Processing characteristics and rheological properties of mechanically separated chicken and chicken breast meat

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This body of work is dedicated to the inspiring, uplifting, and empowering words spoken and written to me by countless individuals. I am continuously reminded how impactful simple words of encouragement can be. If their lips had not parted and the ink had not flown from their pens, I would not have been able to achieve the authorship of this thesis. I have learned if your thought is kind, say it, you will never regret it and you might just give a cowardly lion the courage to slay their giant spider.

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NOMENCLATURE

LMM	Light Meromyosin
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TBA	Thiobarbituric Acid
BT	Chicken Breast Trim
MSC	Mechanically Separated Chicken
TPA	Texture Profile Analysis
MLC	Myosin Light Chain
MHC	Myosin Heavy Chain
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
G'	Storage Modulus
G"	Loss Modulus
δ	Phase Angle
LVR	Linear Viscoelastic Range
BSE	Bovine Spongiform Encephalopathy
MSP	Mechanically Separated Poultry
MST	Mechanically Separated Turkey
PER	Protein Efficiency Ratio
C-PER	Calculated Protein Efficiency Ratio
FMSC	Functionalized Mechanically Separated Chicken
MSCS	Mechanically Separated Chicken Surimi
TPP	Tripolyphosphate

AMR	Advanced Meat Recovery
MSM	Mechanically Separated Meat

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ABSTRACT

Mechanically separated chicken (MSC) reduces the cost of processed meat products; however, it also modifies their texture, flavor, and color. MSC production practices are very diverse, resulting in differences in proximate composition. However, little modern literature has studied the variation in MSC functionality compared to whole muscle chicken raw materials. The objectives of the present work were to assess two types of MSC and chicken breast trim as frankfurter raw materials and to identify rheological attributes of their myofibrillar proteins during gelation. MSC variation was obtained from two separation methods (MSC1, Beehive separator, aged bones; MSC2, Poss separator, fresh bones) and compared to chicken breast trim. Three replications of frankfurters were manufactured from each chicken raw material and pork back fat. Myofibrillar protein solutions 2.8% (w/v) (0.6 M NaCl, 50 mM sodium phosphate, pH 6) from each chicken raw material were evaluated for rheological properties during thermal gelation and cooling.

MSC2 frankfurters were greatest in fat and least in moisture content ($P<0.05$). Both MSC frankfurters had significantly darker (L*), and redder (a*) external and internal color than BT frankfurters with MSC2 being the darkest and reddest treatment ($P<0.05$). Greater hardness, cohesiveness, resilience, and chewiness values were observed in MSC2 frankfurters than in BT and MSC1 frankfurters. All myofibrillar solutions exhibited gelation with increased temperature. A peak, decline, and increase was observed in all treatments both in G' and G". G' slopes on both sides of the peak (S2, S3) and final

increase (S4) were significantly different ($P<0.05$) between BT and both MSCs. BT S2 and S3 were significantly different from MSC treatments in G" ($P<0.05$), but not S4.

The data demonstrate that the physical properties of myofibrillar proteins from MSC and chicken breast meat differ during thermal gelation. This indicates a different myofibrillar protein profile that can be explained by both muscle source and by modification during the production of MSC. The data reveal that properties of raw material produced from dissimilar MSC processing can result in significant variation in finished product quality, underscoring the importance of understanding the features of raw materials that affect processing functionality.

CHAPTER 1. GENERAL INTRODUCTION

Local food deserts and global protein deficiencies are of increasing concern as populations soar. The meat industry has the ability to alleviate the pressure of protein shortages as well as many vitamin and mineral deficits with continued efforts to increase efficiency. Producers of further processed meat products have been utilizing rapidly diversifying methods to reduce total product cost; including the manipulation and addition of non-meat ingredients and the addition of lower cost meat ingredients. Non-meat ingredients are useful tools to manipulate final product attributes; however, with recent consumer concerns, industry-wide staples such as modified food starch and sodium phosphates are being removed from processed meats. Now more than ever, it is important to understand how manipulation of the meat portion of the formula can affect the final product outcome.

Mechanically separated meats are defined as paste-like or batter-like raw materials produced by forcing bones, after desirable parts and whole muscle cuts are removed, against a sieve that separates the soft meat material from the bone and cartilage. With the switch of consumer preference from whole carcass in the 1960s to cut parts and processed products in the 1980s and 1990s the market for mechanically separated chicken was developed (Petracci, Mudalal, Solglia, & Cavani, 2015). Currently, MSC is the most widely used raw material in formulations of mixed meat frankfurters and bologna and is used in many ground poultry products such as chicken nuggets and chicken patties (Paulsen & Nagy, 2014).

Whereas the mechanical separation process recovers great amounts of nutritionally valuable protein, it has been well-documented to reduce protein

functionality and quality characteristics. Addition of MSC to processed meat products impacts final color, texture, and oxidative stability (Paulsen & Nagy, 2014). During mechanical separation from bone, proteins are exposed to increased heat (5−8˚C) and pressure (>6.2 MPa), which are known to modify myofibrillar protein structure (Grossi et al., 2016; Liu et al., 2008). Independently, the high pressure and the increased temperature may not be severe enough to impact functionality; however, in combination they could be causing damage beyond communition. MSC has typically been viewed as having little functionality and therefor little attention has been paid to its production method. Processing factors have been manipulated based on yield improvements, compositional consistency, and production flow efficiency with little focus on MSC functionality. Variation in proximate composition has been extensively studied based on ingoing chicken parts but also has been shown to vary due to separation equipment (Froning, 1970; Paulsen & Nagy, 2014). However, protein functionality differences; including solubilization, and gel structure formation, between MSCs and their whole muscle counter parts are less understood. Strategic processing may be able to improve MSC functionality and allow for higher quality finished products. The first objective of the following research was to identify quality differences between MSC and chicken breast trim when used as frankfurter raw materials. The second was to evaluate the effect of two distinct types of MSC as frankfurter raw materials. The final objective was to identify and compare rheological attributes of their myofibrillar proteins during gelation to understand fundamental differences in their functional meat proteins. It is hypothesized that MSC raw materials will produce frankfurters with a softer texture, and greater

redness than breast trim raw materials as well as their myofibrillar proteins will have different rheological characteristics compared to those from chicken breast trim.

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CHAPTER 2. REVIEW OF LITERATURE

Introduction

The category of processed meats is broad, but is generally described by the addition of non-meat ingredients with a desired goal of preservation, safety or the modification of a meat cut's organoleptic properties. In all processed meat products each ingredient, meat and nonmeat, has a defined purpose and therefore a function. The term functional ingredient, however, relates directly to ingredients having purpose beyond strictly flavor enhancement. Manipulation of texture, flavor, shelf-life, and color is popular when designing a product for specific consumers. Increasing cook yields and adding moisture to a product with added starches, gelatin, or polysaccharides are a few of the many examples of functional ingredients. Non-meat ingredients are useful tools to manipulate final product attributes but, with current consumer concerns, the use of industry-wide staples such as modified food starch and sodium phosphates is being questioned. Therefore, now more than ever, it is important to understand how manipulation of the meat ingredient portion of the formula can change the final product. Added ingredients provide a level of complexity to the structure of processed meats; however, the meat proteins and how they are affected by processing also have a fundamental impact on the final eating quality and shelf-life of a processed meat product.

When designing meat products, it is very important to understand the basic structure of their final form. Most, to varying degrees, can be described as gels. Gels can be defined as networks or systems of filamentous polymer chains that interact and crosslink, to trap other more fluid components, and give the final product a solid-like

structure (Walstra, 2002). Meat systems containing high concentrations of protein polymers are strategically manipulated by heat, acid and other agents to change protein structure irreversibly. The ability of muscle proteins to first be solubilized and dispersed in the form of a batter and then set in new rigid structures allows them to trap water and fat while giving the products their bite and form. The action of a polymer dispersing in a solution, undergoing structural change, and causing the solution to behave like a solid is known as the process of gelation. According to Ashgar, Samejima, Yasui, & Henrickson (1985) "[g]elation of proteins is a phenomenon which takes place in all structured meat products during thermal processing" which is critical to creating a desirable product for the consumer.

Proteins Involved in Gelation

In meat systems, the filamentous polymer chains which interact with each other and the fluid systems around them are a class of proteins known as myofibrillar proteins. Myofibrillar proteins are important in meat gelation because of their abundance (60% of total muscle protein) and their ability to undergo gelation in the temperature ranges of meat product manufacturing (Kauffman, 2001). Stromal and sarcoplasmic proteins have little gelling potential during heating of processed meat products (Sun & Holly, 2010). Depending upon quantity and type, stromal and sarcoplasmic proteins can be both additive in gel strength by aggregating alongside the myofibrillar components or subtractive through inhibition of critical myofibrillar protein-protein interactions. Collagen when hydrolyzed can set to a firm gel however when crosslinked can have a dilution effect or interruptive effect on a porcine semimembranosus myofibrillar gel

structure. With an increase in collagen content, myofibrillar gels were reported to reduce in solid-like characteristics (Doerscher, Briggs, & Lonergan, 2004).

Myosin and actin are the major contributors to gel structure formation in muscle food systems (Sun & Holley, 2011). Myosin, which makes up approximately 43% of myofibrillar protein, has been studied extensively to understand when, how and at what temperatures it forms gel structures (Kauffman, 2001). A filamentous protein composed of 4500 amino acids with a molecular weight of 500 kDa, myosin can form a gel without a secondary gelling agent (Clark, McElhinny, Beckerle, & Gregorio, 2002). Myosin's long rope-like structure has extensive surface area to interact and form cross-bridging with itself. The resulting three-dimensional lattice structure forms a cage that traps water and fat once set through thermal denaturation. Myosin's subunits and how they participate in gelation have been thoroughly investigated. Figure 2.1 depicts a simplified schematic of a myosin heavy chain molecule. The heavy chain of myosin, in muscle, is the work horse whose rowing-like motion causes shortening of a sarcomere. In a gel system the heavy chain is also the most important. The myosin heavy chain can be split into two distinct regions. The heavy portion, known as heavy-meromyosin (HMM), contains the myosin heads (S1) and the rod segment (S2) involved in the hinging mechanism in living muscle tissue. The light-meromyosin (LMM) portion is composed of the remainder of the helical rod (Ishioroshi, Samejima, & Yasui, 1982; Samejima, Hashimoto, & Fukazawa, 1969; Samejima, Ishioroshi, & Yasui, 1981).

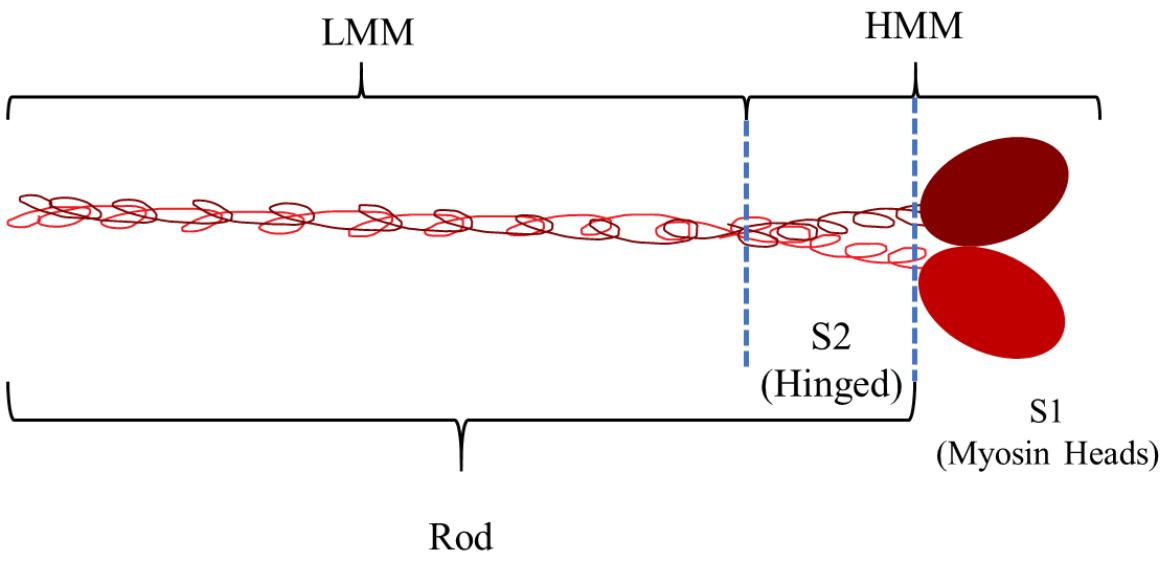

Figure 2.1 Structure of myosin heavy chain; LMM: light meromyosin, HMM: heavy meromyosin

At 22% of total myofirillar protein content, actin is the second most abundant myofibrillar protein (Kauffman, 2001). Actin is a globular protein (G-actin) that is polymerized to form F-actin and plays a supporting role in gel formation. During gel formation, the globular structure is unable to entrap fat and water through extended protein-protein interactions and tends to aggregate along the fibrous network of myosin (Samejima, Hashimoto, Yasui, & Fukazawa, 1969). Also, evidence of actin being expressed during the gelation process has been reported. Using SDS-PAGE, bands at the molecular weight of actin were identified in the expressed liquid of thermally induced gels and not found at the molecular weight characteristic of myosin (Camou & Sebranek, 1991). Such findings support the theory that it is indeed myosin that is forming the gel and actin is playing a supportive role in final gel structure. Actin has been shown to

increase gel strength in combination with myosin but is unable to form a gel on its own (Samejima, Hashimoto, Yasui, & Fukazawa, 1969).

Solubilization and Emulsion Formation

Meat batters are often described as emulsions, although they are not considered true emulsions. Emulsions can be defined as the distribution and stable suspension of two immiscible liquids (Friberg, 2004). They have a continuous phase and a dispersed phase. The continuous phase surrounds the dispersed phase and is completely connected throughout the system interrupting and isolating the dispersed phase. While this description often describes very simple systems of oil and water, a frankfurter batter indeed has many similar qualities. Fat in solid form (during batter formation) is surrounded by water with solubilized components such as salt, sugars and other flavoring components. Emulsions remain stable overtime by the use of surfactants, also known as emulsifiers. Surfactants are molecules that have hydrophobic and hydrophilic components that allow them to interact with both polar and non-polar substances. This interaction reduces surface tension or repulsion between the two immiscible phases, stabilizing the overall emulsion. Proteins are common emulsifiers, as the majority have both polar and nonpolar regions. In a frankfurter batter the muscle proteins, specifically the myofibrillar proteins, serve as system stabilizers in both batter and cooked forms. Currently debate is underway as to whether the myofibrillar proteins interact with and emulsify the fat in a batter or whether they simply surround and physically entrap fat globules. Recent evidence suggests that the structure of protein-protein interactions in raw and cooked forms physically entraps fat and moisture (Liu, Lanier, & Osborne, 2016).

Regardless of which theory of meat batter structure is correct, myofibrillar proteins are major components of the continuous phase. Once solubilized, myosin, in particular, is able to interact with itself and to surround fat globules. Solubilization of myofibrils is essential in batter formation to release myosin from the intricate and well-organized sarcomere structure. Wu & Smith (1987) found beef myofibrillar proteins to become increasingly more soluble with increased salt concentration and increased time in the salt solution. Myosin heavy chain was detected by SDS-PAGE to be soluble only at the highest salt concentration tested (0.3 M) and to have greatest solubility at 72 h. In meat processing it is commonly understood that concentrations around 0.6 M are needed to extract functional myofibrillar proteins (Xiong & Brekke, 1991) and that the quantity of solubilized myofibrils is critical to provide enough surface area to surround the fat particles in the batter. If proper coating does not occur, as temperature increases the solid fat melts and migrates, causing a cap of fat outside of the protein matrix. A complete breakdown of the emulsion structure upon heating will cause significant fat and moisture loss, as well as loss of product.

Mechanism of Gelation

The mechanism behind the shift from a fluid system to a solid-like gel in processed meat products is complex. The fundamental hypothesis focuses around the central idea that proteins denature or unravel, after which new protein-protein interactions form a stable structure. To develop this hypothesis of denaturation followed by crosslinking, gelation of muscle has been studied in simple systems as well as in complex products.

Myosin gelation on its own follows the pattern depicted in Figure 2.2 Heavy-meromyosin is the first portion of myosin to unfold, in the temperature range of 35–45 ˚C (Ishioroshi, Samejima, & Yasui, 1982). Following denaturation of the myosin heads, these begin to aggregate and form star-like patterns which have been experimentally seen in electron micrographs by Yamamoto (1990). Aggregation then occurs due to exposure, through denaturation, of hydrophobic regions on the myosin heads. From 45–55˚C the light-meromyosin portion unfolds, allowing for the rod portion of myosin to interact. Above 55˚C the final gel matrix is formed and begins to further set.

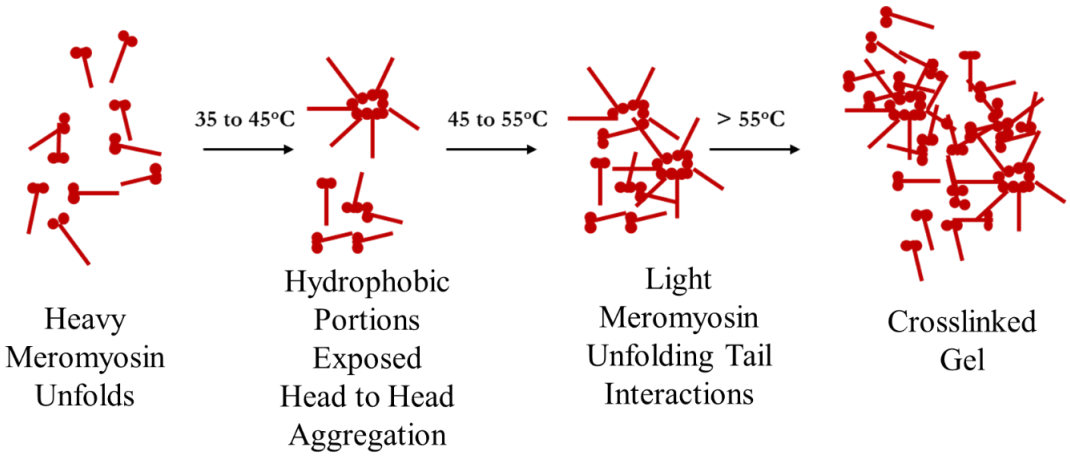

Figure 2.2 Myosin gelation (adapted from Xiong, 2007).

Secondary structure plays a major role in protein gelation. As temperature increases, myosin undergoes a change in secondary structure, from alpha helices to beta sheets with an increase in temperature (Liu et al., 2008). At low temperatures (5˚C) Liu et. al. (2008) found that over 80% of the secondary structure of myosin was alpha helical. In the 35–45˚C temperature range a drastic decrease in alpha helices is reported, decreasing from around 80% to 70% of total secondary structure. A corresponding increase in random coiling occurs as the hydrogen bonding, which forms alpha helices in

the myosin heads, is disrupted. When temperatures reach the 50–55˚C range alpha helices continue to decrease and there is a reduction of random coiling. The total secondary structure begins to shift toward beta sheets, until leveling off at 70˚C. The increase in beta sheets at 55˚C coincides with the gelation temperature of light-meromyosin observed by Ishioroshi, Samejima, and Yasui (1982). Beta sheet formation between rod portions of myosin above 55˚C fits the gelation model and demonstrates a conversion of alpha helices to beta sheets is necessary for myosin gel formation. In a processed meat system free myosin is not common; however, during thermal treatment myofibrils reflect a similar pattern to denature prior to structure formation indicating a high influence of myosin on gelation of extracted myofibrils (Xiong, 2001).

Heat, Pressure, and Oxidation

As previously discussed, heat causes irreversible changes in protein structure. Exposure to heat prior to the completion of the desired gelation process can impact later processing qualities. Specifically, the secondary structure of myosin has been shown to shift towards beta sheets at temperatures as low as 15˚C (Liu et al., 2008). At pH 7 and 5˚C, the secondary structure of myosin is over 80% alpha helices. However, at pH below 7, the alpha helices are destabilized and decrease in total content to 60% at 15˚C (Liu et al., 2008). This indicates an interaction between temperature and pH. Myosin is extremely susceptible to heat-induced changes. Cheng & Parrish (1979) found myosin heavy chain to be the first myofibrillar protein fraction to become insoluble as temperature increases. Pressure has also been shown to induce protein denaturation and even gelation, through different structural changes than those observed with heat (Lee et al., 2007). One way to measure denaturation of both myosin and actin is to measure

myosin's enzymatic activity. Based on enzyme activation assays (Ca-ATPase, Mg-ATPase), the inactivation rate of Ca-ATPase was slower than Mg-ATPase with increased pressure (100 to 500 MPa) but, was faster than that of Mg-ATPase as temperature increased from 40 to 55 ˚C (Lee et al., 2007). At just 150 MPa, Ca-ATPase activity was reduced by 50% in fish myofibrillar proteins (Ashie, Lanier, & MacDonald, 1999). High pressure has been shown to also reduce myosin solubility at pressures above 300 MPa (Lee et al., 2007). Zhang et al. (2015) showed a decreased sulfhydryl content with an increase in pressure from 100 to 500 MPa indicating high pressure can induce disulfide bond formation. Because of differences in how heat and pressure modify myofibrillar proteins, when in combination they could have an additive damaging impact on myofibrillar protein structure.

