Rheological characteristics of mechanically separated chicken and chicken breast trim myofibril solutions during thermal gelation

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ABSTRACT

Mechanically separated chicken (MSC) was obtained by two different separation methods (MSC1, Beehive separator, 3–5 d-old bones; MSC2, Poss separator, fresh bones) and compared to chicken breast trim (CBT). Rheological attributes of myofibrillar protein solutions during thermal gelation and cooling were evaluated. All sources exhibited gelation with increased temperature (decreased δ). In all three treatments, a peak, decline, and subsequent increase in both the G′ and G″ was observed in the 50–55 °C range, with peak values being higher for CBT than for both MSCs. G′ slopes on both sides of the peak (S2, S3) and following the decline (S4) were significantly different between CBT and both MSCs (P < 0.05) and indicated greater instability of the solid-like structure in the temperature range of 50–55 °C (myosin rod denaturation). Myofibrillar protein profiles confirmed fiber type differences among materials, as well as greater myosin fragmentation or modification in the MSC samples.

1. Introduction

It is well known that MSC, compared to intact muscle sources, can impact the eating quality of further processed products, by softening texture, introducing grittiness, increasing off-flavors and increasing redness (Daros, Masson, & Amico, 2005; Froning & Johnson, 1973; Paulsen & Nagy, 2014). In comminuted sausages, replacement of beef and pork raw materials with MSC in excess of 40% decreased both compressive and tensile strength of the product (Daros et al., 2005). Research categorizing MSC behavior in a fundamental system has been limited to MSC surimi and has not been compared to the whole muscle alternatives (Cortez-Vega et al., 2015; Smyth & O’Neill, 1997). Many attempts to improve the functionality of MSC involve post-separation techniques to reduce bone, fat, and connective tissue content, such as washing, surimi-like, processes (Cortez-Vega et al., 2015; Smyth & O’Neill, 1997). During mechanical separation, proteins undergo increased heat (5–8 °C) and pressure (> 6.2 MPa), known to affect myofibrillar protein structure (Grossi et al., 2016; Liu, Zhao, Xiong, Xie, & Qin, 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 by shifting secondary structure towards beta sheets at temperature as low as 15 °C (Liu et al., 2008). The secondary structure of myosin, the primary gel-forming protein, converts from alpha helix to beta sheets as temperature increases (Liu et al., 2008), with myosin heavy chains becoming insoluble at 55 °C (Cheng & Parrish, 1979). High hydrostatic pressure has also been shown to induce protein denaturation and even gelation, through structural changes different than those caused by heat (Lee, Kim, Lee, Hong, & Yamamoto, 2007). Zhang, Yang, Tang, Chen, and You (2015) showed a decrease in sulphydryl content with an increase in pressure from 100 to 500 MPa, indicating these high pressures have the ability to induce disulfide bond formation. These pressures, however, are much lower than those encountered during the mechanical separation process.

Dynamic oscillatory rheological measurements are able to determine 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). It has been observed that, during thermal gelation of...
myofibrillar proteins, as temperature increases G' (measure of solid-like characteristics) and G" (liquid-like characteristics) typically increase, then decrease sharply, before increasing once again. This behavior has been consistently observed across species and muscle types, as well as at varying ionic strength, pH, protein concentration and presence of phosphate (Egelandsdal, Fretheim, & Samejima, 1986; Lesiów & Xiong, 2001; Smyth & O'Neill, 1997; Westphalen, Briggs, & Lonergan, 2005, 2006; 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.

The objective of this study was to evaluate the extent to which two different commercial mechanical separation processes affect the rheological and functional behavior of the resulting chicken raw material. The first process (MSC1) was a high-speed and high-yielding one which utilized cages and necks held in a cooler for 3–5 d prior to separation. The second process (MSC2) was designed to reduce separation 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. Myofibrillar extracts from both of these materials were compared to those of whole muscle chicken breast. We hypothesized that chicken breast myofibrillar proteins would produce greater storage modulus than both MSCs and that 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.

