.4 M sodium chloride, 0.1 M sodium tripolyphosphate and 0.05 M sucrose. The formula of Brine B was provided by Cooper Farms (St. Henry, OH), which contained: 83.25% ice and water, 9% (1.85 M) of sodium chloride, 5% (0.3 M) dextrose and 2.75% phosphate at 28°F.
B.2.3. Myosin/actomyosin Extraction

As previously described in Chapter 5, an ionic strength of 0.6 M or higher is required to achieve optimal myosin/actomyosin extraction and solubility (Whiting, 1988). The principle of myosin/actomyosin extraction and purification is based on the solubility of myosin and actomyosin in different ionic strength environments (Figure B.2).

MDTM Extraction

Salt soluble proteins were extracted from MDTM at a meat: brine A ratio of 1:3 (w/w). The meat and brine were mixed for 1 h at 4°C. The salt-soluble proteins were recovered in the supernatant following centrifugation at 10,000 x g, 4°C, 15 min in a Sorvall® RC-5B Refrigerated Superspeed Centrifuge (Sorvall Instrument, Newton, CT). Fat, collagen, and insoluble meat residue pellets were separated from the supernatant. The supernatant was then sieved through a 1/16-inch (1.6 mm) mesh filter to further remove any solidified fats and meat residues. The same procedures of MDTM extraction were described in Chapter 5, session 5.3.3.

Myosin/Actomyosin Extract (by High Salt/Low Salt Suspension)

Most salt-soluble proteins, including myosin and actomyosin, were recovered in the MDTM extract. An approximate 80% of recovery rate of original weight of MDTM and brine A was obtained from the MDTM extraction procedures as described in Chapter 5, Figure 5.3. The ionic strength of MDTM reduced to below 0.2 M by the addition of seven volumes of cold deionized water. The solution was allowed to settle overnight to
precipitate myosin and actomyosin. Myosin/actomyosin was recovered in the pellet following centrifugation at 10,000 x g. 4°C. 10 min. The myosin/actomyosin pellets were then resuspended with an equal volume of Brine B. The procedures of myosin/actomyosin extraction were summarized in Figure B.3.
Figure B.2: Myosin/actomyosin solubility at different salt concentration (Samejima, 1985).
Fresh MDTM

Mix MDTM: Brine A 1:3 (w/w) at 4°C, 1 h

Centrifuge 10,000 x g, 15 min

Sieve, 1/16-inch mesh → Discard pellet

Collect supernatant

Mix MDTM extract: D.W. 1:7 (v/v) at 4°C, overnight

Centrifuge 10,000 x g, 10 min

Collect myosin/actomyosin pellet → Discard supernatant

Resuspend myosin/actomyosin pellet: Brine B 1:1 (v/v) at 4°C

Figure B.3: The procedures of myosin/actomyosin extraction
B.2.4. Turkey Loaf Manufacturing

Turkey breast samples were randomly assigned into two groups. One was pumped with 20% green weight (original meat weight) of Brine B, and the other was pumped with 20% green weight of myosin/actomyosin extract. Turkey breasts were sliced into 3–4 pieces prior to the turkey loaf manufacture for easy stuffing. After tumbling in a Hollymatic® tumbler (Hollymatic Corporation, Countryside, IL) for 1 ½ hrs. approximate 7–8 kg of turkey breasts from each treatment were stuffed into cellulose fiber casings (length/diameter = 8–10 inch: 4.5 inch) manually.

B.2.5. Cooking Loss

Turkey loaves were cooked in an automatic time/temperature control smokehouse (DEC International Inc., Lodi, WI) until the internal temperature had reached 160°F at a relative humidity of 100% (R.H. = 100%). The cooking loss (%) of each treatment was determined as described in Chapter 5. Section 5.3.7, calculated by dividing (the final cooked product weight) by the difference between stuffed weight and cook weight. The formula for calculation of cooking loss % was shown below.

\[
\text{Cooking loss } % = \frac{(\text{Stuffed wt.} - \text{Cooked wt.})}{\text{Stuffed weight}}
\]
B.3 RESULTS AND DISCUSSION

Results demonstrated that a 20% green weight of myosin/actomyosin extract added as an ingredient during the manufacture of turkey loaf effectively reduced the cooking loss (Table B.1) from 9.2% to 6.7%. The reduction in the cooking loss exhibited in the turkey loaf pumped with myosin/actomyosin extract is most likely due to the increased concentration of the functional myofibrillar proteins, myosin and actomyosin.

The functional properties of myofibrillar proteins are critical to the development of a three-dimensional matrix necessary to the manufacture of high quality products. The addition of myosin/actomyosin in the preblend produces a value-added cooked meat product by increasing the concentration of myofibrillar. These data further support our hypothesis that purified myosin/actomyosin from traditionally underutilized, low-value meat by-products has the potential to lend functionality to processed meat. Thus, inclusion of a myosin/actomyosin extract from MDTM could have a great contribution to the real industrial practice.
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<tr>
<td></td>
<td>(Kg)</td>
<td>(Kg)</td>
<td>(Kg)</td>
<td>(Kg)</td>
<td>%</td>
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<tr>
<td>Turkey loaf w' Brine B</td>
<td>8.7</td>
<td>10.44</td>
<td>10.12</td>
<td>9.2</td>
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<td>Turkey loaf w'</td>
<td>7.6</td>
<td>9.12</td>
<td>8.84</td>
<td>8.25</td>
<td>6.7</td>
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<td>Myosin/actomyosin extract</td>
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<td></td>
<td>N = 2</td>
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</table>

Cooking loss % = \frac{\text{Stuffed wt.} - \text{Cooked wt.}}{\text{Stuffed weight}} \times 100

**Table B.1**: Addition of myosin/actomyosin extract from mechanically deboned meat affects cooking loss of turkey loaves
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