Protein oxidation is another factor (that modifies protein functionality) that the mechanical separation process has the potential to promote. Under a variety of environments, lipid and protein oxidation in fresh meat have been shown to increase simultaneously in response to exposure to oxygen over time, and to be reduced by antioxidants (Xiao, Zhang, Lee, & Ahn, 2013). The covalent modification that oxidation exerts on myofibrillar proteins (myosin being most susceptible) has been shown to impact gelation properties (Zhou et al., 2014b; Zhou et al., 2014a; Zhou, Sun, & Zhao, 2015; Vossen & De Smet, 2015). In a series of three studies, Zhou et al. (Zhou et al., 2014b; Zhou et al., 2014a; Zhou, Sun, & Zhao, 2015) reported that under moderately oxidative environments there was an increase in myofibrillar protein gel strength, including increased hardness, springiness, chewiness, and cohesiveness. At high amounts of oxidative stress, however, they reported significantly reduced chewiness, cohesiveness,

and hardness (Zhou et al., 2014b; Zhou et al., 2014a; Zhou, Sun, & Zhao, 2015). These researchers attribute the observed increase in gel stability to a moderate amount of covalent interactions, which are stronger than hydrophobic interactions. However, excessive amounts of covalent bonding will limit the formation of a broader protein network and the proteins will aggregate as clusters rather than as a fibrous network. The myofibrillar proteins of MSC have the potential to be extremely oxidized. They have increased surface area due to particle size reduction, which make them more susceptible to the effects of the oxygen already present. MSC is also known to be susceptible to accelerated lipid oxidation. The increased amount of lipid oxidation generates and exposes proteins to high concentrations of free radicals, further accelerating oxidation.

Principles of Rheology

Rheology is defined as the study of the flow of materials. Rheological experimentation is a useful tool in analyzing the physical properties of liquids, solids, and semisolids. In dynamic oscillatory rheological testing a strain is applied to a substance by a rotating head. Phase angle (δ), storage modulus (G') and loss modulus (G") are recorded and used to describe the elastic or fluid nature of the substance being tested, under controlled conditions. The basic principle of dynamic oscillatory rheological testing is the application of strain to a substance in a sinusoidal wave, and recording the responsive force provided by the substance, also known as the stress. How the substance reciprocates the energy can be calculated by the wave of stress recorded in relation to time. The rheometer detects if the stress is out of phase (a lagging response) or in phase (immediate response) with the strain applied. When a substance responds in phase, this substance is reacting like a completely elastic substance or a solid. If the substance responds exactly

90˚ out of phase compared to the strain applied it is considered completely fluid (Rao, 2014). If the phase or degree of lagged response is between 0 and 90˚ the substance is viscoelastic meaning it has both solid and fluid characteristics. Figure 2.3 shows the strain response of a solid, liquid, and viscoelastic substance.

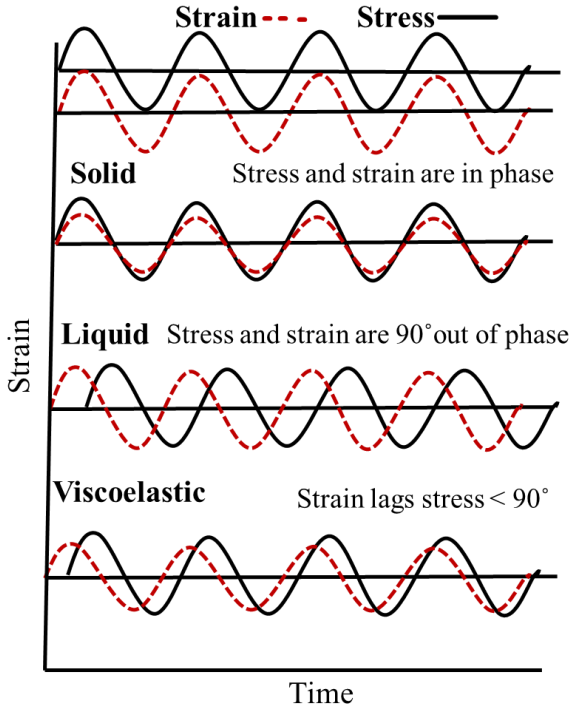

Figure 2.3 Stress versus strain response of a perfectly elastic solid, a Newtonian liquid, and a viscoelastic liquid in dynamic tests. Adapted from Rao (2014).

The frequency and amplitude of the oscillating strain applied to the substance also impact the substances' behavior. The Frequency (ω) is the length of time between the complete range of strain applied to the substance. Strain amplitude (γ_0) controls the height of the wave or how far the substance is deformed each cycle. The data produced from dynamic oscillatory rheological testing are converted into two measurements known as storage modulus (G') and loss modulus (G"). Storage modulus illustrates the elastic

response or the amount of energy stored by the substance, while the G" describes the viscous response or the amount of energy lost through flow of the substance. Both measurements are calculated by the phase angle (δ) or degree of difference between the stress and the strain. Equations 1 and 2 show the relationship between stress (σ_0), strain (γ_0), and phase angle (δ) (Rao, 2014).

Equation 1.

$$G' = \left[\frac{\sigma_0}{\gamma_0}\right] cos\delta$$

Equation 2.

$$G'' = \left[\frac{\sigma_0}{\gamma_0}\right] sin\delta$$

Prior to, performing dynamic oscillatory rheological experiments the linear viscoelastic range (LVR) of a substance must be determined (Tunick, 2011). The LVR is the range of amplitude, or effectively the strain at a set frequency, where the structure of the substance being tested is unaffected. In other words, when experimenting within the LVR of a substance, the act or force applied is non-destructive. To determine the LVR of a substance an amplitude sweep, measurement of response over a range of increasing amplitudes at a set frequency, is performed. The LVR is the range of amplitudes where response characteristics act independently of the amplitude applied. Storage modulus is particularly sensitive to the LVR and is often the response characteristic used to determine the LVR (Tunick, 2011). The amplitude, measured by percent strain at which the storage modulus begins to decrease, is the outside parameter of the LVR; all subsequent testing should be performed below this percent strain.

Another critical aspect of dynamic oscillatory rheological testing is the selection of the head and equipment measuring system. There are two types of settings used for rheological testing: controlled stress and controlled strain. Modern rheological equipment accurately measures substance response in both settings; however, controlled strain is better suited for high frequency testing. The head in contact with the sample may be one of three main geometries, each of which have specialized uses. The cone-plate geometry is the most widely used in the experimentation of food systems due to its ability to test liquid substances to semi-solids in an extremely sensitive manner. The parallel plate geometry is useful in the measurement of characteristics of semi-solids to soft solids. The flat plates tend to be hatched or grooved to prevent slipping of the substance on the plate surface. The cone-plate geometry has a uniform strain rate across the surface area of the sample but requires a fixed gap based on the truncation (height) of the cone. A parallel plate, on the other hand, provides the ability to be set at any gap but does not have an equal strain across the entire sample. The third geometry is the couette cell, which is adept to low viscosity fluids. Careful selection of head geometry based on desired sample experimentation, sample type, and amount of sample is critical for obtaining quality results.

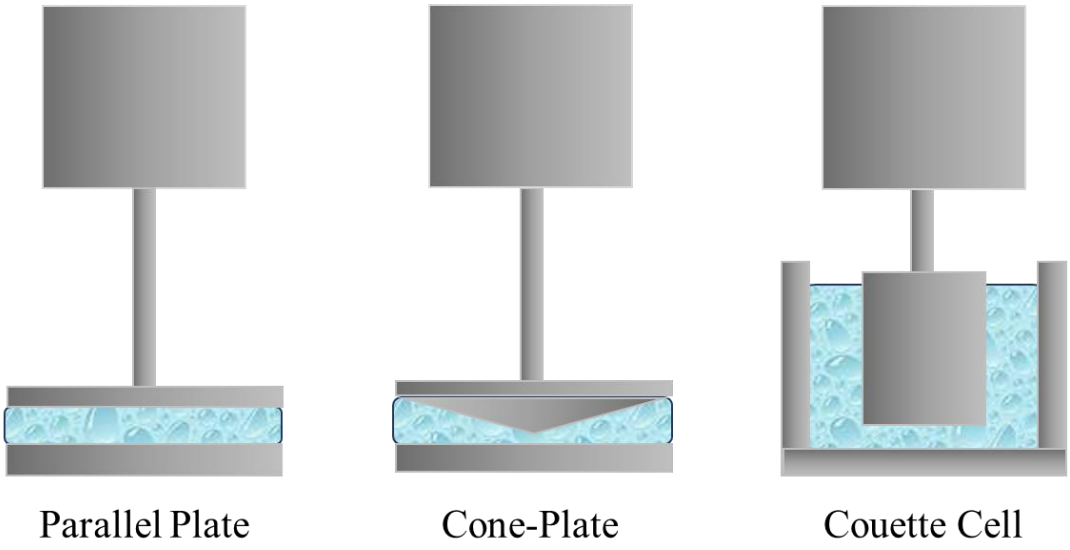

Figure 2.4 Common rheological instrument geometries

Rheology of Myofibrillar Protein Systems

Dynamic oscillatory rheology has been used extensively to study the physical behavior of myofibrillar proteins in various environments. An understanding of when structures form and dissolve during gelation has been deduced from rheological measurements taken over a span of time and temperature also known as temperature sweeps. Thermal gelation is the most applicable and widely practiced method of gelation in the meat industry. Modern rheological instruments allow for tight control and manipulation of sample temperature during analysis of the solutions structure. Effectively, a timetable of the protein solution's physical properties during gelation is developed, which allows for a greater understanding of how environmental characteristics and innate properties of a protein affect gelation.

Purified myosin as well as myofibril solutions undergo a characteristic increase, followed by a sharp decrease and subsequent increase of both storage and loss modulus

during thermal gelation. Figure 2.5 shows the rheological behavior of a solution of myofibrillar proteins extracted from chicken breast muscle and solubilized at 0.6 M NaCl. The temperature sweep from 25–75˚C shows increased G' below a temperature of 50˚C. Increased G' indicates the ability of the myofibrillar protein solutions to store more energy with an increase in temperature, producing characteristics of a solid. Following this increase, G' the decreases between 50–55˚C consistently across species, ionic strength, presence of phosphate, pH, muscle fiber type, and protein concentration (Westphalen, Briggs, & Lonergan, 2006; Westphalen, Briggs, & Lonergan, 2005; Egelandsdal, Fretheim, & Samejima, 1986; Smyth & Oneill, 1997; Xiong, 2001; Xiong & Blanchard, 1994b).

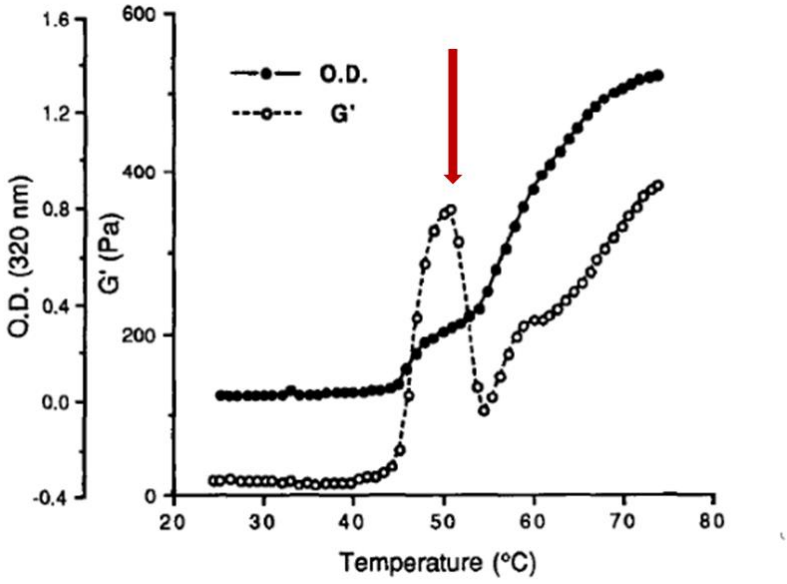

Figure 2.5 Changes in turbidity, optical density (O.D.), and storage modulus (G') during linear heating of chicken breast myofibrillar protein suspensions (20 mg/ml, 0.6 M NaCl, 50 mM sodium phosphate, pH 6.0). Adapted from (Xiong, 2001).

Transitions in gel characteristics have been explained by the denaturation temperatures of myofibrillar proteins, especially myosin. Figure 2.6. and Table 2.1. outline the denaturation temperatures of each portion of the myosin molecule determined by enzymatic cleavage and separation studied utilizing differential scanning calorimetry (Smyth, Smith, Vegawarner, & Oneill, 1996). Specifically, the LMM portion of myosin transitions at 51.6˚C and aligns well with the characteristic decline in storage modulus at this temperature. The entire rod portion shows four denaturation temperatures indicating it has impact on the micro structure of the gel across the broader range of 45–65˚C.

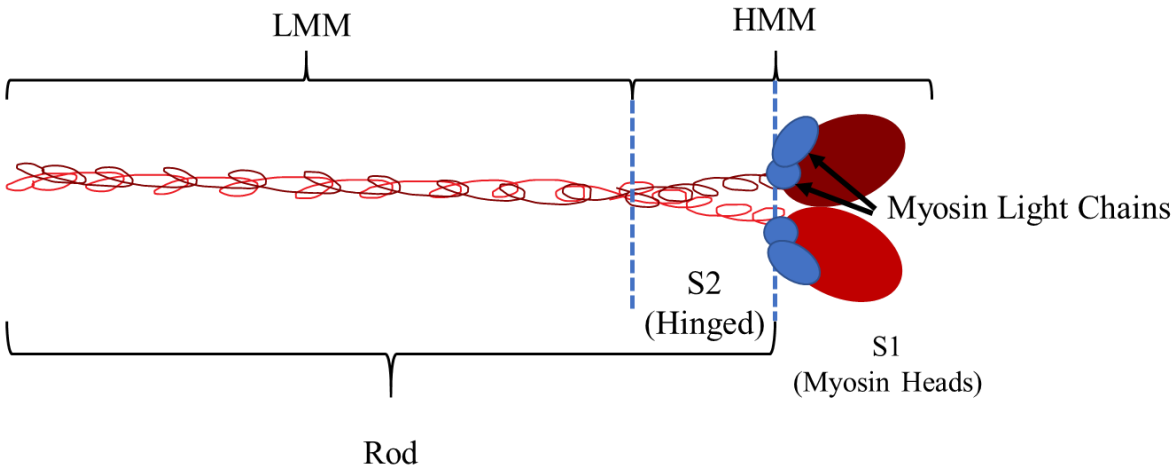

Figure 2.6 Myosin structure; LMM= light-meromyosin; HMM= heavy-meromyosin

Table 2.1 Transition temperatures (C˚) (T1, T2, T3, T4) of myosin sub-fragments (Smyth, Smith, Vegawarner, & Oneill, 1996). LMM: light-meromyosin; MLC: myosin light chain; MHC: myosin heavy chain

Myosin Fragment	T1	T2	T3	T4
S1	47.4			
S2	54.1			
Rod	44.5	50	55.7	63.3
LMM	51.6			
MLC	48.3	57.6		

Another heavily studied rheological response of myofibrillar protein solutions is phase angle. The general trend observed is a decrease in phase angle with an increase in temperature or time at temperatures above 43˚C (Xiong, 2001; Westphalen, Briggs, & Lonergan, 2006; Westphalen, Briggs, & Lonergan, 2005). The reduction in phase angle occurs as the fluid proteins in solution begin to denature, crosslink and aggregate with temperature, forming a solid. The more solid the system the lower the phase angle. Figure 2.7 shows the effect of both time and temperature on phase angle of chicken breast myofibrillar protein solutions.

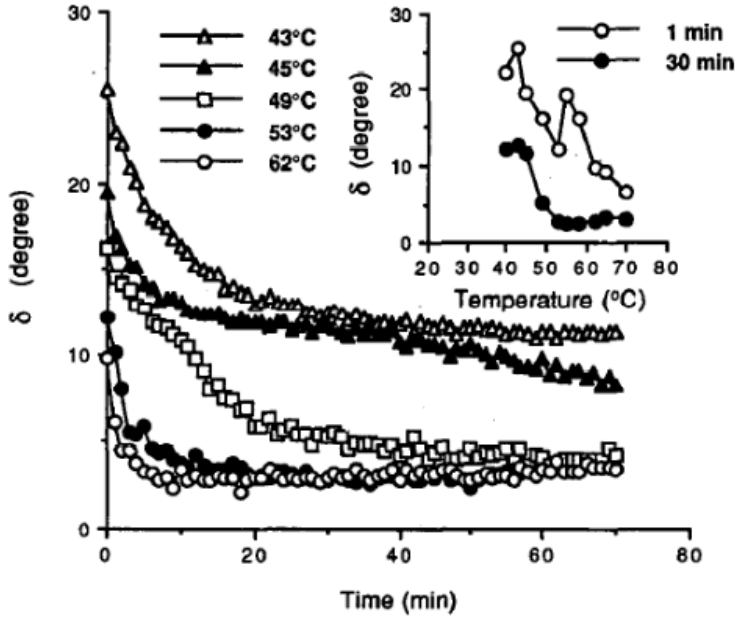

Figure 2.7 Changes in stress-strain phase angle (δ) of gelling myofibril suspensions (20 mg/ml, 0.6 M NaCl, 50 mM sodium phosphate, pH 6.0) during heating at constant (isothermal) temperatures. Insert: δ- temperature plots for samples heated for 1 and 30 minutes. Adapted from (Xiong, 2001).

Rheological Differences Due to Protein Source and Environment

Many innate characteristics of muscle proteins cause differing gel structures and paths of thermal gelation. Myofibrillar proteins from differing animal species have been shown to result in divergent rheograms. Liu et. al. (2007), evaluated silver carp dorsal myosin and porcine longissimus dorsi myosin pastes at a temperature range of 5–90˚C, and observed very different results in G', G", and δ. Fish myosin had the greatest G', (approximately 6000 Pa greater than that of the porcine myosin paste). However, both porcine and fish myosin showed a decline in G' in the 35–51˚C range. The porcine myosin G' declined to a greater extent (approximately 2000 Pa) than the fish myosin G' (approximately 500 Pa). This can be explained by differences in myosin structure and, therefore, denaturation pattern. Liu et al. (2007) also showed that fish myosin alpha-helix

denaturation rate is greatest at lower temperatures than porcine myosin, indicating fish myosin is more susceptible to thermal denaturation.

Fiber type and muscle source also have a significant effect on the rheological characteristics of myofibrillar proteins. In a study by Westphalen, Briggs, & Lonergan (2006), myofibrils from three porcine muscles were evaluated [semimembranosus (SM), longissimus dorsi (LD), and the psoas major (PM)] at pHs of 6.0 and 6.5 over a temperature range of 20–85˚C. All myofibril samples showed a decrease in storage modulus around 52˚C and an increase at 60˚C except SM and PM at a pH of 6, which plateaued in that region. These results indicate that pH within muscle, and muscle type, both influence the gelation pattern of myofibrillar proteins. The effect of pH on rheological characteristics of myofibrillar thermal gelation was also evaluated by Westphalen, Briggs, & Lonergan (2005) in a study in which porcine semimembranosus myofibrils were solubilized and adjusted to four solutions of different pH (7.0, 6.5, 6.0 and 5.6). At pH 5.6 and 6.0 G' plateaued at around 50˚C; however, at pH 7 and 6.5 there was a characteristic decrease in G' at around 50˚C. Myofibrillar proteins at a lower pH, were also found to result in a higher ultimate G' indicating pH can greatly affect gelation structure.

Muscle type has been further evaluated in chicken. Xiong & Blanchard (1994a) observed differences in rheological characteristics during thermal gelation between myofibrils isolated from the pectoralis major and minor, thigh, and drumstick at four different pH levels (5.87, 6.19, 6.38 and 6.53). All samples showed decreases in G' at 50˚C and subsequent increase to ultimate G'. However, dark meat myofibrils did have a more extended decrease, increasing only after reaching 60˚C at pH above 6. G' of

pectoralis muscles increased at temperatures below 55˚C. The magnitude of the decrease in G' was affected by pH to a greater extent in myofibrils from the thigh and drumstick compared to those from the pectoralis muscles. The thigh and drumstick myofibrils had a lower ultimate G' than the pectoralis muscles, which was attributed to reduced solubility of thigh and drumstick myofibrils (Xiong & Blanchard, 1994a).

The environment of muscle proteins during gelation directly affects the shape of the protein network formed. Temperature, ion concentration, mechanical action, added ingredients, and the proteins' physical make up, all influence the final gel structure. Ionic strength, in particular, greatly affects the rheological characteristics of myofibrillar proteins, due to their salt-soluble nature. Greater ultimate G' is observed at higher ionic strengths. Liu & Xiong (1997) found that greater concentrations of sodium chloride resulted in the greatest amount of soluble protein. They also evaluated phosphate type in the same study, and found a dynamic relationship between sodium chloride concentration, solubility and G'. At lower ionic strength conditions phosphate was able to increase solubility and cause the characteristic decrease in G' at 50˚C and subsequent increase, even though ultimate G' did not reach its peak until 0.6M NaCl. At higher salt concentrations the increased solubility due to phosphate was diminished. The effects of these treatments on storage modulus during thermal gelation are shown in Figure 2.8.

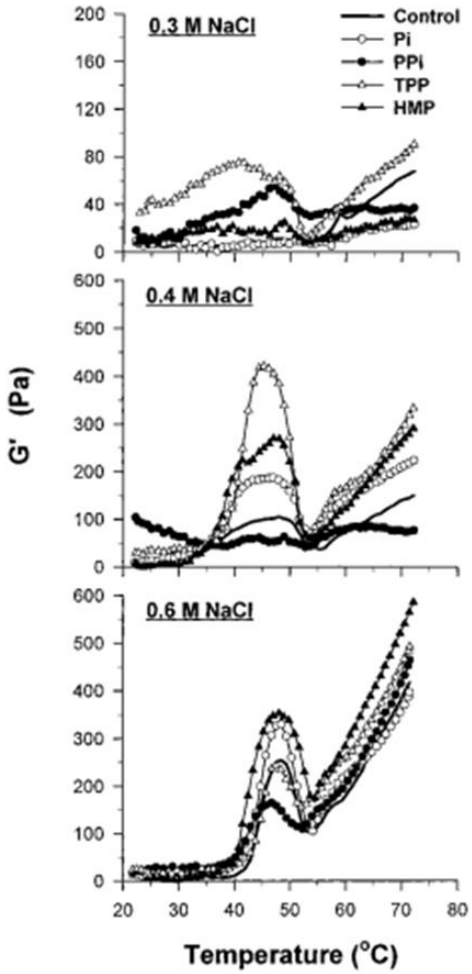

Figure 2.8 Changes in storage modulus (G') during thermal gelation of chicken breast myofibrils treated with various phosphates at different salt concentrations (ortho- (Pi), pyro- (PPi), tripoly- (TPP), or hexametaphosphate (HMP). From Liu & Xiong (1997).