2. Materials and methods

2.1. Raw materials

2.1.1. Raw material sourcing

All poultry raw materials were sourced from commercial facilities. Two types of mechanically separated chicken (MSC1 and MSC2) materials made under different processing conditions were used and individually compared to chicken breast trim (pectoralis major; CBT) (breast trim was selected due to its origin from the main shell of the bird rather than the dark leg meat portions, its consistency, and its wide industrial application). The 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. CBT was sourced from commercial broiler chickens (Cobb 500, Gallus domesticus) approximately 42 d of age at time of harvest, and was sampled from one production lot to limit variation in poultry fat content. All poultry raw materials were packaged in 18.2-kg boxes, 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.

2.1.2. Raw material analysis

2.1.2.1. Proximate analysis. Raw materials were prepared by grinding to homogeneity in a food processor (KFP715WH2, KitchenAid, St. Joseph, Michigan, USA). Protein content was determined by the CEM Sprint Rapid Protein Analyzer (CEM Corporation Matthews, NC, USA) (Official Method 2011.04, AOAC International), moisture content by the CEM SMART 6 system (CEM Corporation Matthews, NC, USA) (Official Method 2008.06, AOAC International) and fat content by the CEM ORACLE system (CEM Corporation Matthews, NC, USA) (Official Method 2008.06, AOAC International). All analyses were done in duplicate and averaged.

2.1.2.2. pH. Ten g of sample 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 and the electrode placed in the liquid that accumulated in the cone. The pH of the filtrate was measured using a Mettler Toledo SevenMulti pH meter (Columbus, OH, USA) with an InLab Solids Pro-ISM electrode. All measurements were done in duplicate.

2.1.2.3. Lipid oxidation. Lipid oxidation was measured by the 2-thiobarbituric acid procedure according to Tarladgis, Watts, Youngathan, and Dugan (1960). Briefly, 10 g of sample were weighed into round-bottom flasks and mixed with 1 mL of HCl (1:2 concentrated HCl: H2O) and 97 mL of distilled water. The flasks were attached to a distillation apparatus and heated on a burner until 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 at 532 nm was measured using a Beckman DU 640 spectrophotometer (Model 4320940, Beckman Instruments, Inc., Fullerton, CA, USA). Absorbance values were converted to μg of malondialdehyde per g of sample by multiplying by a factor of 7.8. Analyses were performed in duplicate and results were averaged.

2.2. 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 (Doerscher, Briggs, & Lonergan, 2004; Westphalen et al., 2005, 2006). All steps were done 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 (100 mM Tris, 10 mM EDTA, pH 8.3) using a Kinematic Polytron Homogenizer (Brinkman Instruments, Inc., Westbury, NY). Samples were then centrifuged at 1000 × 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 K2HPO4 /KH2PO4, 2 mM MgCl2, 1 mM EGTA, 1 mM NaNO3, pH 7). The samples were then resuspended in 4 volumes of a standard salt solution with 1% Triton X-100 and centrifuged at 1500 × g for 10 min between washes. The resulting pellet was then resuspended in 4 volumes of a standard salt solution with 1% Triton X-100 and centrifuged at 1500 × g for 10 min 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 (added for cryoprotection). Samples were then stored in 50-mL centrifuge tubes at −20 °C until needed.

2.3. Preparation of myofibril solutions

Myofibrils suspended in the glycerol buffer were equilibrated to 4 °C and then diluted with four volumes (w/v) of standard salt solution and centrifuged at 3000 × g for 10 min. Pellets resuspended four times in one volume of 50 mM sodium phosphate monobasic buffer (pH 6) each time and centrifuged at 3000 × g for 10 min after each wash to remove any glycerol. The protein content of the resulting pellets was determined using the Bio-Rad DC Protein Assay (Hercules, CA, USA). The pellets were then diluted to 5.6% protein using 50 mM sodium phosphate buffer at pH 6. Pellets were subsequently diluted by half using 1.2 M NaCl, 50 mM solution at pH 6 for a final NaCl 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 them. The samples were mixed thoroughly and stored at 4 °C for rheological measurements within six days.