The oxidation state of myofibrillar proteins has also been shown to affect gelation properties. In a study by Chen et al. (2016) porcine longissimus myofibril solutions were evaluated at three different oxidative states. Myofibril solutions that had been oxidized had a lesser decrease in G' at 50˚C compared to a control but were more similar to the control when oxidized in the presence of an antioxidant. Oxidation at low and intermediate levels resulted in an ultimate G' similar to the control. Only when oxidized for 5 h was there a major decrease in ultimate G'. Similar results were also reported by

Zhou et al. (2014b) on porcine myofibrils subjected to oxidative stress due to proxy radical generator 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) they reported a greater ultimate G' than control with low levels of oxidation (4.5 nmol/mg protein carbonyl content).

Mechanically Separated Chicken Regulations

Mechanically separated poultry is defined as "any product resulting from the mechanical separation and removal of most of the bone from attached skeletal muscle and other tissue of poultry carcasses and parts of carcasses that has a paste-like form and consistency" (USDA, 1995a). The regulations require that the species it was sourced from, such as chicken, be identified in the product name. Regarding mechanically separated poultry regulations specifically, bone particle size and calcium content are tightly regulated to guarantee a high-quality product for consumers. Bone solids in mechanically separated poultry may not exceed 1% and 98% of those bone particles must be less than 1.5 mm at their largest dimension, with no particle exceeding 2 mm (USDA, 1995a). The quality of mechanically separated poultry is also regulated by calcium content which is not allowed to exceed 0.235% (USDA, 1995a). Product exceeding the maximum allowable calcium levels or bone particle size is labeled as ‘‘Mechanically Separated Chicken for Further Processing,’’ and FSIS mandates that this product be limited to extractives such as fat, broths and stocks (USDA, 1995a).

Mechanically separated poultry can be applied in the formulation of any meat or poultry product as long as its permitted by product standards of identity, and must be labeled as "Mechanically Separated (Poultry type)" (USDA, 1995b). For many food label conscious consumers "Mechanically Separated" on the ingredient label raises concerns

for product safety and nutritional value. Present consumer preference patterns cause an unbalanced market in poultry products, in which the demand for whole breast meat continues to soar while its “by products” and dark meat have settled in the value tier market of lunch meats and frankfurters.

Processing of Mechanically Separated Chicken

The basic processing steps begin by choice of ingoing raw material. Not all MSCs are the same and the variation of processing parameters can allow for the tailoring of specific product attributes. In general, the ingoing raw material for mechanically separated chicken would be the chicken carcass once meat from the breast, legs, thighs, and wings has been removed. However, the process becomes more complicated when specific fat targets or other customer requirements must be met. By combo load, parts such as necks, backs, cages (ribcage following breast muscle removal), and when financially advantageous whole parts such as wings and legs, are blended to provide the customer’s desired final composition. Each part of the bird imparts final compositional differences. Hamm & Young (1983) performed an evaluation of mechanically deboned poultry meat from commercial plants across the country in 1983 and reported variations in fat, moisture, and protein with changes in ingoing poultry parts. They found that fat content ranged from 14.7% in MSC from broiler breast frames without skin to 22.9% fat in MSC produced from 50% broiler necks with skin and 50% broiler necks with-out skin. Once the ingoing raw material is identified, the chicken carcasses are either added to a hopper and gravitationally fed into an auger or ground prior to entry into the auger. The auger rotates and pushes the chicken parts towards a series of sieves. The pressure builds as the chicken is forced up against progressively smaller sieves. Adjustment of

mechanical rotations per minute, the auger bevel, and sieve hole diameter can be made to alter the calcium and bone content of the final product. The friction and pressure cause increases in temperature of 5–8˚C or more and therefore a cooling process immediately follows. In-process heat exchangers are commonly used to bring the final temperature of the MSC to below 4.4˚C. MSC has a very short fresh shelf-life and is further-processed as soon as possible. Due to the high mechanical action, increased iron and calcium, and elevated processing temperatures, MSC is highly susceptible to lipid and protein oxidation.

Composition and Nutritional Content of Mechanically Separated Chicken

MSC is a unique processed meat substrate, with many compositional differences when compared to other muscle protein sources. Though high in nutritious protein, with an amino acid profile comparable to that of whole muscle (Froning, 1976), it is considered substantially less functional than other raw materials derived from whole muscle poultry products (Daros, Masson, & Amico, 2005). However, the production of highly nutritious muscle protein by-products increases the financial and environmental, efficiency of the meat industry.

Babji, Froning, & Satterlee (1980) evaluated the nutritional quality of mechanically separated chicken, raw and cooked, using the standardized protein efficiency ratio (PER) in rats as well as invitro to measure the calculated protein efficiency ratio (C-PER). They found PER values and C-PER values of mechanically separated chicken in cooked and in raw forms were comparable to those of casein (PER value of 2.5). In agreement with other research, they also observed that the cooked MSC had a higher PER than raw MSC; however, there was a decrease in sulfur-containing

amino acids in cooked MSC compared to raw (Macneil, Mast, & Leach, 1978). The total amount of essential amino acids in mechanically separated chicken was found to be favorable when compared to other meat proteins (Babji, Froning, & Satterlee, 1980).

MSC varies significantly in composition depending on processing equipment used and ingoing carcass parts (Hamm & Young, 1983; Satterlee, Froning, & Janky, 1971; Froning, 1970). Fat is the most variable component and is typically adjusted to meet specific product and customer needs. However, all types of mechanically separated chicken have much higher amounts of total fat when compared to chicken breast meat and even chicken thigh meat. This difference is attributed to the chicken skin, bone marrow, and abdominal fat that are part of the ingoing poultry cages, necks, and backs (Trindade, De Felicio, & Castillo, 2004). Specific parts of the chicken carcass upon meat removal also vary in fat content. Chicken skin, in particular is extremely high in fat (56%) (Satterlee, Froning, & Janky, 1971) and is one of the few fat sources on a poultry carcass. The fat content of MSC has been found to be positively correlated with the percent chicken skin included with the in ingoing carcass parts (Satterlee, Froning, & Janky, 1971). In order to reduce fat content, lean portions of the chicken carcass can be mixed in, such as skin-less necks and backs.

Total fat content is not the only difference in composition that can occur within types of MSC as compared to whole muscle. A higher cholesterol content is also associated with mechanically separated chicken. Ang & Hamm (1982) compared hand-deboned broiler meat to mechanically separated broiler meat made from necks with skin, necks without skin, whole backs and upper backs, and found the cholesterol content of all MSC to be 14% higher (ranging from 94.2–129.1 mg/ 100 g) than the hand-deboned

meat. The cholesterol content of chicken breast meat with skin (64 mg/100 g) is substantially lower cholesterol as well (USDA, 2018). Bone marrow and spinal columns are high in cholesterol content. During the mechanical separation process the cholesterol can be expelled into the meat, explaining the greater cholesterol content of MSC. However, it is important to remember that total fat content is also a factor when comparing either deboning process with whole muscle. The fat content of both hand-deboned and mechanically-deboned chicken (ranging from 11 to 29%), is much greater than in chicken breast (< 3% fat) (Ang & Hamm, 1982).

Iron and calcium content are much greater in MSC than in whole muscle. MSC contains elevated levels of iron, which originates from hemoglobin in bone marrow released when bones are crushed (Ang & Hamm, 1982). The increased iron impacts final product quality, giving processed meat products formulated with MSC a redder and darkened appearance (Daros, Masson, & Amico, 2005). Greater iron content also affects shelf-life by increasing lipid oxidation. Another important mineral found in mechanically separated chicken is calcium. MSC contains significantly more calcium than whole muscle chicken meat. Up to 3.5 times more calcium in mechanically separated chicken than in whole muscle chicken has been reported (Ang & Hamm, 1982). These elevated calcium levels are due to the crushing and grinding of bones during the separation process, which allows for calcium to become incorporated into the finished MSC. Calcium is also the major regulatory compositional component for MSC. When levels are too high processors are required to adjust processing settings to reduce pressure and decrease bone incorporation. Other micronutrients, such as phosphorus, are also greater in MSC but not to the extent that iron and calcium are.

The connective tissue and total collagen content of MSC is highly variable. MSC has been found to be lower in connective tissue than hand-deboned meat from the same raw material (collection using a wizard knife) (Paulsen & Nagy, 2014). This reduction of connective tissue and collagen is attributed to the ability of the mechanical separating sieves to block passage of large tendons and cartilage. However, in multiple studies the opposite has been observed (Trindade, De Felicio, & Castillo, 2004). Hamm & Young (1983) demonstrated that while most collagen and connective tissue is concentrated in the bone cake, some still passes through, resulting in collagen content of MSC ranging from 1.7% to 2.53%. MSC is therefore greater in collagen content than chicken breast meat (1.3%) (Massimiliano, Samer, Elena, & Claudio, 2014) and thigh meat (1.34%) (Maurer & Baker, 1966). Many researchers have attempted to wash MSC with a washing technique similar to that used in surimi processing (made from mechanically separated fish) to reduce fat, calcium, and iron (Yang & Froning, 1992b; Sh Haji, Hoseini, Behmadi, & Pazhand, 2013; Smyth & Oneill, 1997; Cortez-Vega, Fonseca, & Prentice 2015). However, collagen content was found to be higher in washed MSC than in mechanically separated fish, 21 mg/g and 9.5 mg/g respectively (Yang & Froning, 1992a). Once the fat is washed away, the connective tissue and myofibrillar proteins are both concentrated, making MSC surimi less functional and desirable than surimi produced from seafood.

Functionalized Mechanically Separated Chicken (FMSC)

Mechanically separated chicken is inexpensive and widely utilized in the processed meat industry. Many processes have been attempted to functionalize MSC, including a washing surimi-like process, alkaline and acid extraction methods, and pH

shifting. Surimi is traditionally produced from mechanically separated or “minced” fish. Intensive washing and dewatering are used to create a concentrated myofibrillar protein product yielding high gel functionality with little color and flavor (Park, Graves, Draves, & Yongsawatdigul, 2013). A pseudo surimi process has been recently applied to other mechanically separated meats to produce a highly nutritious product with improved gelation abilities from an inexpensive by-product.

Smyth & Oneill (1997) evaluated the gelation properties of mechanically separated chicken meat after washing with a sodium bicarbonate solution at varying pH, temperature, heating rate, protein and sodium tripolyphosphate (TPP) concentrations. During the washing procedure, they removed fat and connective tissue in the two-phase washing at pH 8.5 and 7.5 in the first and second step, respectively. A 0.5% solution of sodium bicarbonate was used during the washing and sieving process, and then the washed MSC fractions were suspended and washed with a 0.2% NaCl solution. The mechanically separated chicken surimi (MSCS) was then solubilized in a 2.4 M NaCl, 0.2M sodium phosphate buffer and stored overnight. Utilizing this production process, the MSCS had a significantly lower fat, protein, and collagen content (Smyth & Oneill, 1997). The washing process removed soluble calcium and bone fragments as well. Surimi is essentially a semi-purified myofibrillar extraction. When analyzed using SDS-PAGE the bands at the myosin heavy chain and actin were more concentrated in the MSCS than in the MSC. These differences in composition translated to significant differences in the rheological properties of the MSC and MSCS gels. Measured using compression rheology, MSCS produced a significantly higher gel strength than MSC at 8% (w/w) protein, pH 6.2, 2.5% (w/w) NaCl heated to 80 C° at 1.75 C°/min (Smyth & Oneill,

1997). These results support the theory of composition having a significant impact on functional and therefore rheological characteristics of mechanically separated chicken.

Concentration of the functional portion of MSC is just one technique attempted to increase the functionality of MSC. Researchers have also looked at the effects of pH manipulation on mechanically separated meat proteins. Hrynets, Omana, Xu, & Betti (2010) evaluated the effects of extracting proteins from mechanically separated turkey (MST) at 4 different pH levels (2.5, 3.5, 10.5 and 11.5). The idea of pH shifting to both high and low pH has been identified as a method to manipulate protein structure. MST was solubilized at the designated pH and then centrifuged. Soluble protein portions were separated and brought back to their isoelectric point and centrifuged. All functionality tests were performed on the pellet. With both alkali and acid extractions, cook loss was decreased compared to raw mechanically separated turkey. Extraction at pH 11.5 resulted in the highest emulsion stability. However, proteins extracted at pH 3.5 resulted in greater storage modulus. Hrynets, Omana, Xu, & Betti (2010) highlight the impact of pH on the resulting structural properties of proteins. The ability to manipulate functionality by adjusting pH has been well documented in other less functional raw materials, such as meat recovered from spent hens (Wang, Wu, & Betti, 2013).

Mechanically Separated Meat in Processed Meats

Mechanically separated meats, particularly chicken, are widely used in processed meat products. MSC's low cost and high protein content make it ideal when formulating value tier products. In the United States, annual production of mechanically separated poultry exceeds 318,000,000 kg, 182,000,000 kg of which is used in frankfurters and bologna and 136,000,000 kg in used in chicken patties, nuggets, and poultry rolls

(Paulsen & Nagy, 2014). Mechanically separated pork is used in sausages and, when separated from only the femur, can be used in the binder portion of composite hams since it originates from the hind leg of the pork carcass. Beef produced using an advanced meat recovery system (AMR) is mainly used in pizza toppings, taco meat, and restructured jerky (Paulsen & Nagy, 2014). The economic and environmental advantages of mechanically separated meats make them very popular and have contributed to significant cost reductions in processed meat products.

Quality characteristics affected by the addition of mechanically separated meat, especially poultry, are color, texture, and oxidative stability (Paulsen & Nagy, 2014). Mechanically separated poultry (MSP) causes an increase in lipid oxidation in all processed meat products due to the increase in heat during the separation process, disruption of muscle cell structure, exposure to oxygen, and higher levels of polyunsaturated fatty acids in poultry fat in comparison to pork and beef fat (Baker & Kline, 1984; Paulsen & Nagy, 2014; Mielnik et al., 2002; Olsen et al., 2005). In addition, the pro-oxidant heme iron is found in a much greater quantity in MSP than in whole muscle and ground meat due to bone marrow extraction during the mechanical separation process (Lee et al., 1975). Rapid freezing methods and in line heat exchangers, however, rapidly reduce the temperature of MSP, causing lipid oxidation to be reduced and limiting its effects on final product quality (Barbut, Kakuda, & Chan, 1990).

The specific poultry parts used in the separation process have been shown to affect the texture of the final processed meat product. Baker & Kline (1984) reported that frankfurters produced from poultry neck MSP were “mushier” than those made from mechanically separated backs alone and in combination with necks, as measured by

sensory evaluation. When added to products with other meat blocks such as pork sausages MSC had a negative impact on overall texture. Specifically, tension strength was significantly reduced at application levels above 40% MSC and compressive strength was significantly reduced at levels above 5% MSC (Daros, Masson, & Amico, 2005). Sodium chloride also has a substantial impact on the textural and functional properties of frankfurters produced with mechanically separated poultry. Horita et al. (2014) observed frankfurters produced with half the sodium chloride of the control were significantly harder; an effect that they attributed to emulsion instability (loss of moisture and fat) in the reduced salt treatment.

Finished product color is also highly affected by the addition of mechanically separated meats. During the extraction process hemoglobin from the bone marrow becomes incorporated into the MSP and causes noticeable reddening. This darker and redder color carries over into finished products. With the current consumer preference for white meat this color difference is most often considered undesirable. Heme iron concentration has been reported to be three times greater in MSC than in hand-deboned meat (Froning & Johnson, 1973). The color of mechanically separated meats has been shown to also be dependent on species, processing, and poultry part, with greatest impact being due to species (Mielnik et al., 2002). Mielnik et al. (2002) reported mechanically separated turkey sausages as being darker, redder, and less yellow than MSC sausages.

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CHAPTER 3. EVALUATION OF MECHANICALLY SEPARATED CHICKEN AND CHICKEN BREAST TRIM AS FRANKFURTER RAW MATERIALS

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Abstract

Mechanically separated chicken (MSC) has been utilized to reduce the cost of processed meat products particularly as the lean component in emulsified meat formulations. However, its addition modifies the texture, flavor, and color of processed meat products. Mechanically separated chicken processing methods vary greatly in the industry but little is known about how these differences in affect the functionality of the MSC. In this study MSC raw material was obtained from two different separation methods (MSC1, Beehive separator, aged bones; MSC2, Poss separator, fresh bones) and compared to chicken breast trim (BT). Frankfurters were manufactured from each chicken raw material along with pork fat. Three replications were produced and analyzed for texture, color and lipid oxidation over a 98d shelf life. All raw materials were significantly different in moisture and fat content (P<0.05). Both MSC raw materials contained greater fat and less moisture than BT. Frankfurters produced with MSC2 were greatest in fat and lowest in moisture content (P<0.05). Both MSC frankfurters had

darker (L*), and redder (a*) external and internal color than BT frankfurters with MSC2 being the darkest and reddest treatment ($P<0.05$). MSC1 was significantly greater ($P<0.05$) in lipid oxidation than BT and MSC2 throughout the 98-d shelf life. Greater hardness, cohesiveness, resilience and, chewiness values were greater in MSC2 frankfurters than in BT and MSC1 frankfurters. The data show that properties of different MSC can result in significant variation in finished product quality, underscoring the importance of understanding the properties of raw materials that affect processing functionality.

Introduction

Producers of further processed meat products have been utilizing rapidly diversifying methods to reduce total product cost; including the manipulation and addition of non-meat ingredients and the addition of lower cost meat ingredients. Non-meat ingredients are useful tools to manipulate final product attributes however, with recent added consumer concern, industry wide staples such as modified food starch and sodium phosphates are being removed from processed meat products. These commonly used nonmeat ingredients provide water binding, protein extraction, and an increase in pH, all functions which improve overall stability and yields of processed meats. The absence of useful nonmeat ingredients presents a problem for processors to be able to produce quality products and maintain efficiency. Therefore, it is important to understand how to manipulate meat ingredients to reduce cost but retain product quality.

Mechanically separated meats are defined as paste-like or batter-like raw materials produced by forcing bones, after the whole muscles are removed, against a sieve that separates the soft meat material. This unique protein-rich by-product is a major

dietary source of protein around the world. Specifically, MSC consumption has increased significantly since the 1970's due to increased consumption of poultry parts and a steady increase in total chicken consumption (Petracci, Mudalal, Solglia, & Cavani, 2015). Currently, MSC is the most widely-used raw material in the formulations of mixed meat frankfurters and bologna and is used in many ground poultry products such as chicken nuggets and chicken patties. Mechanically separated poultry production exceeds 318,000,000 kg annually, 182,000,000 kg of which is used in frankfurters and bologna and 136,000,000 in chicken patties, nuggets, and poultry rolls annually in the United States (Paulsen & Nagy, 2014).

While the mechanical separation process produces high amounts of nutritionally valuable protein, it has been well-documented to reduce protein functionality and quality characteristics of finished products. Addition of MSC to processed meat products impacts final product color, texture, and oxidative stability (Paulsen & Nagy, 2014). It is well known that MSC has a negative impact on eating quality of processed products by modifying texture, introducing grittiness, increasing off-flavors, and increasing redness (Horita et al., 2014; Froning & Johnson, 1973; Daros, Masson, & Amico, 2005; Paulsen & Nagy, 2014). In one study, tension strength was significantly reduced when used at levels above 40% MSC and at levels above 5%, MSC significantly reduced comprehensive strength in sausages (Daros, Masson, & Amico, 2005). Production of MSC is diverse, with the variation in processing methods and ingoing raw material there is potential for differences in raw material performance. Little modern literature has looked at the quality of differing MSC types and compared them to whole muscle chicken. In this study two very different processing methods used to produce MSC

(MSC1 Beehive separator, aged bones; MSC2 Poss separator, fresh bones) were chosen to compare to a whole muscle chicken breast meat raw material. This study aimed to determine quality differences between MSC and chicken breast trim when used as frankfurter raw materials and to evaluate the effect of two distinct types of MSC as frankfurter raw materials. We hypothesized the two MSC raw materials would behave differently in a frankfurter system. Due to freshness of bones and reduced processing speed, we also hypothesize the MSC2 raw material would behave more similarly to chicken breast trim.

Materials and Methods

Raw Materials

All chicken raw materials were sourced from commercial facilities. Two types of mechanically separated chicken (MSC1 and MSC2), processed under different processing conditions were used and compared to chicken breast trim (BT). MSC1 was prepared using broiler frames produced 3–5 d following breast meat removal on a Beehive S88 mechanical separator (Provisur Technologies, Mokena, IL, USA) with sieve sizes of 1.5, 9.9, and 7.4 mm. MSC2 was produced from frames of broiler carcasses separated immediately following breast meat removal by a Poss separator (Poss Design Limited, Oakville, ON, Canada). The composition of MSC can be variable due to variability of in-going raw material; therefore, to obtain a representative understanding of its properties, three lots produced on three consecutive days of mechanical separation were sampled. However, to limit within-block variation in frankfurter formulations pooled samples were used for frankfurter manufacturing. Chicken breast trim was obtained from commercial broilers and sourced from one production lot to limit variation in poultry fat content in

the frankfurter formulations. Three boxes of breast trim from the same lot were randomly assigned to each frankfurter processing day. All poultry raw materials were packaged in 18.2-kg boxes and blast frozen at -44.4˚C for 72 h and held for 19, 18, and 17 d at -17.7˚C to -23.3˚C. Boxes were then packed in dry ice and shipped overnight to the Iowa State University Meat Laboratory, Ames, IA. Upon arrival, poultry raw materials were stored at -20˚C. Pork back fat was sourced from the Iowa State University Meat Laboratory and frozen at -20˚C until used. All raw materials were thawed at 0˚C for 3 d and then at 4˚C for 2 d before processing.