2.4. Dynamic oscillatory rheology

2.4.1. Temperature sweep experiment

Rheological experiments were done using a Discovery Hybrid Rheometer HR-2 running TRIOS software version 4.2.2.36612 (TA Instruments, New Castle, DE, USA). 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 performed 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. Heating and cooling rate and temperature range settings were determined based on previous research investigating gelation of muscle proteins (Doerscher et al., 2004; Westphalen et al., 2005, 2006; Xiong & Blanchard, 1994b). The temperature ramp was from 20 °C to 85 °C at a rate of 1 °C min⁻¹. The measurements started once the sample reached 20 °C and there was a soak time of 3 min at 85 °C to ensure the entire 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 also taken during cooling, at a starting temperature of 85 °C, final temperature of 5 °C, and a rate of 5 °C min⁻¹. 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 dehydration during experiment. The experiment was replicated 3 times and rheological measurements collected in triplicate.

2.4.2. 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, then cooled to 21 °C. The top plate was then lowered to the surface of the gel and an amplitude sweep was performed from 0 to 4% strain at a frequency of 1 Hz.

2.4.3. Rheological slope calculations

Slopes of subsequent linear portions of the rheograms were obtained by linear regression of segments of the curve delimited by the inflection points of the transition temperatures. Inflection points were determined by the second derivative of the curves. These slopes were compared to understand the relationship between time and temperature, and storage (G') and loss (G″) moduli values. The data points from each rheological run (3 per replication, 9 per treatment) were graphed in Microsoft Excel with G' and G″ on the y-axis and temperature on the x-axis. Once graphed the inflection points at each transition were identified and the slopes between them were calculated. Slopes between treatments were then analyzed statistically. These slopes are graphically illustrated in Fig. 1A.

2.5. SDS-PAGE

Myofibrillar proteins were separated 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 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 for a final protein concentration of 4 mg/mL. Forty µg protein were loaded into individual wells of a 10 cm × 12 cm × 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 a solution 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.

2.6. Protein identification

SDS-PAGE was performed using the same process as described above and unique bands were excised for identification. 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). Peptides from the extracted bands were analyzed by tandem mass spectrometry (MS/MS) using a Thermo Scientific Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Thermo Scientific’s Proteome Discoverer Software (Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the raw data for identification of matched proteins and peptides against publicly available or user-provided databases. Sample fragmentation patterns were compared to known 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).

2.7. Experimental design and statistical analysis

The experiment was replicated three times. An MSC replication consisted of a separate production lot of each raw material. Production lots were from three consecutive production days. Myofibril isolation from each of these lots was conducted on three consecutive days. Chicken breast trim products from three separate boxes were randomly assigned to each of three replication days. Rheological data were analyzed using the PROC MIXED procedure of the Statistical Analysis System (SAS v. 9.4, SAS Institute, Cary, NC, USA) with treatment (MSC1, MSC2, CBT) as fixed factors, and day (day samples were tested on the rheometer) and day × treatment as random factors. Significance was determined at P < 0.05.

3. Results and discussion

3.1. Raw material analyses

The composition of the chicken raw materials used in this study is shown in Table 1. All raw materials were significantly different (P < 0.05) from each other in moisture and fat. Protein content was higher in CBT than in both MSC materials. Moisture, fat, and protein contents were similar to those previously reported for similar raw materials (Ang & Hamm, 1982; Hamm & Young, 1983; Li et al., 2015; Perlo, Bonato, Teira, Fabre, & Kueider, 2006; Soglia et al., 2016). pH of
3.2. Dynamic viscoelastic properties

3.2.1. Thermal gelation

All sources displayed typical transitions in storage modulus ($G'$) and loss modulus ($G''$) over the temperature range of 20 °C to 85 °C (Lesiów & Xiong, 2001; Liu & Xiong, 1996; Westphalen et al., 2005; Xiong & Blanchard, 1994a) as shown in Fig. 1. 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 peak patterns 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 2 and 3 respectively.

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 (Lesiów & Xiong, 2001). The observed S1 region transition temperature of 47.4 °C coincides with previously observed increases in storage modulus (Smith, Smith, Vega-Warner, & O’Neill, 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. Peak $G'$ for CBT, however, was over 100% higher ($P < 0.05$) and occurred at slightly lower temperature (for CBT than for both MSCs, indicating formation of a stronger elastic gel network, which could be attributed to the higher proportion of white muscle fibers found in the CBT material, as reported elsewhere. Myosin from chicken breast can produce longer filaments and aggregate more. Following the peak, a sharp decline in $G'$ occurs from 50.4–51.9 °C to 56.6–58.0 °C. This abrupt decrease in solid-like structure is attributed to the denaturation of the light meromyosin (LMM) portion of the myosin filament, as it coincides with the transition temperature of LMM, reported as 51.6 °C (Lesiów & Xiong, 2001). Following 56.6–58.0 °C, all samples continued to rise in $G'$. Storage modulus of CBT was significantly greater ($P < 0.05$) at all points of interest than that of MSC2 and was significantly greater ($P < 0.05$) than MSC1 at the identified peak and final storage modulus, indicating formation of a stronger elastic gel network. MSC2 had lower $G'$ ($P < 0.05$) at the first inflection point than both MSC1 and CBT but was not significantly different ($P < 0.05$) from MSC1 at any other point of interest. The temperature of CBT at the first inflection point was significantly lower by 5 °C, indicating it is less thermally stable than the MSC sources. This trend was also seen in the identified peak temperature with $G''$ being 2 °C lower than the MSC sources. However, the second inflection point for MSC1 occurred at a significantly higher ($P < 0.05$) temperature than for MSC2 and CBT. This could indicate a difference in the thermal stability of the myofibrillar proteins that are denatured in that temperature range, particularly the LMM portion (Lesiów & Xiong, 2001; Xiong & Blanchard, 1994b).

Calculated slopes of the $G'$ and $G''$ rheograms are shown in Tables 2 and 3. The slope 1 of MSC2 was higher than that of CBT.

Lipid oxidation levels were different ($P < 0.05$) for all three materials, being highest for MSC1 (4.59), followed by MSC2 (0.49) and CBT (0.22). The much higher value for MSC1 was most likely due to the higher amount of physical disruption (reduced particle size) and mechanical energy (which increases pressure and heat generation) applied during its obtainment process, which can make fat and pro-oxidant minerals (e.g., iron) more available (Froning & Johnson, 1973; Paulsen & Nagy, 2014).

### Table 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>pH</th>
<th>TBARS (μg MDA/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBT</td>
<td>74.4±a</td>
<td>2.4±c</td>
<td>23.4±a</td>
<td>5.88b</td>
<td>0.22±c</td>
</tr>
<tr>
<td>MSC1</td>
<td>68.35±c</td>
<td>16.17±a</td>
<td>14.40±b</td>
<td>6.82±a</td>
<td>4.59±c</td>
</tr>
<tr>
<td>MSC2</td>
<td>71.00±b</td>
<td>14.83±b</td>
<td>14.00±b</td>
<td>6.70±b</td>
<td>0.49±b</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.34±</td>
<td>0.16±</td>
<td>0.14±</td>
<td>&lt; 0.01</td>
<td>1.15±</td>
</tr>
</tbody>
</table>

* a-c Means in the same column with different superscripts are significantly different ($P < 0.05$).
S.E.M.: standard error of mean.

MSC1 and MSC2 was higher than that of CBT.

Lipid oxidation levels were different ($P < 0.05$) for all three materials, being highest for MSC1 (4.59), followed by MSC2 (0.49) and CBT (0.22). The much higher value for MSC1 was most likely due to the higher amount of physical disruption (reduced particle size) and mechanical energy (which increases pressure and heat generation) applied during its obtainment process, which can make fat and pro-oxidant minerals (e.g., iron) more available (Froning & Johnson, 1973; Paulsen & Nagy, 2014).