Frankfurter Manufacture

The frankfurter formulations used were comparable to commercial products containing chicken (Table 3.1). Batch sizes were 16.22 kg (BT), 14.32 kg (MSC1), and 12.27 kg (MSC2). The chicken breast trim and pork fat were ground to 12.7 mm, using a grinder (The Biro Manufacturing Co., Marblehead, OH, USA). Lean meat was added to a bowl chopper (KILIA-Fleischerei-und Spezial Maschinen-Fabrik GmbH, Neumünster, Germany) along with half of the ice and water, salt, corn syrup solids, seasoning, and sodium nitrite. Batters were chopped to 8.3˚C, after which the fat and remainder of water and ice was added. Chopping was continued under vacuum to 12.7˚C. Batters were then stuffed into 25-mm cellulose casings (Viscofan, Danville, IL, USA) using an automatic linker and vacuum stuffer (Handtmann VF 608 Plus, Lake Forest, IL, USA). Frankfurter links were then hung on smoke truck sticks (approximately 3 per treatment) and weighed. Sticks from the same treatment were spread out on the smoke-truck to account for oven variation. All treatments were cooked on the same smoke truck in a single truck Alkar oven (DEC International, Inc., Lodi, WI, USA) following the smoke cycle shown in

Table 3.2 Frankfurters were smoked using hickory smoke generated from hickory chips (Chips n' Chunks Hickory All-Natural Wood Chips, Smokehouse, OR, USA) pyrolyzed by a smoke generator (Alkar Smokemaster, DEC International Inc., Lodi, WI, USA). Internal temperatures were monitored with temperature probes throughout the cook cycle. Frankfurters were transferred to a -1˚ cooler for approximately 18 h until packaging. Prior to packaging, the frankfurters were weighed and casings were removed using an automatic frank peeler (Townsend 2600, Townsend Engineering, Des Moines, IA, USA). Frankfurters were then packaged by placing 4 frankfurters inside 10.16 cm x 25.4 cm plastic bags (oxygen transmission rate of 3–6 cm^3 per m^2 per 24 h at 23°C, 0% RH; Cryovac Sealed Air Corp., Duncan, SC, USA) and vacuum-sealed (Ultravac UV 2100, UltraSource LLC, Kansas City, MO, USA). Packages were shrink-wrapped by dipping for 2s in a hot water bath at 195˚C, placed in cardboard boxes, and stored at 1.1 °C for the duration of the study.

Table 3.1 Frankfurter formulations

	Chicken Breast Trim (%)	MSC1 (%)	MSC2 (%)
Chicken breast trim	47.30	-	-
MSC1	-	69.46	-
MSC2	-	-	79.78
Pork backfat	22.59	9.72	12.64
Salt	1.48	1.46	1.48
Corn syrup solids	3.52	3.71	3.70
Modern Cure (6.25% $NaNO_2$)	0.17	0.16	0.18
Dextrose	0.76	0.76	0.78
Sodium tripolyphosphate	0.39	0.41	0.41
Sodium erythorbate	0.03	0.03	0.04
Spices (black pepper, coriander, garlic powder, red pepper,)	0.53	0.48	1.04
Water	23.24	13.84	0.00

Table 3.2 Thermal processing cycle

	Step Time (min)	Dry Bulb Temperature (˚C)	Wet Bulb Temperature (˚C)	Relative Humidity (%)	Exhaust Fan
Cook	10	43.3	40.5	84	Off
Cook	20	54.4	0	0	On
Smoke	15	54.4	0	0	Off
Smoke	30	62.8	57.2	75	Off
Cook	30	68.3	0	0	On
Cook	15	74.0	62.8	59	On
Steam Cook	10	79.4	79.4	100	On
Cold Shower	30	10	0	0	On

Light Display Storage

Following packaging frankfurters were stored under fluorescent display lights (2300 lux) at 1.1˚C. They were randomly placed under four separate light sources which were positioned 30.5 cm from the surface of the frankfurters. Packages were moved weekly to minimize the effect of light storage locations.

Texture Profile Analysis

Texture profile analysis (TPA) was performed using a TA-XT2i Texture Analyser (Texture Technologies Inc., Scarsdale, NY, USA) on days 0, 14, 28, 42, 56, 70, 84, and 98 post packaging. One randomly selected package of frankfurters from each treatment group was analyzed each day. Frankfurters were equilibrated to room temperature for at least 5 h prior to texture analysis. A 2.54-cm long section was cut from the center of each frankfurter, placed on a flat end and compressed twice to 50% of its original height with a 5.08 cm (diameter) x 20 mm (height) aluminum probe (Texture Technologies Inc., Scarsdale, NY, USA). Compression speed was set to 5mm/s. The TPA parameters

measured were firmness, cohesiveness, chewiness, springiness, and resilience. Three measurements from each package were averaged.

Color

Color was evaluated at days 0, 14, 28, 42, 56, 70, 84, and 98 post packaging. One package of frankfurters was randomly selected from each treatment group for each day of analysis. From that package three frankfurters were imaged. Both internal and external color (CIE L*a*b*) were measured by a LabScan XE colorimeter (Model LS 1500, Hunter Associated Laboratories, Inc., Reston, VA, USA) using illuminant D65 (daylight at 6500K) and a 10° observer angle. For internal color frankfurters were sliced in half lengthwise and two measurements taken in the center with an aperture size of 6.35 mm diameter. External color was measured in two different locations on each frankfurter's light-exposed surface using a 3.3 mm aperture setting, avoiding abnormal spots and ensuring the entire aperture was covered. Measurements from the same package were averaged.

Lipid Oxidation

Lipid oxidation was measured on days 0, 14, 28, 42, 56, 70, 84, and 98 post packaging using a modified 2-thiobarbituric acid (TBA) method for meat products containing sodium nitrite (Zipser & Watts, 1962). Three frankfurters from one package were homogenized in a food processor (KFP715WH2, KitchenAid, St. Joseph, MI, USA). Ten g of the homogenized frankfurter sample were mixed with 1 mL of HCL (1:2 concentrated HCl: H_2O), 2 mL of sulfanilamide reagent (2.9×10^{-2} M sulfanilamide dissolved in a 1:4 HCl: H_2O solution) and 97 mL of distilled water. The mixture was heated on a burner and 50 mL of distillate were collected. Five mL of the TBA reagent

(2.0×10^{-2} M 2-TBA in distilled water) were mixed with 5 mL of the sample distillate. Samples were then boiled for 35 min and placed in a cold-water bath to chill for at least 10 min. Absorbance was measured at 532 nm using a spectrophotometer (Model 4320940, DU 640, Beckman Instruments, Inc., Fullerton, CA, USA). Absorbance values were converted to mg of malonaldehyde per 1000 g of sample using the following equation:

Equation 3.

$$\text{mg of malonaldehyde per 1000 g of sample} = (\text{Absorbance})_{532} \times 7.8$$

Analyses were performed in duplicate and results were averaged.

pH

Initial pH was measured on each raw material. Raw materials were first ground using a food processor (KFP715WH2, KitchenAid, St. Joseph, Michigan, USA) after which 10 g of each were diluted with 90 mL of distilled, deionized water. The mixture was mixed with a glass stirring rod for 30 s, after which a cone made of 11-µm–filter paper (Whatman Grade 1, GE Healthcare Life Sciences, Pittsburgh, PA, USA) was submerged in the beaker. The pH of the filtrate was measured using a Mettler Toledo SevenMulti pH meter (Columbus, OH, USA) with an InLab Solids Pro-ISM electrode. Each raw material pH was measured in duplicate.

Proximate Composition Analysis

Proximate composition was determined on all raw materials, frankfurter batters, and finished products to verify product formulation and analyze raw material differences. Samples were first homogenized using a food processor (KFP715WH2, KitchenAid, St. Joseph, Michigan, USA).

Protein content was determined using the CEM Sprint Rapid Protein Analyzer (CEM Corporation Matthews, North Carolina, USA) (AOAC, 2005a; Moser & Herman, 2011), which is based on the binding of Crocein Orange dye to cationic groups of the basic amino acid residues (histidine, arginine, and lysine) followed by absorbance measurement. Protein content was measured in duplicate and averaged.

Moisture content was determined by the CEM SMART 6 system (CEM Corporation Matthews, North Carolina, USA) (AOAC, 2005b) and fat content was measured using the CEM ORACLE system (CEM Corporation Matthews, North Carolina, USA) (AOAC, 2005b ; Leffler et al., 2008). Both moisture and fat content were recorded in duplicate and averaged.

Hydroxyproline

All poultry raw materials were analyzed for hydroxyproline content by Nestle Purina Analytical Laboratories (St. Louis, MO, USA). Samples were frozen and shipped overnight to their facility. The sample was first mixed with 6 N hydrochloric acid in a modified Kjeldahl flask. Oxygen was then removed from the sample by pulling a vacuum and repeated freezing and thawing. The flask was then heat-sealed and placed in a 110˚C oven for 24 h to hydrolyze the protein. The flasks were then cooled, mixed with an internal standard and adjusted to a pH of 2.2. Samples then were run on a sodium cation exchange column and separated by pH gradient elution with a temperature gradient of 53˚C to 90˚C. The separated amino acids were subsequently reacted with ninhydrin, and measured spectrophotometrically. Fractions were then injected into a Biochrom amino acid analyzer (Cambridge, UK). The concentration of hydroxyproline in the sample was compared to a standard solution of known concentration, which was injected into the

amino acid analyzer under the same conditions (Zarkadas, 1992; Lin, 1982; Lee & others, 1978).

Calcium and Iron

All poultry raw materials were analyzed for calcium and iron content by Nestle Purina Analytical Laboratories (St. Louis, MO, USA). Samples were frozen and shipped overnight to their facility. Ten g of each sample were ashed in a muffle furnace to remove organic material and then dissolved in acid and an ionization suppressant. Atomic absorption spectroscopy was used and sample spectrums were compared to standards of iron and calcium to determine concentration (Williams, 1972; Elwell, 1971; AOAC, 2005c; AOAC, 2005d; AOAC, 2005e; AOAC, 2005f).

Frankfurter Batter Stability

Frankfurter batter stability was tested on all treatments on the same day of manufacture following the method of Rongey (Rongey, 1965). Approximately 25 g of batter was inserted into Wierbicki tubes (Wierbicki, Cahill, & Deatherage, 1957). Samples were measured in duplicate and percent batter stability was measured using the following equation:

Equation 4.

$$\% \text{ Batter Stability} = [1 - \text{purge loss/sample weight}] * 100.$$

The larger this percentage is, the greater the batter's stability.

Experimental Design and Statistical Analysis

The experiment was replicated three times, with each replication corresponding to a separate frankfurter manufacturing day. All data were analyzed with SAS 9.4 mixed procedure (SAS Institute Inc., Cary, NC, USA). For single time point measurements, the

fixed effect was treatment (MSC1, MSC2, BT) and the random effect was replication. For the multiple timepoint measurements, fixed effects were treatment, day and treatment*day, and the random effect was replication. The multiple time point measurements were also corrected with a Tukey's adjustment and an autoregressive order 1 covariate. Significance was determined at *P*-value of < 0.05.

Results and Discussion

Raw Material, Frankfurter Batter, and Frankfurter Composition

It is well documented that MSC composition can vary and is different from whole muscle chicken (Hamm & Young, 1983; Ang & Hamm, 1982; Satterlee, Froning, & Janky, 1971; Paulsen & Nagy, 2014). Table 3.3 demonstrates the wide variation in moisture, fat and, protein between each type of raw material.

Table 3.3 Composition of chicken raw materials.

	Raw Composition						
Treatment	Moisture %	Fat %	Protein %	pH	Hydroxyproline g/100g	Calcium %	Iron ppm
BT	74.41^{a}	2.40^{c}	23.48^{a}	5.88^{c}	0.08^{c}	0.01^{c}	5.75^{c}
MSC1	68.35^{c}	16.17^{a}	14.40^{b}	6.82^{a}	0.21^{a}	0.25^{a}	16.57^{b}
MSC2	71.00^{b}	14.83^{b}	14.00^{b}	6.70^{b}	0.14^{b}	0.09^{b}	18.67^{a}
SEM	0.34	0.16	0.14	<0.01	0.01	0.02	0.59

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)

Chicken raw material source had a significant effect on moisture ($P < 0.05$) (BT>MSC2>MSC1). BT was also significantly greater ($P < 0.05$) in protein than both MSC raw materials and significantly lesser ($P < 0.05$) in fat content. Both MSC types had typical compositions compared to literature and commercial data (Ang & Hamm, 1982; Perlo et al., 2006). The composition of the chicken breast trim raw material was very similar to the composition of chicken breast meat reported in recent literature (Soglia et al., 2016; Li et al., 2015). MSC1 was significantly greater ($P < 0.05$) in fat content than

MSC2. The increased moisture and decreased fat content of MSC2 could be attributed to processing differences as MSC2 is directly separated following leg, wing, thigh and breast removal, whereas MSC1 bones were held 3–5 d prior to separation, which could have resulted in loss of moisture. Also, MSC1 had significantly more ($P < 0.05$) fat but similar protein, causing the raw material to be lesser in moisture content.

The pH between all raw materials was significantly different ($P < 0.05$), MSC1>MSC2>BT, as shown in Table 3.3 The pH of chicken breast trim was comparable to that of normal chicken breast reported in recent literature (Li et al., 2015) (BT=5.88). MSC1 had a similar pH to that reported by Rivera, Sebranek, Rust, & Tabatabai (2000). However, MSC2 had a slightly lesser pH than MSC1. Hydroxyproline, calcium, and iron contents were also different among the raw materials (Table 3.3). BT contained the least amount of hydroxyproline, as expected due to low amounts of connective tissue in typical chicken breast meat. MSC1 had a significantly greater ($P<0.05$) hydroxyproline content than MSC2. Calcium is an indicator of bone content used in the literature as well as for regulation of MSC product quality and identity. BT contained the least amount of calcium (0.01% ± 0.02) as expected. Calcium content between MSCs was also significantly different ($P < 0.05$), with MSC1 containing more than twice as much (0.25%±0.02) as MSC2 (0.09%±0.02), indicating a greater amount of bone incorporation. Iron content was approximately 3 times greater in both MSCs than in BT which is consistent with literature reports (Froning & Johnson, 1973). Interestingly, the iron content of MSC2 was significantly greater ($P < 0.05$) than that of MSC1. MSC1 was separated by equipment designed for higher speeds than the equipment used in the separation of MSC2. The increased crushing force exerted on the MSC1 raw material

would explain the greater bone content and hydroxyproline content found in MSC1, but it is counter intuitive that MSC1 would have less iron than MSC2.

Sampling of raw materials prior to formulation allowed for targeted batter moisture, fat, and protein. However, upon analysis there were significant differences in frankfurter batter composition between treatments, as shown in Table 3.

Table 3.4 Batter stability, batter proximate, cooked proximate, yield, and cooked pH.

	Batter Stability		Batter Composition			Cooked Composition				
Treatment	Water Loss %	Fat Loss %	Moisture %	Fat %	Protein %	Moisture %	Fat %	Protein %	Yield %	pH
BT	5.92^{ab}	0.45^{a}	61.77^{b}	21.11^{a}	11.52^{a}	57.23^{b}	23.98^{b}	12.48^{a}	87.12^{b}	6.25^{a}
MSC1	9.37^{a}	0.89^{a}	62.91^{a}	20.06^{b}	10.17^{c}	58.28^{a}	22.77^{c}	11.24^{c}	87.26^{b}	6.69^{b}
MSC2	3.79^{b}	0.30^{a}	61.00^{c}	21.27^{a}	11.11^{b}	55.36^{c}	25.40^{a}	11.81^{b}	87.79^{a}	6.59^{c}
SEM	1.02	0.44	0.24	0.30	0.13	0.26	0.26	0.12	0.38	0.02

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)

The significant differences in frankfurter batter formulations are due to the very similar compositions between replications but, drastic compositional differences between raw materials as discussed above. Although significantly different, batter compositions did not differ in content of any component by greater than 2.0%. Final frankfurter composition was significantly different ($P < 0.05$) between all treatments in moisture (MSC1>BT>MSC2), fat (MSC2>BT>MSC1) and, protein (BT>MSC2>MSC1) as shown in Table

3.4. Some final product compositional differences can be explained by the raw material and batter compositional differences including BT having the greatest protein content. Other differences are explained strictly by batter composition, both cooked frankfurter and frankfurter batter containing increased fat content in MSC2 treatment and increased moisture content in the MSC1 treatment.

Batter Stability and Cook/Chill Yields

Batter stability results are shown in Table 3.4. No significant differences ($P < 0.05$) were found between treatments in percent fat separation. Moisture separation was significantly greater ($P < 0.05$) in the MSC1 treatment than in MSC2 and was intermediate in the BT treatment, which was not significantly different ($P > 0.05$) from either MSC treatment. Total combined cook and chill yield was significantly higher ($P < 0.05$) in MSC2 than in BT and MSC1.

Texture Profile Analysis

Hardness, adhesiveness, resilience, cohesiveness, chewiness, and springiness were calculated from a two-bite compression test and all attributes were found to be significantly affected ($P<0.05$) by treatment, as shown in in Table 3.5.

Table 3.5 Means for effect of treatment on instrumental texture values of frankfurters.

	Hardness N	Resilience %	Cohesiveness %	Chewiness	Springiness %
BT	46.02[b]	36.66[c]	0.69[b]	3077.02	95.40[c]
MSC1	44.15[b]	38.45[b]	0.67[c]	2950.69	97.98[a]
MSC2	54.82 [a]	41.50[a]	0.72[a]	3871.98[a]	96.68[b]
SEM	1.68	0.95	<0.01	135.18	0.31

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$)

Hardness is the measurement of the peak force during the first TPA compression. MSC2 frankfurters were significantly harder ($P < 0.05$) than both MSC1 and BT

frankfurters which were not significantly different (P >0.05) from each other. These results are in contrast with previous literature which shows a decrease in compressive strength with the addition of MSC (Massingue et al., 2018; Daros, Masson, & Amico, 2005). However, in those studies meat from red meat species was replaced with MSC, which would cause a species effect; poultry products are known to produce a softer texture. A study by Meullenet, Chang, & Carpenter (1994) evaluated collagen fiber addition and added water in frankfurters made from MSC and reported an increase in hardness with increased addition of collagen fibers which could explain MSC2 having greater hardness than BT. This was also seen in a study by Pereira et al. (2011) across multiple levels of MDM in the frankfurters. In both studies however, they were utilizing functionalized collagen fibers which would be able to bind water on their own, whereas in the present study the collagen present would not be functional. When collagen that has not been gelatinized is heated it will contract and harden which may more accurately describe the difference in hardness observed between the MSC2 and BT treatments. MSC1 did contain higher amounts of hydroxyproline, therefore greater amounts of collagen than MSC2 but, as indicated by its greater calcium content, MSC1 had a greater bone content and possibly more myofibrillar disruption. The increased damage could prevent the proper gel structure to form, or there could be a threshold beyond which more collagen could disrupt the functional protein network and soften product structure. Greater moisture content can also explain the decreased hardness of both BT and MSC1 frankfurters compared to MSC2. Meullenet, Chang, & Carpenter (1994) reported a decrease in hardness with increased added water. Decreased firmness in beef frankfurters with increased moisture was also reported by Lee, Whiting, & Jenkins (1987). Although

moisture content is lower in MSC2 frankfurters, fat content is much greater (3%), which should result in a softer product (Lee, Whiting, & Jenkins, 1987). Storage did not have a significant impact ($P < 0.05$) on hardness of any treatment.

The understanding of the greater hardness in MSC2 frankfurters plays upon three interactive components of the raw material and final product. The first factor is the ability of the salt-soluble proteins to be both solubilized and interact with each other to form a stable and ridged structure. The second is the amount of fragmentation or damage inflicted on the myofibrillar proteins before use in a product, which directly affects the first factor. The final factor to keep in mind is the composition of the finished product, which can impact finished product texture. MSC1 underwent higher amounts of shear than MSC2 during the recovery process. The added force resulted in greater calcium content and hydroxyproline content (Table 3.3). Although MSC2 underwent greater amounts of shear than BT, it seems to have retained enough functionality to form a rigid network similar in functionality to BT. However, compositional differences could also be attributing to greater hardness of MSC2 frankfurters as they had less moisture. As discussed previously collagen content could cause increased firmness due to shrinking with heat. However, if too much is present, the protein matrix could be disrupted. The objective measurement of cohesiveness demonstrates the break-down of the gel structure by its ability to undergo a second compression. MSC1 was significantly less cohesive than MSC2 and BT. Pereira et al. (2011) report an increase and then a decrease in cohesiveness with added mechanically deboned poultry meat (MDPM), indicating there is a threshold beyond which protein structure is lost. The many factors influencing final

product texture makes these results complex to explain and warrants further research into how specific MSC processing parameters and composition affects frankfurter quality.

Resilience is a measure of the recovery time of the product following the first compression. MSC2 was significantly more resilient ($P < 0.05$) than MSC1 followed by BT. Springiness followed a similar trend with both types of MSC being significantly springier ($P < 0.05$) than BT. This agrees with a study by Massingue et al. (2018) which found an increase in springiness with increasing addition of MSC to lamb sausages. Between the MSC samples, MSC1 was significantly ($P < 0.05$) springier than MSC2. Cohesiveness measures the ability of the frankfurter to retain its structure after the first bite compression by dividing the area under the peak force of the second bite by that of the first. All treatments were significantly different ($P < 0.05$) in cohesiveness, with MSC2 being the most cohesive followed by BT and MSC1. These results align with trends found in hardness of each treatment. In contrast to MSC2 being the most cohesive frankfurter, Pereira et al. (2011) found frankfurter cohesiveness to decrease when 50% added mechanically deboned poultry meat was used. As a whole, textural results of MSC2 frankfurters challenge the understanding of mechanically separated chicken contributing to decreased hardness and causing a less desirable texture in processed meat products. MSC2 had greater hardness, resilience, cohesiveness, and adhesiveness when than whole muscle chicken breast trim frankfurters. Moreover, MSC2 was found to be significantly different from MSC1 in all textural attributes other than chewiness, where no significant differences ($P > 0.05$) were observed between treatments. These results support the hypothesis that processing parameters during the mechanical separation process can directly affect the functional quality of the raw material and the quality of the

ultimate product. Further research to determine how specific parameters (processing speed, pressure, time from harvest, parts, and equipment) impact final product textural attributes is warranted.

Color

Summarized color data over the 98 d shelf-life shown in Table 3.6.

Table 3.6 Means for effect of treatment on L*, a*, b* of frankfurters stored under retail display lights.

	External			Internal		
	L*	a*	b*	L*	a*	b*
BT	59.36[a]	10.83[a]	37.97[a]	81.03[a]	3.84[a]	15.19
MSC1	46.48[b]	15.79[b]	29.74[b]	63.52[b]	11.34[b]	15.86[a]
MSC2	43.33[c]	18.14[c]	28.28[c]	60.72[c]	13.96[c]	15.31
SEM	1.63	0.76	1.06	0.38	0.19	0.14

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$).

Chicken breast trim frankfurters were the lightest treatment, with significantly greater ($P < 0.05$) internal and external L* values than both MSC frankfurters (Tables 3.7 and 3.8).

Table 3.7 Mean external L* value of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	54.97[ax]	58.16[axy]	59.14[ay]	60.69[ay]	59.99[ay]	60.19[ay]	60.77[ay]	61.00[ay]
MSC1	42.30[x]	46.04	46.21	47.18	46.31[b]	48.07[b]	48.08	47.63
MSC2	38.73[x]	42.92	43.76	44.54	42.56[c]	44.42[c]	44.56	45.16

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$).
[x-z] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM = 1.75

Table 3.8 Mean internal L* value of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	81.33[a]	80.76[a]	81.05[a]	81.03[a]	80.60[a]	80.72[a]	81.27[a]	81.44[a]
MSC1	63.43[b]	63.32[b]	63.31[b]	63.79[b]	63.74[b]	63.83[b]	63.68[b]	63.07
MSC2	60.47[c]	60.47[c]	60.54[c]	60.51[c]	60.38[c]	60.29[c]	61.04[c]	62.05

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$).
Means in the same row were not significantly different ($P > 0.05$).
SEM = 0.38

This corresponds with the significantly lesser ($P < 0.05$) iron content of chicken breast trim (Table 3.3). Internal and external L* values were significantly higher ($P < 0.05$) for MSC1 than for MSC2, which can be explained by raw material iron content. All treatments were significantly different ($P < 0.05$) in internal and external a* values at every time point (Table 3.9 and 3.10).

Table 3.9 Mean external a* value of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	13.03^{ax}	11.76^{axy}	11.14^{axy}	9.92^{ay}	10.31^{ay}	10.31^{ay}	10.33^{ay}	9.82^{ay}
MSC1	18.63^{x}	15.89	15.69	15.56	15.71^{b}	15.07^{b}	14.91	14.88
MSC2	20.91^{x}	18.27^{y}	17.84^{y}	17.70^{y}	19.33^{cxy}	17.57^{cy}	17.30^{y}	16.21^{y}

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$).
[x-z] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM = 0.85

Table 3.10 Mean internal a* value of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	3.22^{a}	3.63^{a}	3.60^{a}	3.87^{a}	3.90^{a}	3.89^{a}	3.77^{a}	4.81^{a}
MSC1	10.88^{b}	11.10^{b}	11.40^{b}	11.35^{b}	11.37^{b}	11.22^{b}	11.29^{b}	12.13
MSC2	13.98^{c}	14.16^{c}	14.16^{c}	14.28^{c}	14.27^{c}	14.13^{c}	13.92^{c}	12.80

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$).
Means in the same row were not significantly different ($P > 0.05$).
SEM = 0.36

MSC2 frankfurters had the greatest internal and external a* value, followed by MSC1 and BT. These differences can again be attributed to the iron content. Both myoglobin and hemoglobin contain iron and are the main pigments in mechanically separated meats. Increased myoglobin and hemoglobin content in mechanically separated meats cause both increased a* values and lower L* values of processed meat products (Froning & Johnson, 1973; Mielnik et al., 2002). Externally, all treatments were significantly different ($P < 0.05$) in b* values with BT being the most yellow followed by MSC1 and MSC2 respectively. Internally, MSC1 was significantly greater ($P < 0.05$) in b* value than both MSC2 and BT.

There were no significant differences ($P < 0.05$) in internal color (L*, a*, and b*) over the shelf-life for all treatments. External L* value increased significantly ($P < 0.05$) at day 14 compared to day 0 for both MSC frankfurters and at day 28 for BT frankfurters. L* value did not change significantly for the remainder of the study for any treatment (Table 3.7). External a* values followed a similar trend as external L* values, with a significant decrease ($P < 0.05$) at day 14 compared to day 0 for both MSC frankfurters and at day 42 compared to 0d for BT frankfurters (Table 3.9).

External b* values trended to decrease with display time for all treatments and was significantly decreased ($P < 0.05$) at day 98 compared to day 0 for BT and MSC1 as shown in Table 3.11.

Table 3.11 Mean external b* value of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	41.86^{ax}	40.35^{axy}	38.32^{axyz}	36.37^{az}	37.11^{ayz}	37.18^{ayz}	37.59^{ayz}	34.98^{az}
MSC1	32.14^{x}	31.07^{xy}	29.33^{xy}	29.32^{xy}	29.77^{xy}	29.31^{xy}	29.11^{xy}	27.86^{y}
MSC2	29.37^{xy}	29.24^{xy}	27.70^{xy}	27.68^{xy}	29.90^{x}	28.05^{xy}	28.02^{xy}	26.24^{y}

[a-b] Means in the same column with different superscripts are significantly different ($P < 0.05$).
[x-z] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM = 1.22

Display light and time effects on color for this study are consistent with previous reports of color shelf-life in processed meat products; reduced a* and increased L* values with time (Nannerup et al., 2004; Yen, Brown, Dick, & Acton, 1988; Møller et al., 2003).

Lipid Oxidation

There was no significant effect of time on TBA values for all treatments for the entirety of the day 98 shelf-life (Figure 3.1).

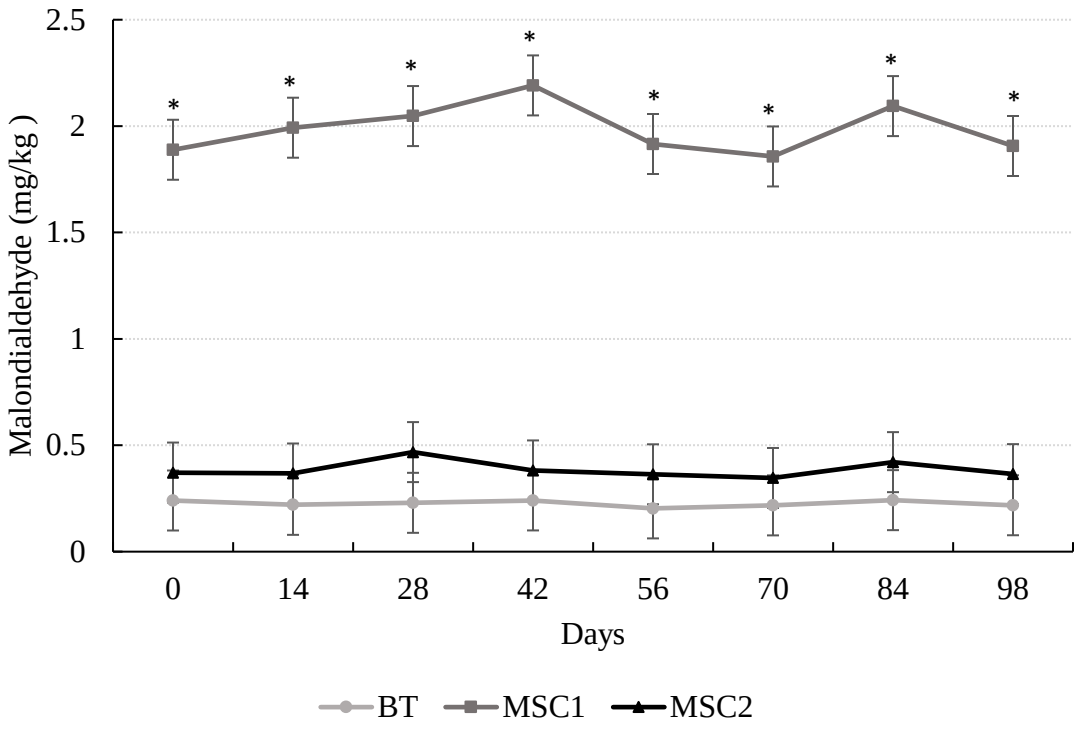

Figure 3.1 TBA values at eight timepoints over a 98d shelf life of frankfurters produced with chicken breast trim (BT) and two types of mechanically separated chicken (MSC1, MSC2). Error bars represent SEM = 0.14. Data within the same time point with (*) are significantly different (P<0.05).

These results are not surprising, due to the powerful antioxidant ability of sodium nitrite and the vacuum storage of the product. TBA values were significantly greater (P < 0.05) in MSC1 than in both BT and MSC2 frankfurters for the entirety of the study. The increased level of lipid oxidation in this product most likely occurred prior to frankfurter manufacture and most likely during the thawing of the MSC. Increased lipid oxidation of MSC compared to whole muscle is well documented (Baker & Kline, 1984; Paulsen & Nagy, 2014; Mielnik et al., 2002; Olsen et al., 2005). The poultry mechanical separation process creates favorable conditions for lipid oxidation: increased iron (lipid oxidation initiator); greater surface area, allowing for more exposure to oxygen; and poultry having greater amounts of poly-and mono-unsaturated lipids than pork or beef (which are more

susceptible to initiation and propagation of lipid oxidation) than pork or beef. TBA values were significantly lower ($P<0.05$) in MSC2 than in MSC1, despite higher iron content (Table 3.3.). This difference can be attributed to the time the bones were held prior to mechanical separation and the aggressiveness of the separation equipment used. Bones for MSC1 were held 3 to 5d prior to separation, which exposed them to more oxygen prior to freezing of the MSC. In contrast MSC2 was produced in-line with the deboning process and was therefore processed much closer to harvest. The mechanical separation process of MSC1 caused an increased susceptibility to further oxidation during thawing prior to frankfurter manufacture.

Conclusions

Generally, mechanically separated chicken is regarded as a highly nutritious and economical by-product of the poultry industry. MSC has been previously reported to cause reduced hardness, increased redness, and increased lipid oxidation in processed meat products (Paulsen & Nagy, 2014). Previous studies have reported a lower quality product was produced from MSC than from whole muscle products. However, in the present study frankfurters produced with MSC2 exhibited equal or greater quality in all textural characteristics and in lipid oxidation when compared to frankfurters produced from whole muscle chicken breast trim. This is in contrast to MSC1, which was equal in hardness, the least cohesive and the most oxidized. Both MSC frankfurters were lesser in L* values and greater in a* values than frankfurters formulated with BT, which can be viewed as a negative or a positive depending on product type. The current hypothesis that MSC functionality can vary based on processing equipment, separation speed, storage, and composition is supported by the results of the present study and warrants further

investigation into specific factors that produce superior MSC functionality. From the data presented, it can be concluded that not all MSCs are equal in functionality and indeed some have the potential to be used to produce products with equal or greater textural quality to whole muscle chicken raw materials.

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CHAPTER 4. RHEOLOGICAL CHARACTERISTICS OF MECHANICALLY SEPARATED CHICKEN AND CHICKEN BREAST TRIM MYOFIBRIL SOLUTIONS DURING THERMAL GELATION

A paper to be submitted to the Journal of Food Science

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Abstract

Mechanically separated chicken (MSC) is widely used in the processed meat industry to reduce the cost of lean raw materials. Whereas the separation process produces nutritionally valuable protein, it has been well documented to soften the texture of processed meat products; thought to be due mainly to composition. MSC was obtained from two different separation methods (MSC1, Beehive separator, aged bones; MSC2, Poss separator, fresh bones) and compared to chicken breast trim. Rheological attributes of myofibrillar protein solutions (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6) during thermal gelation and cooling were evaluated. All treatments exhibited gelation with increased temperature (decreased δ). A peak, decline, and subsequent increase was observed in all 3 treatments at the 50–55˚C range in both the G' and G". G' slopes on both sides of the peak (S2, S3) and following the decline (S4) were significantly different between BT and both MSCs ($P<0.05$). BT's S3 was

significantly steeper indicating a greater instability of the solid-like structure in the temperature range of 50–55˚C (myosin rod denaturation). BT S2 and S3 were significantly different from MSC treatments in G" ($P<0.05$), but not significantly different during S4. Results indicate physical properties of myofibrillar proteins from MSC and chicken breast meat differ during thermal gelation. This indicates a different protein profile that could be explained by muscle source or by denaturation during isolation of the MSC.

Introduction

Since the 1970s a highly nutritional by-product of the poultry industry has been used in value added products to reduce the cost of protein staples. It is well known that MSC has a negative impact on eating quality of processed products by modifying the texture, introducing a grittiness, increasing off-flavors, and increasing redness (Daros, Masson, & Amico, 2005; Paulsen & Nagy, 2014; Froning & Johnson 1973). Specifically, tension strength was significantly reduced at application rates above 40% MSC and above 5% MSC significantly reduced compressive strength in sausages (Daros, Masson, & Amico, 2005). Research categorizing MSC behavior in a fundamental system has been limited to MSC surimi and has not been compared to whole muscle alternatives (Cortez-Vega, Fonseca, & Prentice, 2015; Smyth & Oneill, 1997).

Many attempts to functionalize MSC involve post-separation techniques to reduce bone, fat, and connective tissue content, such as washing surimi-like processes (Cortez-Vega, Fonseca, & Prentice, 2015; Smyth & Oneill, 1997). During mechanical separation, proteins undergo increased heat (5-8˚C) and pressure (>6.2 MPa) which are known to modify myofibrillar protein structure (Grossi et al., 2016; Liu et al., 2008). Independently

the higher pressure and the increased temperature may not be severe enough to impact functionality; however, in combination they could be.

Heat causes irreversible changes in protein structure. Specifically, secondary structure shifts toward beta sheets at temperatures as low as 15˚C (Liu et al., 2008). Myosin has a conversion from alpha helix secondary structure to beta sheets with an increase in temperature (Liu et al., 2008). Myosin, the major gel-forming protein, is extremely susceptible to heat induced changes. Cheng & Parrish (1979) found myosin heavy chain to be the first of the myofibrillar proteins to become insoluble due to temperature increases.

Pressure also has been shown to induce protein denaturation and even gelation through structural changes different than those caused by heat (Lee et al., 2007). Increased pressure has been reported to reduce myosin solubility at pressures above 300 MPa (Lee et al., 2007). Zhang et al. (2015) showed a decrease in sulfhydryl content with an increase in pressure from 100–500 MPa, indicating pressure has the ability to induce disulfide bond formation. Because of differences in how heat and pressure modify myofibrillar proteins, when in combination, it is hypothesized that procedures that include heat and pressure will have an additive damaging effect on myofibrillar protein structure.

Dynamic oscillatory rheological measurements are able to measure changes in the physical structure of substances based on reactions to an applied rotational force. During thermal denaturation of myofibrillar proteins structural changes can be recorded by the rheological measurements of storage modulus (G'), loss modulus (G"), and phase angle (δ). Investigators have been able to document a characteristic increase, followed by a

sharp decrease and subsequent increase in G' (measure of solid-like characteristics) and G" (liquid-like characteristics) during thermal gelation. This graph pattern has been consistently observed across species, ionic strength, presence of phosphate, pH, muscle fiber type, and protein concentration (Westphalen, Briggs, & Lonergan, 2006; Westphalen, Briggs, & Lonergan, 2005; Egelandsdal, Fretheim, & Samejima, 1986; Smyth & Oneill, 1997; Xiong, 2001; Xiong & Blanchard, 1994b). The observed transitions in protein gel physical structure have been explained by the denaturation temperatures of myofibrillar proteins especially myosin. Rheological experiments are extremely useful in testing the physical interactions occurring under controlled conditions.

In the present study two processing methods were used to produce divergent types of commercial MSC. The first separation method (MSC1) was a high-speed and high-yielding process, utilizing cages, necks held in a cooler for 3–5 d prior to separation. The second separation method (MSC2) was a process designed to reduce the speed and increase particle size of the final product and used bones predominantly from the front half of the carcass within 24 h of harvest. Utilizing dynamic oscillatory rheological measurements, the following study addresses the question of how myofibrillar proteins from different types of MSC behave during thermal gelation, compared to whole muscle chicken breast myofibrillar proteins. It was hypothesized that chicken breast myofibrillar proteins would produce greater storage modulus than both MSCs and the two MSC processing methods would produce MSC myofibrillar proteins with different thermal gelation characteristics. Differences in myofibrillar protein profile were also assessed to

better understand if and how the functional proteins of the three types of raw materials differ.

Materials and Methods

Raw Materials

All poultry raw materials were sourced from commercial facilities. Two types of mechanically separated chicken (MSC1 and MSC2) material made under different processing conditions were used and individually compared to chicken breast trim (BT). (breast trim was selected due to its origin from the main shell of the bird and not from the dark leg meat portions, its consistency, and industrial application). MSC1 was prepared using broiler frames produced 3–5 d following breast meat removal on a Beehive S88 mechanical separator (Provisur Technologies, Mokena, IL) with sieve sizes of 1.5, 9.9, and 7.4 mm. MSC2 was produced from frames of broiler carcasses separated immediately following breast meat removal following a proprietary process. The material was generated on three consecutive manufacturing days. Chicken breast trim was sourced from commercial broilers and was sampled from one production lot to limit variation in poultry fat content in the frankfurter formulations. All poultry raw materials were packaged in 18.2 kg boxes and blast frozen at -44.4˚C for 72 h and held at -17.7˚C to -23.3˚C for 19, 18, and 17 d. Boxes were then packed in dry ice and shipped overnight to the Iowa State University Meat Laboratory, Ames, IA. Upon arrival, poultry raw materials were stored at -20˚C. Before use, raw materials were thawed by storing at 0˚C for 3 d, followed by 2 d at 4˚C.

Myofibril Isolation

Once thawed, each raw material was sampled and washed, using the protocol below. Samples were washed following a modified procedure used to purify myofibrils according to a differential centrifugation method (Westphalen, Briggs, & Lonergan, 2005; Westphalen, Briggs, & Lonergan, 2006; Doerscher, Briggs, & Lonergan, 2004). All steps occurred at 4˚C

Two hundred g of MSC1, MSC2, and chicken breast trim were sampled. Excess fat and cartilage were trimmed from the chicken breast trim sample before homogenization. All samples were homogenized in 800 mL of a post rigor extraction buffer (100mM Tris, 10mM EDTA, pH 8.3) using a Kinematic Polytron Homogenizer (Brinkman Instruments, Inc., Westbury, NY). Samples were then centrifuged at 1000 X g for 20 min. Following centrifugation, the supernatant was poured off and the pellet was resuspended in 4 volumes of a standard salt solution (100 mM KCL, 20 mM K_2HPO_4 /KH_2PO_4, 2mM $MgCl_2$, 1 mM EGTA, and 1 mM NaN_3, pH 7). The samples were homogenized again, and centrifuged at 1000 X g for 20 min. This step was repeated 3 times. The resulting pellet was then resuspended in four volumes of a standard salt solution with 1% Triton X-100 twice and centrifuged at 1500 x g for 10 min. between washes. The pellet was then resuspended in four volumes of standard salt solution and centrifuged at 1500 x g for 10 min twice to remove residues of Triton X-100. The final pellet was resuspended in 150 mL of 100 mM KCl, 5 mM Tris buffer pH 7.0 and 150 mL glycerol. Samples were then stored in 50-mL centrifuge tubes at -20˚C until needed.

Preparation of Myofibril Solutions

Frozen myofibril samples were equilibrated to 4˚C and then diluted by four volumes (w/v) of standard salt solution and centrifuged at 3000 x g for 10 min. The pellet was resuspended four times in one volume of 50 mM sodium phosphate monobasic buffer pH 6 and centrifuged at 3000 x g for 10 min after each wash to remove any glycerol. The protein concentration of the resulting pellet was determined using the Bio-Rad RC DC Protein Assay (Hercules, CA, USA). The pellet was diluted using 50mM sodium phosphate buffer at a pH of 6 to 5.6% protein. The pellet was diluted by half using 1.2 M NaCl, 50 mM solution at pH 6 for a final sodium chloride concentration of 0.6 M. Final sample protein concentration was 2.8%. All myofibril samples were adjusted to pH 6 and 1 mM sodium azide was added to preserve samples. The samples were thoroughly mixed and stored at 4˚C for rheological measurements within six days.

Dynamic Oscillatory Rheology

Temperature sweep experiment

A Discovery Hybrid Rheometer HR-2 (TA Instruments, New Castle, DE, USA) was utilized for all rheological experiments. A 40-mm parallel plate geometry with a cross hatched bottom and top plate was used for all rheological testing. The temperature sweep experiments were done with a gap of 1500 µm, a trim gap offset of 50 µm, and a loading gap height of 4500 µm. An oscillation temperature ramp was performed on 2.8 g of each sample per run. The temperature ramp was from 20°C to 85°C at a rate of 1°C/ min. The measurements started once the sample reached 20°C and there was a soak time of 3 min at 85°C to ensure the whole sample reached the final temperature of 85°C. The sampling interval was set to 20 s with a 0.25% strain and a frequency of 1 Hz.

Measurements were taken during cooling as well at a starting temperature of 85°C and final temperature of 5°C at a rate of 5°C/min. Heating and cooling rate and temperature range settings were determined based on previous research investigating gelation of muscle proteins (Xiong & Blanchard, 1994b; Westphalen, Briggs, & Lonergan, 2006; Westphalen, Briggs, & Lonergan, 2005; Doerscher, Briggs, & Lonergan, 2004). Frequency and amplitude were determined based on data collected from amplitude sweeps on gels thermally set to 85˚C to ensure gels were tested within their linear viscoelastic range (LVR). Mineral oil was used to coat the exposed surface of 0.6 M NaCl solutions to prevent moisture loss from the sample during experimentation.