3.2.2. Phase separation during thermal gelation as the more liquid-like structure is attributed to the denaturation of the light meromyosin (LMM) portion of the myosin filament, as it coincides with the transition temperature of LMM, reported as 51.6 °C (Lesiów & Xiong, 2001). Following 56.6–58.0 °C, all samples continued to rise in $G'$. Storage modulus of CBT was significantly greater ($P < 0.05$) at all points of interest than that of MSC2 and was significantly greater ($P < 0.05$) than MSC1 at the identified peak and final storage modulus, indicating formation of a stronger elastic gel network. MSC2 had lower $G'$ ($P < 0.05$) at the first inflection point than both MSC1 and CBT but was not significantly different ($P < 0.05$) from MSC1 at any other point of interest. The temperature of CBT at the first inflection point was significantly lower by 5 °C, indicating it is less thermally stable than the MSC sources. This trend was also seen in the identified peak temperature with $G''$ being 2 °C lower than the MSC sources. However, the second inflection point for MSC1 occurred at a significantly higher ($P < 0.05$) temperature than for MSC2 and CBT. This could indicate a difference in the thermal stability of the myofibrillar proteins that are denatured in that temperature range, particularly the LMM portion (Lesiów & Xiong, 2001; Xiong & Blanchard, 1994b).

Calculated slopes of the $G'$ and $G''$ rheograms are shown in Tables 2 and 3. The slope 1 of MSC2 was significantly smaller ($P < 0.05$) prior to the first inflection point than in MSC1 and CBT. For the remaining three slopes, CBT myofibrillar proteins exhibited significantly steeper ($P < 0.05$) transitions, particularly the slopes on either side of the identified peak. Slope 2 and slope 3 of CBT were 3 and 4 times steeper, respectively, than for both MSC sources. $G''$ displayed a pattern with inflection points and a peak at similar temperatures as the $G'$, as shown in Tables 2 and 3.

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 CBT. Phase angle of MSC1 was also significantly larger ($P < 0.05$) at the denaturation temperature (51.6 °C) of LMM than that of MSC2 and CBT, indicating a less solid gel structure at that temperature. More interestingly, δ of CBT (47.4 °C) was significantly lower ($P < 0.05$) than for both MSCs, indicating CBT 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 CBT.

Much of the difference observed in rheological properties of...
myofibrillar protein between different treatments in the present study may be attributed to differences in muscle fiber type. Chicken breast meat has for years been selected for large and rapid muscle growth and has therefore been shifted to almost entirely white fiber type (Petracci, Mudalal, Solgia, & 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 are known to contain predominantly red fiber types (Suzuki, Tsuchiya, Ohwada, & Tamate, 1985), and their proteins have been shown to exhibit lower G′ and G″ than those of chicken pectoralis muscles, with their G′ transition temperatures, but not peak G′, being pH-dependent (higher transition temperature at higher pH, observed in measured pH range of 5.87 to 6.53) (Xiong & Blanchard, 1994a). Both differences in magnitude of G′ and transition temperatures of thigh/leg (red fiber) and breast meat (white fiber) were also observed by Liu and Xiong (1996) in chicken myofibrillar proteins. In the present study, the rheological patterns of both MSC and CBT 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 is the state of degradation, oxidation, or fragmentation of the myofibrillar proteins. The mechanical separation process exposes muscle tissue to factors that could potentially affect the structure of myofibrillar proteins. The process, as previously stated, applies pressure, increases heat, reduces particle size and makes fat and pro-oxidant minerals such as iron more available (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. Liu and 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 for leg meat. However, in the presence of antioxidants, G′ was not affected by the addition of iron and fat. They also found that the extent of lipid oxidation in breast and leg muscle myofibrils did not have a major influence in their rheological properties, an observation that our results agree with, considering the variability in TBARS values between MSC1 and MSC2 (Table 1) and the similarities in their peak G′ and G″ values (Tables 2 and 3). 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, Diao, Li, Chen, & Kong, 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 (Cao, True, Chen, & Xiong, 2016; Chen et al., 2016; Zhou, Sun, & Zhao, 2015; Zhou, Zhao, Su et al., 2014; Zhou, Zhao, Zhao et al., 2014).