Amplitude sweep experiment

Myofibrils were washed in the same way as the samples used during the temperature sweep. A composite sample of the three replications of each type of raw material at both NaCl concentrations was used for determining the linear viscoelastic range of each type of gel. The solutions were prepared in the same way as the temperature sweep samples, loaded on the rheometer and heated to 85˚C with the parallel plate on the surface of the sample to prevent moisture loss. Once temperature was reached the sample was held for 3 min and cooled to 21˚C. The top plate was then lowered to the surface of the gel and an amplitude sweep was performed from 0–4 % strain at a frequency of 1 Hz.

SDS-PAGE

Myofibrillar protein composition was measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Each myofibrillar pellet was solubilized and adjusted to a protein concentration of 6.4 mg/mL, using the Bio-Rad RC

DC Protein Assay (Hercules, CA, USA). The samples were then further diluted into gel samples using [3 mM EDTA, 3% (w/v) SDS, 30% (w/v) glycerol, 0.003% (w/v) pyronin Y, and 20 mM Tris–HCl, pH 8.0] and 0.1 vol. 2-mercaptoethanol (Wang, 1982) for a final protein concentration of 4 mg/ml. Forty mg samples were loaded into individual wells of a 10 cm x 12 cm x 1.5 mm 10% polyacrylamide separating gel [10% acrylamide/bis (100:1 acrylamide: bisacrylamide)], 0.375 M Tris–HCl, pH 8.8, 0.1% (w/v) sodium dodecyl sulfate (SDS), 0. 05% (w/v) ammonium persulfate and 0. 05% (v/v) N,N,N',N'-Tetramethylenediamine (TEMED). The 10% separating gel had a 5% polyacrylamide stacking gel [5% acrylamide/bis (100:1 acrylamide: bisacrylamide)], 0.125 M Tris–HCl, pH 6.8, 0.1% (w/v) SDS, 0.125% (v/v) TEMED, and 0.075% ammonium persulfate). The running buffer was 192 mM glycine, and 0.1% SDS (w/v), 25 mM Tris, pH 8.3. Gels were run at 120 V for approximately 150 min at room temperature on a Hoefer SE 260B Mighty Small II running unit (Pharmacia Biotech, San Francisco, CA, USA). Proteins were visualized by staining for a minimum of 12 h in an excess of 0.1% (w/v) Coomassie brilliant blue R-250, 40% (v/v) ethanol, and 7% (v/v) acetic acid. Gels were de-stained in distilled deionized water for 24 h. An image of the gel was collected with an Alpha Innotech FluorChem 8800 imaging system (Alpha Innotech Corporation, San Leandro, CA, USA). Gels were run in duplicate.

Protein Identification

SDS-PAGE was performed using in the same process as described above and unique bands were excised for identification. Protein identification was performed using a Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer from Thermo Fisher Scientific (Waltham, MA, USA). Proteins from nine different locations were excised,

frozen at - 80˚C and stored until analysis. Samples were first enzymatically digested with trypsin on a Genomic Solutions Investigator ProGest automated digester (Ann Arbor, MI, USA) and then separated by liquid chromatography before entering the dual mass spectrometer. Sample fragmentation patterns were compared to know peptide fragments. Raw data were analyzed using Thermo Scientific's Proteome Discoverer Software (Waltham, MA, USA) and compared to Mascot and Sequest HT against Uniprot-gallus gallus (Waltham, MA, USA).

Experimental Design Statistical Analysis

MSC composition can be variable due to variability of in-going raw material; therefore, to obtain a representative understanding of MSC properties we choose to sample three lots produced on three consecutive days of mechanical separation and myofibril isolation was conducted on three consecutive days. Chicken breast trim product from three separate boxes were randomly assigned to each replication day. All data were analyzed with SAS 9.4 mixed procedure. Rheological data were analyzed with fixed effect of treatment (MSC1, MSC2, BT), and random effect of day (day samples were tested on the rheometer) by treatment. Significance was determined by a *P*-value of < 0.05.

Results & Discussion

Raw Material Composition and pH

Chicken raw material characteristics for this study were previously reported in a companion study in Table 3.3, chapter 3 of this thesis (Miller 2018). All raw materials were significantly different from each other ($P < 0.05$) in moisture and fat. BT was greatest in protein content with no difference between MSC raw materials. All raw

materials had expected moisture, fat, and protein from literature (Ang & Hamm, 1982; Perlo et al., 2006; Soglia et al., 2016; Li et al., 2015). Raw material pH was comparable for all treatments to previous reports with both MSC raw materials having a greater pH than BT. Hydroxyproline, calcium, and iron content reflected the differences between MSC processing methods and whole muscle raw material. Chicken breast trim contained the least amount of hydroxyproline, calcium, and iron. Hydroxyproline and calcium were significantly greater in MSC1 than MSC2 indicating a greater incorporation of connective tissue and bone during the MSC1 separation process.

Dynamic Viscoelastic Properties

Thermal gelation

All treatments displayed typical transitions in storage modulus (G') and loss modulus (G") over the temperature range of 20 to 85˚C (Xiong & Blanchard, 1994a; Liu & Xiong, 1996; Xiong, 2001; Westphalen, Briggs, & Lonergan, 2005) as shown in Figures 4.1 and 4.2.

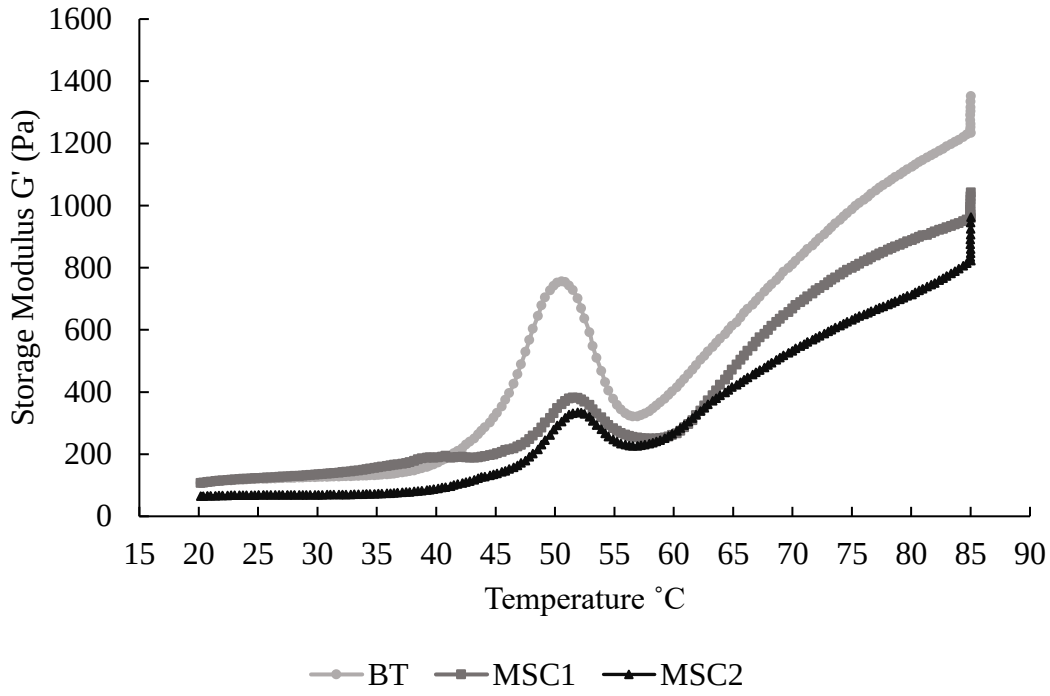

Figure 4.1 Storage modulus of myofibrillar protein solutions (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6) during thermal gelation (20 to 85˚C).

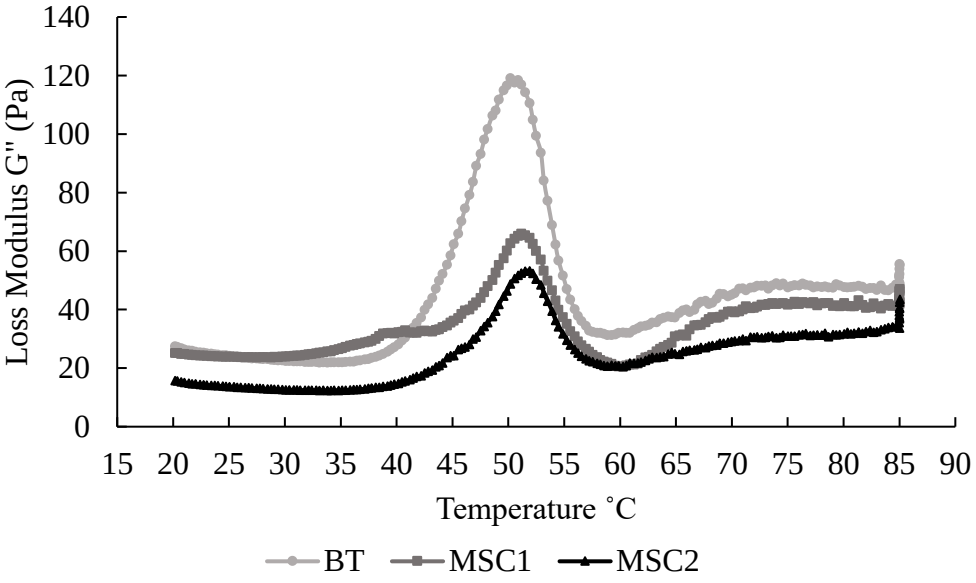

Figure 4.2 Loss modulus of myofibrillar protein solutions (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6) during thermal gelation (20 to 85˚C).

A steady decrease of phase angle with increase in temperature was observed which is typical of myofibrillar protein solutions during thermal gelation as the more liquid-like solution of myofibrillar proteins aggregate and denature to form a solid gel. All treatments showed similar transition peaks in G" and G'. Two inflection points and a peak were identified for each treatment as well as the temperatures at which these points of interest occurred for both G' and G", as shown in Tables 4.1 and 4.2 respectively.

Table 4.1 Means for effect of treatment on storage modulus inflection points and temperatures of myofibrillar protein solutions heated from 20 to 85˚C at 1˚C/min (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6.0).

	Initial G' (Pa)	Initial T (C˚)	Inflection 1 G' (Pa)	Inflection 1 T (C˚)	Peak G' (Pa)	Peak T (C˚)	Inflection 2 (Pa)	Inflection 2 T (C˚)	Final G' (Pa)	Final T (C˚)
BT	125.19^{a}	20.15	181.07^{b}	39.90^{a}	863.67^{a}	50.44^{a}	346.16^{a}	56.64^{b}	1351.57^{a}	85.00
MSC1	108.03^{ab}	20.16	203.19^{b}	45.20^{b}	371.48^{b}	51.74^{b}	245.27^{ab}	58.03^{a}	1041.77^{b}	84.99
MSC2	64.36^{b}	20.17	106.14^{a}	44.97^{b}	315.62^{b}	51.94^{b}	216.35^{b}	56.74^{b}	963.00^{b}	85.00
SEM	16.80	0.01	16.70	1.36	97.46	0.23	39.02	0.28	58.06	0.01

T=Temperature; $^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)

Table 4.2 Means for effect of treatment on loss modulus inflection points and temperatures of myofibrillar protein solutions heated from 20 to 85˚C at 1˚C/min (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6.0).

	Initial G" (Pa)	Initial T (C˚)	Inflection 1 G" (Pa)	Inflection 1 T (C˚)	Peak G" (Pa)	Peak T (C˚)	Inflection 2 G" (Pa)	Inflection 2 T (C˚)	Final G" (Pa)	Final T (C˚)
BT	31.63^{a}	20.15	30.51^{b}	39.51	142.52^{a}	50.63^{a}	36.42	59.42	55.28^{a}	85.00
MSC1	25.14^{ab}	20.16	30.29^{b}	40.81	65.05^{b}	51.16^{ab}	20.73	59.88	47.05^{ab}	84.99
MSC2	15.75^{b}	20.17	13.64^{a}	41.75	50.21^{b}	51.72^{b}	19.10	59.15	43.50^{b}	85.00
SEM	3.98	0.01	4.16	0.97	19.96	0.21	6.42	0.41	3.20	0.01

T=Temperature; $^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)

A slight rise in G' was observed from 20˚C to 39.9–45.2˚C followed by a sharp increase until 50.4–51.9˚C depending on treatment. The initial increase is attributed to the aggregation and clumping of myosin heads followed by a sharp increase as they begin to fully denature (Xiong, 2001). The S1 region transition temperature of 47.4˚C coincides with the previously observed increases in storage modulus (Smyth, Smith, Vegawarner, & Oneill, 1996; Xiong & Blanchard, 1994b). The first inflection point also closely aligns with the first transition temperature reported for both the myosin rod as well as myosin heavy chain, indicating conformational changes of these fragments could be contributing to gel structure in this range as well. Following the peak, a sharp decline in G' occurs from 50.4–51.9˚C to 56.6–58.0˚C. This abrupt deacease in solid-like structure is attributed to the denaturation of the light-meromyosin portion of the myosin filament, as it coincides with the transition temperature of LMM, reported as 51.6˚C (Xiong, 2001; (Smyth, Smith, Vegawarner, & Oneill, 1996). Following 56.6–58.0˚C, all samples continued to rise in G'. Storage modulus of BT was significantly greater ($P < 0.05$) at all points of interest than that of MSC2 ($P < 0.05$). Storage modulus of BT was also significantly greater ($P < 0.05$) than MSC1 at the identified peak and final storage modulus. MSC2 had lesser G′ ($P < 0.05$) at the first inflection point than both MSC1 and BT but was not significantly different ($P < 0.05$) from MSC1 at any other point of interest. BT's temperature at the first inflection point was significantly lower by 5˚C indicating it is less thermally stable than the MSC treatments. This trend was also seen in the identified peak temperature with G′ being 2˚C lower than MSC treatments. However, the second inflection point for MSC1 occurred at a significantly higher ($P < 0.05$) temperature than for MSC2 and BT. This indicates that there could be a difference in the

thermal stability of the myofibrillar proteins that are denatured in that temperature range or there maybe differences in crosslinking ability of the gel due to oxidation or fragmentation of myosin particularly the LMM portion. Four slopes were then calculated and values compared across treatments, as shown in Table 4.3.

Table 4.3 Means for effect of treatment on the calculated slopes of storage modulus of myofibrillar protein solutions heated from 20 to 85˚C at 1 ˚C/min (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6.0).

	Slope 1 (Pa/˚C)	Slope 2 (Pa/˚C)	Slope 3 (Pa/˚C)	Slope 4 (Pa/˚C)
BT	3.32[b]	60.16[a]	-81.44[a]	36.36[a]
MSC1	3.77[b]	28.25[b]	-20.82[b]	29.53[b]
MSC2	1.78[a]	32.21[b]	-20.26[b]	26.13[b]
SEM	0.31	5.07	8.24	1.69

[a-b] Means in the same column with different superscripts are significantly different (P < 0.05)

MSC2 exhibited a significantly lesser ($P < 0.05$) slope prior to the first inflection point than MSC1 and BT. For the remaining 3 slopes BT myofibrillar proteins exhibited significantly steeper ($P < 0.05$) transitions, particularly the slopes on either side of the identified peak. BT slope 2 and slope 3 were 3 and 4 times steeper, respectively, than for both MSC treatments. G″ displayed a pattern with inflection points and a peak at similar temperatures as the G′, as reported in Table 4.4.

Table 4.4 Effect of treatment on the calculated slopes of loss modulus of myofibrillar protein solutions heated from 20 to 85˚C at 1 ˚C/min (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6.0).

	Slope 1 (Pa/˚C)	Slope 2 (Pa/˚C)	Slope 3 (Pa/˚C)	Slope 4 (Pa/˚C)
BT	-0.07[b]	9.79[a]	-11.54[a]	0.91
MSC1	0.22[a]	3.35[b]	-5.08[b]	1.06
MSC2	-0.08[b]	3.68[b]	-4.22[b]	0.91
SEM	0.08	1.34	1.19	0.17

[a-b] Means in the same column with different superscripts are significantly different (P < 0.05)

Phase angle (δ) in all treatments decreased as temperature increased. The initial δ for MSC1 was significantly smaller ($P < 0.05$) than for MSC2 and BT. δ of MSC1 was also significantly larger ($P < 0.05$) at the denaturation temperature (51.6˚C) of LMM than that of MSC2 and BT, indicating a less solid gel structure at that temperature. More interestingly, δ of BT was significantly smaller ($P < 0.05$) at 47.4˚C, indicating BT was more solid at the transition temperature of the myosin S1 head region. This difference in head interaction could be related to premature crosslinking of the tail region of MSC treatments due to modification during the mechanical separation process. The interactions could be preventing the aggregation of the myosin heads, resulting in a more fluid system. This difference could also be due to differences in myosin isoforms between the MSC and BT.

Many differences observed in rheological properties of myofibrillar protein between treatments in the present study may be attributed to differences in muscle fiber type. Chicken breast meat has been selected for large and rapid muscle growth for years and has therefore been shifted to almost entirely white fiber type (Petracci, Mudalal, Solglia, & Cavani, 2015). MSC muscle tissue is found predominantly close to the bone and in-between ribs and neck bones of the animal. As these muscles are utilized for stabilization, the myosin isoforms would be intermediate and red fiber type rather than white fiber type. Chicken leg and thigh meat is known to be predominantly red fiber type. In one study the rheological properties at four different pHs of chicken breast and leg muscle myofibrillar protein solutions were compared (Xiong & Blanchard, 1994a). Breast muscle exhibited a greater G′ at all temperatures than thigh and leg meat (Xiong & Blanchard, 1994a). Thigh and leg treatments were also found to transition at higher

temperatures than breast muscle myofibrils indicating more stable secondary structure. Both differences in magnitude of G' and transition temperatures of thigh/leg (red fiber) and breast meat (white fiber) were observed in another study by Liu & Xiong (1996). In the present study, the rheological patterns of both MSC and BT were similar to those previously reported for muscles with predominantly red fiber type and breast meat respectively (Liu & Xiong, 1996; Xiong & Blanchard, 1994a).

Another factor that could explain the observed differences due to source is the state of degradation, oxidation, or fragmentation of the myofibrillar proteins. The mechanical separation process exposes muscle tissue to factors that could potentially damage myofibrillar proteins. The process applies pressure, increase heat, reduces particle size and increases concentrations of fat and pro-oxidant minerals such as iron (Froning & Johnson, 1973; Paulsen & Nagy, 2014) all of which are known to cause damage to myofibrillar proteins particularly myosin. Crosslinking has been reported to occur in the myosin tail region under oxidative stress (Ooizumi & Xiong, 2006) and its occurrence prior to gelation temperatures causes changes in the gelation process which could detrimentally affect final gel strength. Lui & Xiong (1996) induced oxidative stress on breast meat by adjusting iron and fat content to levels naturally present in leg muscle and observed a significant reduction in the peak G' of breast meat myofibrils compared to control, although still greater than leg meat. However, in the presence of antioxidants, G' was not affected by the addition of iron and fat. In myofibrils extracted from porcine longissimus muscle, oxidation induced for 1, 3, and 5 h resulted in a reduction in the peak G' and a plateau effect resulting in equal and greater G' at 1 and 3 h, respectively (Chen et al., 2016). However, at the longest treatment’s (5 h) ultimate G' was less than the non-

oxidized control. Again, in the presence of an antioxidant, rheological traits were less divergent from the non-oxidized control. These as well as other studies indicate oxidative stress can impact gel-forming properties (Chen et al., 2016; Cao, True, Chen, & Xiong, 2016; Zhou, Sun, & Zhao, 2015; Zhou et al., 2014a; Zhou et al., 2014b).

Other studies have also demonstrated that pH, ionic strength, and temperature greatly impact rheological properties (Westphalen, Briggs, & Lonergan, 2005; Liu et al., 2007; Liu & Xiong, 1997; Liu et al., 2008). Lower pHs (<6.0) cause secondary structure to shift towards beta sheets and a plateau in G' at the temperatures where LMM denatures (Westphalen, Briggs, & Lonergan, 2005; Liu et al., 2008). In the present study the pH of the myofibril solutions was adjusted to pH 6.0. However, if the destabilization of the LMM alpha helices is responsible for a more gradual decline in G' beyond 50˚C, damage of the LMM region due to mechanical action, could destabilize those same alpha helices and contribute to the observed plateau in this region for MSC treatments, a phenomenon that is absent in the BT. The temperature at which the final sharp increase in G' of MSC1 was observed was significantly higher ($P < 0.05$) than for both MSC2 and BT. This could be explained by an increased amount of crosslinking already present in the more aggressively processed MSC, causing the final crosslinking to be more gradual.

Cooling

During cooling from 85 to 5˚C at a rate of 5˚C/ min all myofibrillar proteins gradually increased in G', G", and δ as the gels set. Ultimate G', G" and slopes were significantly greater ($P < 0.05$) in BT. No significant difference ($P < 0.05$) was found for any δ points during cooling of the myofibril gels. Myofibrillar proteins from BT resulted

in a stronger gel, as expected, when compared to MSC treatments. However, MSC treatments were not significantly different ($P < 0.05$) from each other in ultimate G'.

Myofibrillar Protein Profile

Distinct and repeatable myofibrillar protein profiles were identified for each treatment, as depicted in a representative gel in Figure 4.3. Prominent dark bands in the region of myosin heavy chain (223 kDa) and actin (42 kDa) were detected for all treatments and replications. The majority of bands excised were from the BT treatment, as depicted in Figure 4.5.

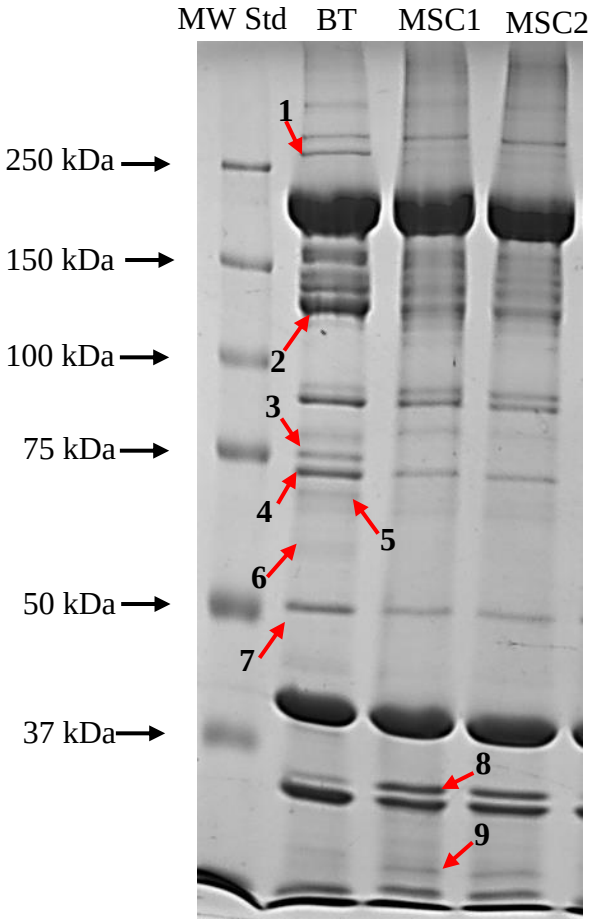

Figure 4.3 Representative SDS-PAGE gel (10% polyacrylamide separating gel) containing each treatment chicken breast trim (BT), mechanically separated chicken 1 (MSC1), and mechanically separated chicken 2 (MSC2) (40 mg of protein). Differing bands were excised and identified denoted by the arrows and number (1–9).

Table 4.5 Proteins identified in each band from myofibrillar protein samples separated using SDS-PAGE[1.]

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	PI	Number of Peptides	Coverage (%)	Mowse Score
1	nebulin-like	*Gallus gallus*	XP_015145654.1	229.1	8.73	72	46.8	3148
	nebulin	*Gallus gallus*	XP_015145352.1	328.8	9.14	31	13.2	1620
	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	35	19.3	904
	myosin, heavy chain 1C, skeletal muscle	*Gallus gallus*	NP_001107181.1	223	5.86	26	14.7	482
2	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	116	43.5	32897
	myosin, heavy chain 1A, skeletal muscle	*Gallus gallus*	NP_001013414.1	222.8	5.8	99	39.2	24044
	myosin, heavy chain 1C, skeletal muscle	*Gallus gallus*	NP_001107181.1	223	5.86	96	36.9	23679
	myosin-3	*Gallus gallus*	XP_001231409.1	222.9	5.86	95	38.3	19673
	myosin, heavy chain 1B, skeletal muscle	*Gallus gallus*	NP_989559.2	223.3	5.81	94	36.6	21497
3	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	20	11.2	1557

Table 4.5 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	PI	Number of Peptides	Coverage (%)	Mowse Score
4	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	47	25.2	4713
	myosin, heavy chain 1A, skeletal muscle	*Gallus gallus*	NP_001013414.1	222.8	5.8	40	22.3	3689
	myosin-3	*Gallus gallus*	XP_001231409.1	222.9	5.86	33	18.9	2435
	myosin-13	*Gallus gallus*	XP_015150680.1	223.5	5.76	16	8.6	1156
5	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	40	20.7	6795
	myosin, heavy chain 1A, skeletal muscle	*Gallus gallus*	NP_001013414.1	222.8	5.8	32	17.3	5107
	myosin-3	*Gallus gallus*	XP_001231409.1	222.9	5.86	27	14.6	3696
	alpha-actinin-2	*Gallus gallus*	NP_990654.1	104.2	5.39	13	17.3	458
	phosphorylase b kinase regulatory subunit alpha, skeletal muscle isoform isoform X1	*Gallus gallus*	XP_004940655.2	138.6	6.57	13	12.1	599
6	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	32	17.1	5584

Table 4.5 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	PI	Number of Peptides	Coverage (%)	Mowse Score
	myosin, heavy chain 1A, skeletal muscle	*Gallus gallus*	NP_001013414.1	222.8	5.8	28	15.5	4382
	myosin-binding protein H	*Gallus gallus*	NP_001026199.1	58.6	7.53	9	16.0	641
	actin, alpha skeletal muscle	*Gallus gallus*	NP_001026234.1	42	5.39	6	18.6	355
	phosphoglucomutase-1	*Gallus gallus*	NP_001033782.2	61.5	7.01	8	14.1	172
7	myosin-binding protein H	*Gallus gallus*	NP_001026199.1	58.6	7.53	25	39.1	3246
	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	16	9.2	717
	myosin heavy chain, skeletal muscle, adult isoform X1	*Gallus gallus*	XP_015150707.1	226.3	5.96	12	6.6	482
	calsequestrin-2 precursor	*Gallus gallus*	NP_989857.1	47.1	4.23	7	13.1	931
	tubulin beta-3 chain	*Gallus gallus*	NP_001074329.2	49.8	4.86	10	25.2	186
	tubulin beta-2 chain	*Gallus gallus*	NP_001004400.1	49.9	4.89	7	17.8	141
8	tropomyosin beta chain isoform X4	*Gallus gallus*	XP_015132749.1	32.8	4.72	25	62.0	4096

Table 4.5 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	PI	Number of Peptides	Coverage (%)	Mowse Score
	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	7	4.5	568
	desmin	*Gallus gallus*	XP_015145578.1	53.5	5.38	8	17.0	502
	tropomyosin alpha-1 chain	*Gallus gallus*	NP_990732.1	32.9	4.78	8	26.8	831
9	troponin T, fast skeletal muscle isoforms isoform X27	*Gallus gallus*	XP_015142046.1	32.4	6.95	16	29.9	1813
	troponin T, fast skeletal muscle isoforms isoform X1	*Gallus gallus*	XP_015142017.1	35.7	6.29	16	27.1	1668
	actin, alpha skeletal muscle	*Gallus gallus*	NP_001026234.1	42	5.39	12	35.5	1947
	myosin heavy chain, skeletal muscle, adult isoform X2	*Gallus gallus*	XP_003642358.1	223.1	5.9	15	8.8	1246
	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	5.82	16	8.9	1271
	capping protein (actin filament) muscle Z-line, beta isoform 1	*Gallus gallus*	NP_990768.1	31.3	5.59	12	41.2	1494

Table 4.5 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	PI	Number of Peptides	Coverage (%)	Mowse Score
	capping protein (actin filament) muscle Z-line, beta isoform 2	*Gallus gallus*	NP_001167000.1	30.6	6.01	11	37.9	1242

[1] Band locations are shown on an SDS-PAGE gel in Figure 4.3.

The predominant protein in band 1 was a fragment of nebulin, a large protein (600 kDa) involved in sarcomere structural stabilization and resting tension along the actin filament (Horowits, Kempner, Bisher, & Podolsky, 1986). Band 1 is completely missing from the MSC treatments. One possibility is that further myofibrillar fragmentation took place during the separation process, which would result in smaller fragments causing them to migrate further down the gel. Bands 2-7 were predominantly fragments of myosin heavy chain. Fragments identified were of fast myosin heavy chain which are highly abundant in chicken breast muscles. One explanation for bands predominantly myosin heavy chain to be darker and present in the BT but less in MSC could be due to the inability of myosin fragments to enter the gel due to the mechanical separation process denaturing them and reducing their solubility. Interestingly, band 2 is a tight dark band in breast trim, a tight but lighter band in MSC2 and a lighter smeared band in MSC1. These observable differences may be associated with fiber type as well as damage or fragmentation during the separation process, causing MSC1 to display a variety of peptide lengths in this region. Bands 3 and 4 were also present (band 3) and darker (band 4) in all BT

replications compared to both MSC treatments. Myosin fragments were the predominant proteins present and were associated with fast myosin isoforms. Differences in this region can be explained by fiber type of the raw materials. Bands 5 and 6 contain proteins associated with glycolytic metabolism including phosphorylase b kinase regulatory subunit and phosphoglucomutase-1. Because these bands are darker in the BT samples than MSC the data suggest MSC samples contain less glycolytic metabolites related closely with white fibers. This supports the previously discussed fiber type differences between breast trim and MSC. Band 7 contains high amounts of myosin binding protein-H which is associated with binding myosin into the thick filament (Alyonycheva, Mikawa, & Fischman, 1996). The lower intensity of this band region in MSC samples could indicate greater damage of the protein during mechanical separation causing insolubility or smaller degradation products of myosin binding protein-H.

Band 8 was consistently darker in the MSC treatments than in the BT treatment. The major protein found in this band was tropomyosin, specifically more peptides from the beta chain (25) than alpha chain (8) which is associated with red fiber types (Billeter, et al., 1981). The majority of MSC myofibrillar proteins originate from muscles close to the bone which are often more red type muscles used for long term muscle stabilization therefore, the presence of greater amounts of proteins associated with red fiber type is expected.

Band 9 was darker in MSC treatments than in BT. The majority of peptides detected were associated with fast skeletal isoforms of troponin T. MSC is a mixture of fiber types so it is reasonable that it should contain proteins associated with white as well as red fibers. Troponin T present at 27 and 30 kDa is most likely proteolytic degradation

products (Carlson et al., 2017). Greater degradation could be associated with the elevated temperatures MSC undergoes during processing and/or the extended ageing time prior to being frozen.

Conclusions

Mechanically separated chicken (MSC) is a common ingredient, in many cases as the main source of protein in the product. From bologna to chicken nuggets, MSC is used in numerous consumer products due to high nutritional value and very low cost. However, the addition of MSC to processed meat products has been reported to reduce overall texture of products. The results from the present study reveal that this in part can be explained by differences in the gelation structure of MSC myofibrils. MSC myofibrils exhibit a similar overall rheological pattern to chicken breast trim with a peak, decline, and subsequent increase in storage and loss modulus with increasing temperature. However, slopes as well as total magnitude were significantly reduced in both types of MSC myofibrils. MSC myofibrils exhibited rheological patterns more similar to myofibrils of dark meat and to myofibrils subjected to oxidation. Distinct protein band patterns were observed between MSC and BT myofibrillar proteins. The myofibrillar protein profiles corroborate fiber type differences and provide evidence that fragmentation or modification of myosin may also be contributing to overall differences between MSC and BT. These results suggest that MSC composition (decreased protein content and increased collagen content) is not the only factor contributing to differences in final product texture between MSC and more whole muscle raw materials. Further research evaluating specific processing methods to increase MSC functionality is warranted.

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CHAPTER 5. GENERAL CONCLUSIONS

MSC process variations resulted in significant differences in frankfurter texture but little to no difference in myofibril gelation characteristics. Further research evaluating specific processing methods to increase MSC functionality is warranted and should be best tested in applied formulations as well as at the myofibrillar protein level to better understand how processing methods affect the properties of MSC.

Generally, mechanically separated chicken (MSC) is regarded as a by-product of the poultry industry with little functionality. MSC has been previously reported to cause reduced hardness, increased redness, and increased lipid oxidation in processed meat products (Paulsen & Nagy, 2014). However, in the present study frankfurters produced with MSC2 exhibited equal or greater quality in all textural characteristics and in lipid oxidation when compared to frankfurters produced from whole muscle chicken breast trim. In contrast, frankfurters made with MSC1 were of equal hardness to BT, were the least cohesive and, the greatest in lipid oxidation. Both MSC frankfurters were lesser in L* values and greater in a* values than chicken breast trim which can be viewed negatively or positively, depending on product type. The distinct difference between the two types of MSC opens a new research area into the exact processing parameters that create the most functional MSC. It can be concluded that not all MSC are equally functional in processed meat products and some are capable of producing equal or greater textural quality than whole muscle chicken raw materials.

Differences in MSC frankfurter quality did not relate well to myofibrillar protein gelation patterns. MSC myofibrils exhibit a similar overall rheological pattern to chicken breast trim with a peak, decline, and subsequent increase in storage and loss modulus

with increasing temperature. However, rate of each phase (increase and decrease) as well as total magnitude were significantly reduced in myofibrillar protein solutions from both types of MSC. Differences observed between MSC and BT treatments can be attributed to both fiber type as well as changes in the myofibrillar protein structure due to the mechanical separation process. The myofibrillar protein profiles add to the case of fiber type differences and fragmentation or modification of myosin contributing to overall differences observed between MSC and BT myofibrillar proteins. The current study documents distinct and repeatable differences between the protein profiles of MSC and chicken breast meat myofibrils and their physical properties during thermal gelation.

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APPENDIX. ADDITIONAL TABLES AND FIGURES

Table A.1 Mean TPA Hardness (g) of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	4770^{ab}	4562^{ab}	4823	4823^{ab}	4615^{a}	4716^{ab}	4802^{ab}	4414^{a}
MSC1	4288^{a}	4221^{a}	4314	4337^{a}	4283^{a}	4616^{a}	4672^{a}	5282^{ab}
MSC2	5448^{b}	5357^{b}	5358	5646^{b}	5789^{b}	5755^{b}	5831^{b}	5540^{b}

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)
Means in the same row were not significantly different ($P > 0.05$)
SEM = 246.77

Table A.2 Mean TPA Adhesiveness (g/s) of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	-11.33	-11.33	-15.52	-28.13	-23.42	-27.91	-12.17	-13.25
MSC1	-10.20	-22.51	-11.36	-12.10	-16.44	-6.45	-4.22	-23.51
MSC2	-22.57	-23.95	-3.89	-22.46	-26.37	-22.14	-25.42	-32.13

No significant differences were found between means at different time points with-in treatment or between treatment ($P<0.05$)
SEM = 6.97

Table A.3 Mean TPA Resilience (%) of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	36.66	37.03	36.41^{a}	37.07	36.40	36.04^{a}	36.90	36.78
MSC1	38.22	37.01	41.05^{ab}	37.37	36.66	38.62^{ab}	38.93	39.71
MSC2	40.68	41.84	42.39^{b}	40.96	41.43	42.05^{b}	41.78	40.91

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)
Means in the same row were not significantly different ($P > 0.05$)
SEM = 1.34

Table A.4 Mean TPA Cohesiveness (%) of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	0.69	0.70^{ab}	0.69	0.69	0.69	0.68	0.69	0.68
MSC1	0.67	0.65^{a}	0.70	0.65	0.64	0.66	0.67	0.69
MSC2	0.71	0.72^{b}	0.73	0.71	0.71	0.72	0.71	0.72

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)
Means in the same row were not significantly different ($P > 0.05$)
SEM = 0.014

Table A.5 Mean TPA Chewiness of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	3118	2996^{ab}	3166	3164^{ab}	3014^{a}	3073^{a}	3194^{ab}	2891^{a}
MSC1	2805	2694^{a}	2959	2784^{a}	2687^{a}	2994^{a}	3110^{a}	3572^{ab}
MSC2	3713	3703^{b}	3778	3837^{b}	3974^{b}	4036^{b}	4042^{b}	3893^{b}

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)
Means in the same row were not significantly different ($P > 0.05$)
SEM = 200.85

Table A.6 Mean TPA Springiness (%) of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	94.79^{a}	94.51^{a}	95.39^{a}	94.96^{a}	95.27	95.23^{a}	96.09	96.94
MSC1	97.59^{b}	98.19^{b}	98.28^{b}	97.72^{b}	97.38	98.10^{b}	98.80	97.81
MSC2	96.27^{ab}	96.20^{ab}	96.77^{ab}	96.13^{ab}	96.68	97.34^{ab}	97.19	96.84

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$)
Means in the same row were not significantly different ($P > 0.05$)
SEM = 0.55

Table A.7. Mean internal b* value of frankfurters for each treatment at each time point.

	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
BT	14.12^{ax}	15.18^{xy}	15.59^{xy}	15.22^{xy}	15.23^{xy}	15.08^{xy}	14.92^{xy}	16.19^{y}
MSC1	16.05^{b}	15.97	16.30	15.88	15.87	15.66	15.76	15.39
MSC2	15.19^{ab}	15.64	15.48	15.34	15.22	15.08	15.04	15.48

$^{a-b}$ Means in the same column with different superscripts are significantly different ($P < 0.05$).
$^{x-z}$ Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM = 0.31

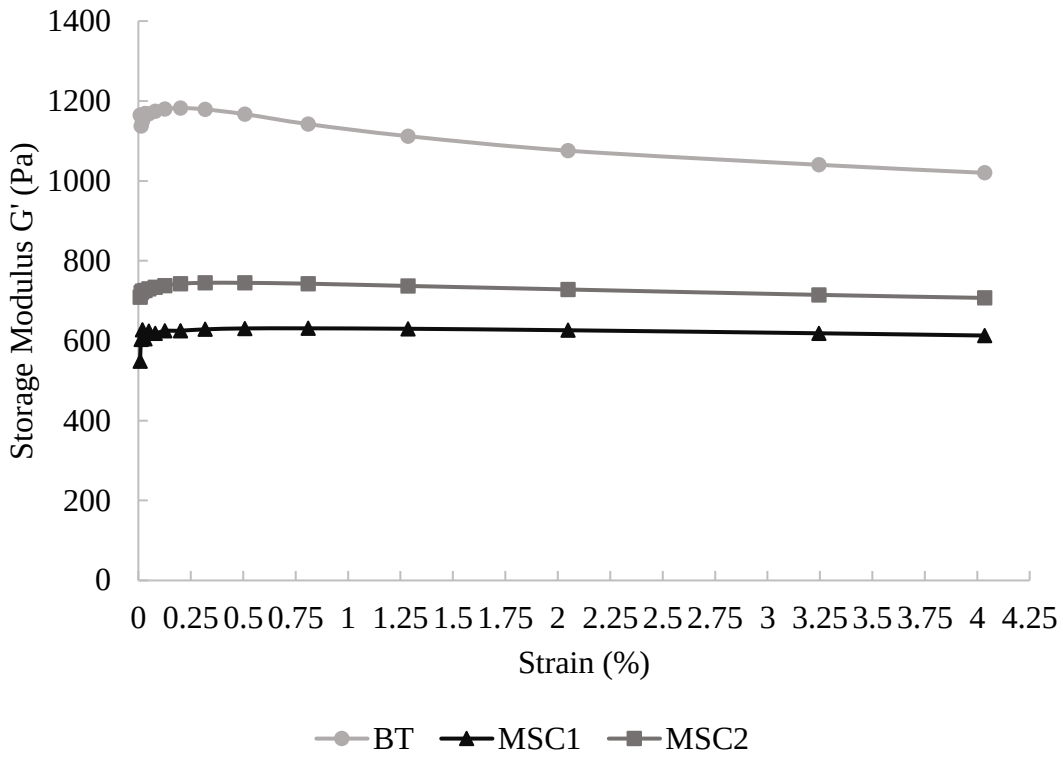

Figure A.1 Amplitude sweep used to determine the linear viscoelastic range (LVR) of the myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) of each treatment (chicken breast trim=BT; mechanically separated chicken 1= MSC1, mechanically separated chicken 2= MSC2)

Table A.8 Means for effect of treatment on storage modulus, loss modulus, and phase angle at myosin fragment transition temperatures myofibrillar protein solutions heated from 20 to 85˚C at 1 ˚C/min) (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6).

	Myosin Fragment[1]	S1	S2	Rod			LMM	MLC		MHC		
	(C˚)	47.4	54.1	44.5	50.0	55.7	51.6	48.3	57.6	46.4	54.1	63.9
G'	BT	628.15^{a}	465.16^{a}	374.58^{a}	858.64^{a}	359.06^{a}	817.03^{a}	710.01^{a}	356.66^{a}	517.61^{a}	465.16^{a}	585.94^{a}
	MSC1	240.08	296.98	205.09^{ab}	338.70	258.07^{ab}	371.22	257.48	247.13^{ab}	225.15	296.98	426.69
	MSC2	157.95	256.57	114.59^{b}	269.22	221.16^{b}	311.56	179.45	222.51^{b}	136.86	256.57	386.88
	SEM	93.98	45.86	69.04	99.04	40.54	85.44	98.61	39.61	86.64	45.86	40.64
G"	BT	112.58^{d}	69.95^{d}	68.74^{d}	141.91^{d}	46.03	132.90^{d}	122.05^{d}	37.70	96.49^{d}	69.95^{d}	42.06^{d}
	MSC1	44.68	42.17^{de}	36.36^{de}	61.83	30.60	64.44	48.29	24.18	41.36	42.17^{de}	27.85^{de}
	MSC2	28.87	34.45^{e}	20.51^{e}	44.74	24.84	49.69	31.65	20.86	24.59	34.45^{e}	23.17^{e}
	SEM	17.68	9.25	12.24	20.17	7.50	16.76	17.99	6.73	15.82	9.25	6.00
δ	BT	10.19^{x}	8.46	10.74^{xy}	9.23	7.17	9.14	9.76^{x}	5.84	10.74	8.46	3.90
	MSC1	10.82	8.28	10.33^{x}	10.57^{x}	6.95	10.04^{x}	10.88	5.75	10.70	8.28	3.76
	MSC2	10.81	7.83	10.79^{y}	9.65	6.56	9.22	10.45	5.49	10.90	7.83	3.50
	SEM	0.16	0.26	0.13	0.27	0.30	0.23	0.20	0.34	0.16	0.26	0.25

$^{a\text{-}b;\ d\text{-}f;\ x\text{-}z}$ Means in the same column with different superscripts are significantly different ($P < 0.05$); [1]Myosin fragment transition temperatures (Smyth and others 1996); S1= myosin heads; S2= myosin rod;
LMM= light-meromyosin; MLC= myosin light chain; MHC= myosin heavy chai

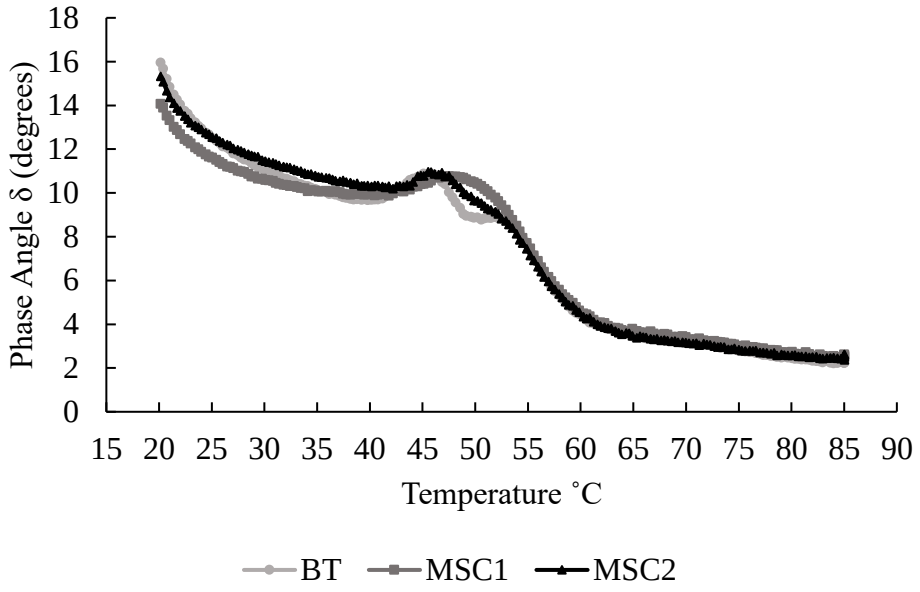

Figure A.2 Phase angle of myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) during thermal gelation (20 to 85˚C) (chicken breast trim=BT; mechanically separated chicken 1= MSC1, mechanically separated chicken 2= MSC2).