Other studies have also demonstrated that pH, ionic strength, and temperature impact rheological properties (Liu & Xiong, 1997; Liu et al., 2008; Liu, Zhao, Xiong, Liu, & Xie, 2007; Westphalen et al., 2005). Lower pH (< 6.0) causes secondary structure to shift towards beta sheets and a plateau in G′ at the temperatures where LMM denatures (Liu et al., 2008; Westphalen et al., 2005). In the present study the pH of the myofibrillar solutions was adjusted and buffered 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 materials, a phenomenon that is absent in the CBT. The temperature of the final sharp increase in G′ of MSC1 was significantly higher (P < 0.05) than for both MSC2 and CBT. 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.

### Table 2
Storage modulus, temperatures, inflection points and calculated slopes of myofibrillar protein solutions during heating.

<table>
<thead>
<tr>
<th>Initial</th>
<th>Inflection 1</th>
<th>Peak</th>
<th>Inflection 2</th>
<th>Final</th>
<th>Slopes (Pa °C⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G′ (Pa)</td>
<td>T (°C)</td>
<td>G′ (Pa)</td>
<td>T (°C)</td>
<td>G′ (Pa)</td>
<td>T (°C)</td>
</tr>
<tr>
<td>CBT</td>
<td>125.19</td>
<td>20.15</td>
<td>181.07</td>
<td>39.90</td>
<td>863.67</td>
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<td>MSC1</td>
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<td>20.16</td>
<td>203.19</td>
<td>45.20</td>
<td>371.48</td>
</tr>
<tr>
<td>MSC2</td>
<td>64.36</td>
<td>20.17</td>
<td>106.14</td>
<td>44.97</td>
<td>315.62</td>
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<td>S.E.M.</td>
<td>18.60</td>
<td>0.01</td>
<td>16.70</td>
<td>1.36</td>
<td>97.46</td>
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</tbody>
</table>

**Means in the same column with different superscripts are significantly different (P < 0.05).**

T: Temperature; S.E.M.: standard error of mean.

### Table 3
Loss modulus, temperatures, inflection points and calculated slopes of myofibrillar protein solutions during heating.

<table>
<thead>
<tr>
<th>Initial</th>
<th>Inflection 1</th>
<th>Peak</th>
<th>Inflection 2</th>
<th>Final</th>
<th>Slopes (Pa °C⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G′ (Pa)</td>
<td>T (°C)</td>
<td>G′ (Pa)</td>
<td>T (°C)</td>
<td>G′ (Pa)</td>
<td>T (°C)</td>
</tr>
<tr>
<td>CBT</td>
<td>36.63</td>
<td>20.15</td>
<td>30.51</td>
<td>39.51</td>
<td>142.52</td>
</tr>
<tr>
<td>MSC1</td>
<td>25.14</td>
<td>20.16</td>
<td>30.29</td>
<td>40.81</td>
<td>65.05</td>
</tr>
<tr>
<td>MSC2</td>
<td>15.72</td>
<td>20.17</td>
<td>13.64</td>
<td>41.75</td>
<td>50.21</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>3.98</td>
<td>0.01</td>
<td>4.16</td>
<td>0.97</td>
<td>19.96</td>
</tr>
</tbody>
</table>

**Means in the same column with different superscripts are significantly different (P < 0.05).**

T: Temperature; S.E.M.: standard error of mean.

1. 0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6.0.
2. 20 °C to 85 °C at 1 °C min⁻¹.

3.2.2. Cooling

During cooling from 85 °C to 5 °C at a rate of 5 °C min⁻¹, 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...
CBT. No significant difference ($P < 0.05$) was found for any δ points during cooling of the myofibril gels. Myofibrillar proteins from CBT resulted in a stronger gel, as expected, when compared to MSC materials. However, MSC materials were not significantly different ($P < 0.05$) from each other in ultimate G’.

### 3.3. Myofibrillar protein profile

Distinct and repeatable myofibrillar protein profiles were identified for each treatment, as depicted in a representative gel in Fig. 2. Prominent dark bands in the region of myosin heavy chain (223 kDa) and actin (42 kDa) were detected for all treatments and replications. Most of the bands excised were from the CBT material, as depicted in Fig. 2.

The predominant protein in band 1 was a fragment of nebulin (Table 4), 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 materials. One possibility is that further myofibrillar fragmentation took place during the separation process, which would result in smaller fragments that 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. Band 2 is a tight dark band in CBT, 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 CBT replications, compared to both MSC materials. 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 CBT samples than in MSC, the data suggest that MSC samples contain less glycolytic metabolites that are 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 (Alcyoncheva, 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 materials than in the CBT. 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 (Billetter, Heizmann, Reist, Howald, & Jenny, 1981). Most of the myofibrillar proteins of MSC 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 materials than in CBT. Most of the peptides detected were associated with fast skeletal isoforms of tropomycin T. MSC is a mixture of fiber types so it is reasonable that it could contain proteins associated with white as well as red fibers. Tropomycin T, present at 27 and 30 kDa, most likely represents proteolytic degradation products (Carlson et al., 2017). Greater degradation could be associated with the elevated temperatures that MSC undergoes during processing and/or the extended ageing time prior to it being frozen.

### 4. Conclusions

Mechanically separated chicken (MSC) is a common ingredient, in many cases as the main source of protein in further processed meat products. It is used in numerous consumer products due to its 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 can partly be explained by differences in the gelation structure of MSC myofibrils. The overall rheological pattern of MSC myofibrils is similar to that of chicken breast trim, with a peak, decline, and subsequent increase in storage and loss modulus with increasing temperature. However, the slopes and total magnitude were significantly reduced in both types of MSC myofibrils. MSC myofibrils exhibited rheological patterns more similar to those of myofibrils of dark meat and to myofibrils subjected to oxidation. Distinct protein band patterns were observed between MSC and CBT myofibrillar proteins. The myofibrillar protein profiles corroborate fiber type differences and provide evidences that fragmentation or modification of myosin may also be contributing to overall differences between MSC and CBT. These results suggest that the composition of MSC (decreased protein content and increased collagen content) is not the only factor that contributes to differences in final product texture between MSC and intact muscle raw materials. Future research should closely evaluate the effects of specific processing methods and variables (e.g., time between deboning and mechanical separation, freezing and thawing protocols) on the functionality of MSC.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgments

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Table 4
Proteins identified in each band from myofibrillar protein samples separated using SDS-PAGE\(^1\).

<table>
<thead>
<tr>
<th>Band</th>
<th>Protein ID</th>
<th>Species</th>
<th>Accession number</th>
<th>Molecular weight (kDa)</th>
<th>pl</th>
<th>Number of peptides</th>
<th>Coverage (%)</th>
<th>Mouse score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>nebulin-like</td>
<td>Gallus gallus</td>
<td>XP_015145654.1</td>
<td>229.1</td>
<td>8.73</td>
<td>72</td>
<td>46.8</td>
<td>3148</td>
</tr>
<tr>
<td>2</td>
<td>nebulin</td>
<td>Gallus gallus</td>
<td>XP_015145352.1</td>
<td>328.8</td>
<td>9.14</td>
<td>31</td>
<td>13.2</td>
<td>1620</td>
</tr>
<tr>
<td>3</td>
<td>myosin, heavy chain 1E, skeletal muscle</td>
<td>Gallus gallus</td>
<td>NP_001013415.1</td>
<td>223</td>
<td>5.82</td>
<td>35</td>
<td>19.3</td>
<td>904</td>
</tr>
<tr>
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<td>Gallus gallus</td>
<td>XP_01517811.1</td>
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<td>5.86</td>
<td>26</td>
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<td>NP_001013414.1</td>
<td>222.8</td>
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<td>22</td>
<td>13.6</td>
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<td>6</td>
<td>myosin, heavy chain 1E, skeletal muscle</td>
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<td>NP_001013415.1</td>
<td>223</td>
<td>5.82</td>
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<td>Gallus gallus</td>
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<td>223</td>
<td>5.82</td>
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<tr>
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<tr>
<td>9</td>
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</tbody>
</table>

\(^1\) Band locations are shown in Fig. 2.

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References


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