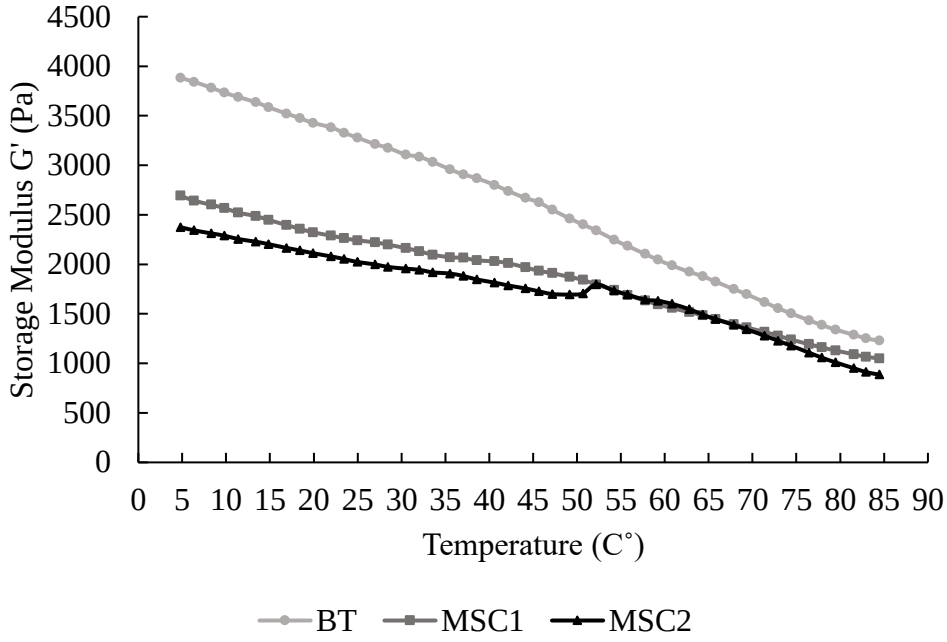

Figure A.3 Storage modulus of myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) during cooling (85 to 5˚C) (chicken breast trim=BT; mechanically separated chicken 1= MSC1, mechanically separated chicken 2= MSC2).

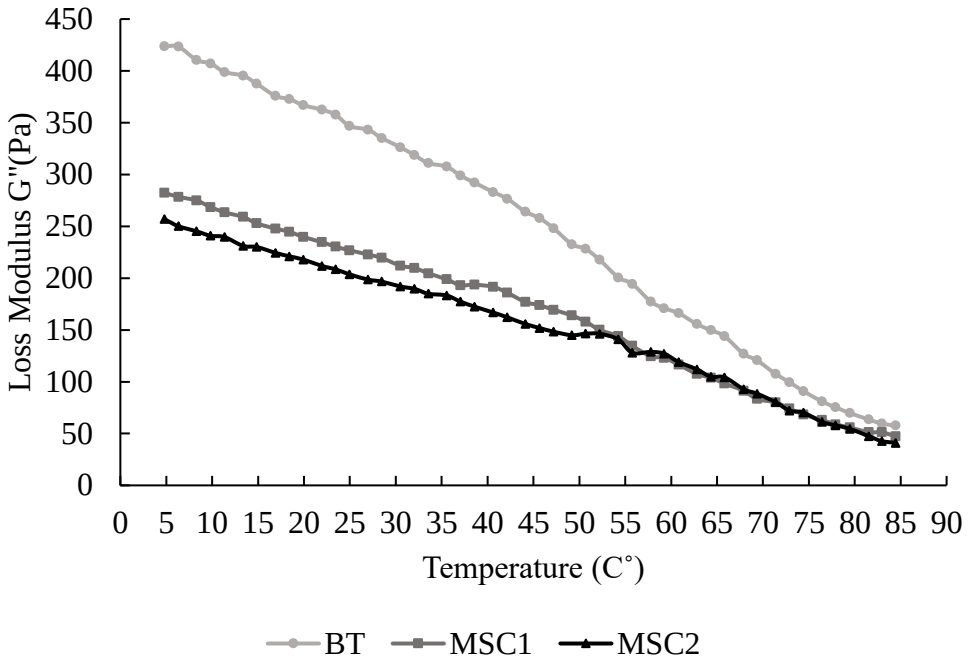

Figure A.4 Loss modulus of myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) during cooling (85 to 5˚C) (chicken breast trim=BT; mechanically separated chicken 1= MSC1, mechanically separated chicken 2= MSC2).

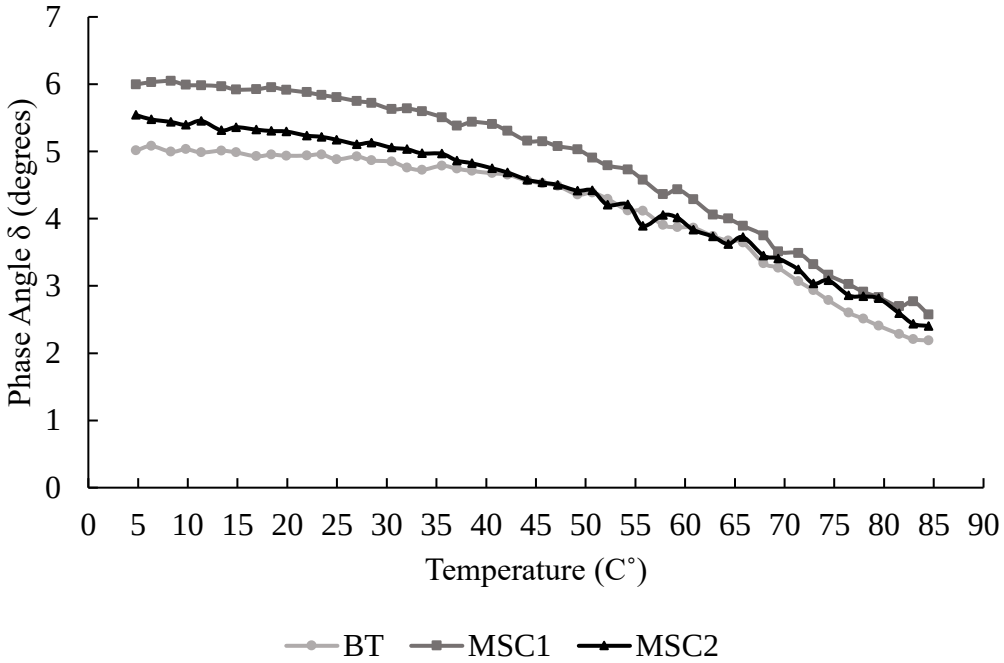

Figure A.5 Phase angle of myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) during cooling (85 to 5˚C) (chicken breast trim=BT; mechanically separated chicken 1= MSC1, mechanically separated chicken 2= MSC2).

Table A.9 Means for effect of treatment on initial and final phase angle of myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) heated from 20 to 85˚C at 1 ˚C/min.

	Initial δ (Pa)	Initial T (C˚)	Final δ (Pa)	Final T (C˚)
BT	15.62	20.15	2.35[a]	85.00
MSC1	14.00[a]	20.16	2.59[ab]	84.99
MSC2	15.36	20.17	2.61[b]	85.00
SEM	0.39	0.01	0.081	0.01

T=Temperature; [a-b] Means in the same column with different superscripts are significantly different (P < 0.05

Table A.10 Means for effect of treatment on storage modulus, loss modulus, and phase angle of myofibrillar protein solutions (2.8% protein, 0.6M NaCl, 50 mM sodium phosphate, pH 6) during cooling from 85 to 5˚C.

	Initial T (C˚)	Initial G' (Pa)	Initial G" (Pa)	Initial δ (˚)	Final T (C˚)	Final G' (Pa)	Final G" (Pa)	Final δ (˚)	Slope G' (Pa)	Slope G" (Pa)	Slope δ (˚)
BT	84.45	1365.34[a]	57.78[a]	2.46	4.74	4319[a]	442[a]	5.73	-37.87[a]	-4.85[a]	-0.04
MSC1	84.45	1049.92	47.22	2.57	4.70	2692	281	6.04	-20.34	-2.93	-0.04
MSC2	84.45	984.37	45.54	2.67	4.74	2638	282	6.14	-20.51	-2.95	-0.04
SEM	0.01	58.35	3.33	0.09	0.02	197	31	0.15	2.40	0.37	0.00

T=Temperature; [a-b] Means in the same column with different superscripts are significantly different (P < 0.05)

Table A.11 The most abundant protein identified with a list of peptides in each band from myofibrillar protein samples separated using SDS-PAGE [1.]

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
1	nebulin	*Gallus gallus*	XP_015145654.1	229.1	46.8	QIASDYK FTSLPDSMPMVLAK FTSITDTPDVVQAK SNYTLTDTPQLDMAR FDAIPIQAAK WTCLPDQNDVVQAR FALGIGK ECQTLVSDVDYR SYIAAWEK AYDLQSDNIYK ECQALVSDVDYR TYLHHWNCSPEEHDVIQAR LYTEAWDADK HYLHQWTCLPDHNDVVHAR GIGWVPIGSLDVEK GIGWSPLGSLDEEK THFSSPVDMLGIVLAK FTSVTDSLEMTLAK GIGWLPNDSLGINHVK CQYILSDLEYR ATGYLLPPDTVQIR	3148

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						YTSVVDTPDIVLAK YTSVVDSPDILLAK TTIHVMPDTPEILLAK TSIHIMPDTPGILLAQQNK IHIPADIMSVVAAK GIGWLPNDSPGVQR LVWFEHAGEIQNDR TSIHVMPDTPTILLAK KCQYILSDLEYR SDLEWLR SDLQWLR TTIHVMPDTPEILLAK CQELVSDVDYR VYDLQSDNVYK HAGDLLNER INSVNQSDLK HANYVNSELK AQEILSDR AGEILSDR HAQDLLSDK AMLLQNDR KAQEILSDR TPIDSVK ASMILSDK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						HSNDVQSELK AGQILSDK EIASDYK SDAIPIQAAK ADAIPIR AHIHMPVDAMSLQAAK AYDLQSDAVYK SGEILSDIK CQELVSDVDYK AYDLQSDNVYK GQYIGSDDTPELNHAR MNAEQLSIPK HYLHQWTCHPDQNDCIQAR KCQELVSDVDYR KVYDLQSDNVYK LYTEAWNK ILNQSLYK KAYDLQSDAVYK XCQTLVSDVDYR SDLEWMR KAYDLQSDNVYK DMSLLYSDK LNALNISNK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						AHIHMPVDAMSLQAAK AETLHFTPVADRVDYVTAK TQCHLPVDMLSIQSAK VKHAQDLLSDK TSIHVMPDTPTILLAK KAYDLQSDNFYK HYLHQWTCLPDQNDVMHAR	
2	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	43.5	TLEDQLSEIK MEGDLNEMEIQLSHANR SRKVAEQELLDATER LDEAEQLALK TKLEQQVDDLEGSLEQEK HADSTAELGEQIDNLQR TPGAMEHELVLHQLR NAYEESLDHLETLKR RANLLQAEVEELR AEDEEEINAELTAK KKHADSTAELGEQIDNLQR DTQIHLDDALR IEDEQALGMQLQK	32897

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						NALAHALQSAR KMEGDLNEMEIQLSHANR MEIDDLASNMESVSK LDEAEQIALKGGK IVESMQSTLDAEIR CNGVLEGIR LAHDSIMDLENDK KDFEISQIQSK AITDAAMMAEELKK TQEDLKEQVAMVER GKQGFTQQIEELKR AITDAAMMAEELK QAEEAEELSNVNLSK RDLEEATLQHEATAAALR LEQQVDDLEGSLEQEKK IQLELNQIK NTQGTLKDTQIHLDDALR DLEEATLQHEATAAALRK QGFTQQIEELK IEELEEELEAER LQNEVEDLMVDVER MKNAYEESLDHLETLKR LEQQVDDLEGSLEQEK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						SELKMEIDDLASNMESVSK DLEEATLQHEATAAALR MKNAYEESLDHLETLK ALQEAHQQTLDDLQVEEDKVNTLTK NALAHALQSAR TKLEQQVDDLEGSLEQEKK VLNASAIPEGQFMDSK VLNASAIPEGQFMDSKK QAEEKDALISQLSR LQDLVDKLQMK ALQEAHQQTLDDLQVEEDK LAHDSIMDLENDKQQLDEK KHADSTAELGEQIDNLQR VAEQELLDATER DFEISQLQSK VQLLHTQNTSLINTK NAYEESLDHLETLK DDKLAEIITR QGFTQQIEELKR VLNASAIPEGQFMDSK RESIFCIQYNVR DIDDLEITLAK KMEGDLNEMEIQLSHANR	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						KAITDAAMMAEELKK ELEGEVDSEQK LQTETGEYSR LEDECSELKK KLEDECSELKK SAESEKEMANMKEEFEK KAITDAAMMAEELKK GSSFQTVSALFR VRELEGEVDSEQKR LEDECSELK ANLLQAEVEELR ELEGEVDSEQKR TKYETDAIQR KEQDTSAHLER EQDTSAHLER EQYEEEQEAK EQVAMVER MINDLNTQR AGLLGLLEEMR EMANMKEEFEK SNAACAALDK SNAACAALDKK ADIAESQVNK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						SELQASLEEAEASLEHEEGK ELTYQCEEDRK KKDFEISQIQSK AGLLGLLEEMRDDK VRELEGEVDSEQK IAEKDEEIDQLK EQYEEEQEAKGELQR KKMEGDLNEMEIQLSHANR MVVLLQEK LEEAGGATAAQLEMNK LEEAGGATAAQIEMNK ADIAESQVNKLR KVAEQELLDATER IAEKDEEIDQLKR AGLLGLLEEMRDDK LQDAEEHVEAVNAK KIQHELEEAEER IQHELEEAEER ELTYQCEEDR ANLLQAEVEELR QKYEETQTELEASQK DEEIDQLKR LEEAGGATAAQIEMNKK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						YEETQTELEASQK LQNEVEDLMVDVER DALISQLSR TEELEEAKK GALEQTER MVVLLQEK ANSEVAQWR KKMEGDLNEMEIQLSHANR MEIDDLASNMESVSK ELEELSER YETDAIQR HLEEEIK ENQSILITGESGAGK EMANMKEEFEK VKELTYQCEEDRK NAYEESLDHLETLKR AITDAAMMAEELK IKEVTER EQVAMVER TEELEEAK NMDQTVK LAEIITR VLYADFK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						VLNASAIPEGQFMDSKK TLEDQLSEIKTK SLSTELFK VGNEFVTK NMDQTVK	
3	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	11.2	INQQLDTK ENQSILITGESGAGK TPGAMEHELVLHQLR VTFQLPAER VIQYFATIAASGEK DPLNETVIGLYQK AAYLMGLNSAELLK LASADIETYLLEK GQTVSQVHNSVGALAK QREEQAEPDGTEVADK EEQAEPDGTEVADK LYDQHLGK IHFGATGK IEAQNKPFDAK	1557

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						TEGGETLTVK VGNEFVTK EDQVFSMNPPK CLIPNETK SSVFVVHPK LTGAVMHYGNLK	
4	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	25.2	KVAEQELLDATER VLYADFK TPGAMEHELVLHQLR LDEAEQIALK AAYLMGLNSAELLK AITDAAMMAEELKK ENQSILITGESGAGK CNGVLEGIR AAYLMGLNSAELLK GQTVSQVHNSVGALAK GQTVSQVHNSVGALAK VAEQELLDATER NKDPLNETVIGLYQK IEDMAMMTHLHEPAVLYNLK	4713

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						VIQYFATIAASGEK SYHIFYQIMSNK VTFQLPAER DPLNETVIGLYQK QGFTQQIEELKR VQLLHTQNTSLINTK DTQIHLDDALR TKYETDAIQR IEAQNKPFDAK ELEGEVDSEQKR LYDQHLGK EEQAEPDGTEVADK IHFGATGK LASADIETYLLEK ANLLQAEVEELR GSSFQTVSALFR MFLWMVIR TEELEEAK INQQLDTK MFLWMVIR QREEQAEPDGTEVADK TEGGETLTVK VGNEFVTK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						IAEKDEEIDQLKR LTGAVMHYGNLK LTGAVMHYGNLK CIIPNETK SSVFVVHPK EDQVFSMNPPK LQDAEEHVEAVNAK LQDLVDK EDQVFSMNPPK CASLEK SYHIFYQIMSNK GALEQTER ADIAESQVNK LMANLR NALAHALQSAR DALISQLSR	
5	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	20.7	SNNFQKPKPAK IQLELNQIK LQQFFNHHMFVLEQEEYKK IEDMAMMTHLHEPAVLYNLK	6795

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						SYHIFYQIMSNK VTFQLPAER VAEQELLDATER DTQIHLDDALR KVAEQELLDATER NKDPLNETVIGLYQK MFLWMVIR RVIQYFATIAASGEK GSSFQTVSALFR ANLLQAEVEELR LASADIETYLLEK AAYLMGLNSAELLK DPLNETVIGLYQK KGSSFQTVSALFR VIQYFATIAASGEK TPGAMEHELVLHQLR LQDAEEHVEAVNAK VGNEFVTK TEGGETLTVK TKYETDAIQR IEAQNKPFDAK IHFGATGK LYDQHLGK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						EEQAEPDGTEVADK INQQLDTKQPR QREEQAEPDGTEVADK ADIAESQVNK INQQLDTK VKVGNEFVTK TPGAMEHELVLHQLR CLIPNETK EDQVFSMNPPK ENQSILITGESGAGK GQTVSQVHNSVGALAK LTGAVMHYGNLK SSVFVVHPK MFLWMVIR AITDAAMMAEELKK	
6	myosin, heavy chain 1E, skeletal muscle	*Gallus gallus*	NP_001013415.1	223	17.1	INQQLDTK IEDMAMMTHLHEPAVLYNLK SYHIFYQIMSNK VTFQLPAER DTQIHLDDALR	5584

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						VAEQELLDATER LDEAEQIALK TPGAMEHELVLHQLR NKDPLNETVIGLYQK GSSFQTVSALFR LASADIETYLLEK AAYLMGLNSAELLK DPLNETVIGLYQK VIQYFATIAASGEK IQLELNQIK VGNEFVTK TEGGETLTVK IEAQNKPFDAK LYDQHLGK IHFGATGK EEQAEPDGTEVADK QREEQAEPDGTEVADK ADIAESQVNK ALCYPR ENQSILITGESGAGK GQTVSQVHNSVGALAK CLIPNETK LTGAVMHYGNLK	

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						SSVFVVHPK VKVGNEFVTK EDQVFSMNPPK MFLWMVIR	
7	myosin-binding protein H	*Gallus gallus*	NP_001026199.1	58.6	39.1	INGAEDK GYSTHLFCSVR DMMEPPKFTQPLTDR AILDIR KPSPFDAGVYTCK AVNPLGEASVDCK DTIFFIR WFTVLER SSLDGYVVEICK NTDKDTIFFIR AISASGTSDPATLEQPVLIR VFSENACGMSETAAVAAGVAHIK DGSTDWTAVNKEPFLSTR EPFLSTR IHDLASGEK TVYQPQK DMMEPPK	3246

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						DNQPLDTSR EITDLPR YQLSVR DGSTDWTAVNK VIERPGPPQNLK TVYQPQKIPER FTQPLTDR IPERDMMEPPK	
8	tropomyosin beta chain isoform X4	*Gallus gallus*	XP_015132749.1	32.8	62.0	HIAEEADR KATDAEAEVASLNRR KATDAEAEVASLNR ATDAEAEVASLNRR LKGTEDEVEKYSESVK GTEDEVEKYSESVK TIDDLEDEVYAQK KLVVLEGELER IQLVEEELDR LVVLEGELER KYEEVAR SLEAQADK LKGTEDEVEK	4096

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						LDKENALDR EAQEKLEQAEK IVTNNLK LATALQK QLEEEQQGLQKK QLEEEQQGLQK EDKYEEEIK MEIQEMQLK AMKDEEKMEIQEMQLK SLEAQADKYSTK LEEAEKAADESER	
9	troponin T, fast skeletal muscle isoforms isoform X27	*Gallus gallus*	XP_015142046.1	32.4	29.9	KAEDDLKK ALSSMGASYSSYLAK IPEGEKVDFDDIQK IPEGEKVDFDDIQKK YDFAEQIK YDFAEQIKR KPLNIDHLNEDKLR KYEIVTLR	1813

Table A.11 (continued)

Band	Protein ID	Species	Accession Number	Molecular Weight (kDa)	Coverage (%)	Peptides	Mowse Score
						RKPLNIDHLNEDK KPLNIDHLNEDK KEEEELVALK KKYEIVTLR RKEEEELVALK VDFDDIQK RKPLNIDHLNEDKLR EEEELVALK	

[1] Band locations can be seen on an SDS-PAGE gel in Figure 4.3.