Rheological Behavior of Chicken Breast Meat and Mechanically Separated Chicken Myofibril Solutions during Thermal Gelation

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Summary and Implications

Mechanically separated chicken (MSC) obtained from two different commercial mechanical separation processes was compared to commercial chicken breast trim. Rheological analyses showed that the gentler mechanical separation process resulted in material with better thermal gelation properties than the more aggressive process, which could result in a more functional raw material. Myofibrils from the more intact breast muscle, however, had better thermal gelation properties than both types of MSC tested. These results indicate that the type of mechanical separation process used can influence the functional properties of the resulting raw material, even when such materials are legally commercialized under the same name and assumed to be equivalent.

Introduction

Mechanically separated chicken (MSC) is widely used in the meat industry as a lower cost source of nutritionally valuable protein, but has been well documented to have a negative impact on the quality of further processed meat and poultry products by softening texture, introducing grittiness, increasing off-flavors, and increasing redness. Research categorizing the behavior of MSC in a fundamental system has been limited to MSC surimi and no comparison has been made to whole muscle alternatives.

During mechanical separation, proteins undergo increased heat (5–8°C) and pressure (>6.2 MPa), which are known to affect myofibrillar protein structure. 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 temperatures as low as 15°C. The secondary structure of myosin, the primary gel-forming protein, converts from alpha helix to beta sheets as temperature increases, with myosin heavy chains becoming insoluble at 55°C. Pressure has also been shown to induce protein denaturation and even gelation, through structural changes different than those caused by heat. Increased pressure has been reported to reduce myosin solubility at pressures above 300 MPa. An increase in pressure from 100 to 500 MPa has been observed to decrease sulfhydryl content, indicating pressure can induce disulfide bond formation. Because of differences in how heat and pressure modify myofibrillar proteins, it is hypothesized that, when applied in combination, they will have an additive damaging effect on myofibrillar protein structure.

In the present study MSC from two different types of separation processes were compared to each other and to chicken breast meat. The first separation method (MSC1) was a high-speed, high-yielding process that utilized cages and necks held in a cooler for 3–5 d prior to separation. The second separation method (MSC2) involved lower separation speeds and pressure in order to increase particle size of the final product and used bones predominantly from the front half of the carcass within 24 h of harvest. The rheological characteristics of myofibril solutions from each of these three materials were analyzed by dynamic oscillatory rheology in order to assess myofibrillar protein behavior during themal gelation and how this behavior is affected by commercial mechanical separation processes. Differences in myofibrillar protein profile were also assessed.

Materials and Methods

MSC samples were obtained from three commercial lots produced on three consecutive manufacturing days. Myofibrils were isolated from two types of MSC (MSC1, Beehive separator, 3–5 d-old bones; MSC2, Poss separator, fresh bones), and from chicken breast (*pectoralis major*) meat (BT) (Table 1) by differential centrifugation. Briefly, 200 g of sample, previously trimmed of excess fat, were homogenized in post rigor extraction buffer (100mM Tris, 10mM EDTA, pH 8.3) and centrifuged for 20 min at 1000 x g. Resulting pellets were resuspended three times 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, 1 mM NaN_3, pH 7) and centrifuged for 20 min at 1000 x g, twice resuspended in 4 volumes of standard salt solution with 1% Triton X-100 and centrifuged for 10 min at 1500 x g, twice resuspended in 4 volumes of standard salt solution and centrifuged for 10 min at 1500 x g (to remove Triton X-100), resuspended in 150 mL of 100 mM KCl, 5 mM Tris buffer (pH 7.0) and 150 mL glycerol (for cryoprotection), and stored in 50-mL centrifuge tubes at -20°C until needed.

To prepare myofibril solutions, myofibril samples were diluted with 4 volumes (w/v) of standard salt solution and centrifuged at 3000 x g for 10 min. Pellets were washed four times by resuspending in one volume of 50 mM sodium phosphate monobasic buffer (pH 6) and centrifuging at 3000

x g for 10 min. After determining their protein content by using the Bio-Rad RC DC Protein Assay (Hercules, CA, USA), pellets were diluted to 5.6% protein in 50mM sodium phosphate buffer (pH 6). Pellets were subsequently diluted by half in a 1.2 M NaCl, 50 mM solution (pH 6) for a final NaCl concentration of 0.6 M. Final sample protein concentration was 2.8%. Samples were adjusted to pH 6, followed by addition of 1 mM sodium azide (to preserve them), and storage at 4°C for up to 6 d.

Rheological measurements were done in triplicate using a Discovery Hybrid Rheometer HR-2 (TA Instruments, New Castle, DE, USA; 40-mm parallel plate geometry, cross-hatched bottom and top plate). Temperature sweep experiments were performed with 1500 µm gap, 50 µm trim gap offset, and 4500 µm loading gap height. An oscillation temperature ramp was performed on 2.8 g of sample. Temperature ramp was from 20°C to 85°C at a rate of 1°C min^{-1}, with a soak time of 3 min at 85 °C to ensure the entire sample reached 85°C. Sampling interval was 20 s, with 0.25% strain and frequency of 1 Hz. Cooling measurements were taken from 85°C to 5°C at a rate of -5°C min^{-1}. Mineral oil was used to coat exposed surface of 0.6 M NaCl solutions to prevent moisture loss. To identify transition temperatures, linear regressions were calculated on segments of linear relationships during the heating and cooling portions of the curves and inflection points were identified as the points where subsequent linear phases intersected. From this information, four distinct slopes (Fig. 1) were calculated (see footnotes of Tables 2 and 3).

Myofibrillar proteins were separated, in duplicate, by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein bands excised from SDS-PAGE gels were analyzed by tandem mass spectrometry (MS/MS) (Thermo Scientific Q ExactiveTM Hybrid Quadrupole-OrbitrapTM Mass Spectrometer, Thermo Fisher Scientific, Waltham, MA, USA) and identified by matching with existing databases using Thermo Scientific's Proteome Discoverer Software (Thermo Fisher Scientific, Waltham, MA, USA).

Myofibril isolation was conducted on three consecutive days, corresponding to their raw material commercial production lots. Rheological data were analyzed using PROC MIXED procedure of the Statistical Analysis System (SAS v. 9.4, SAS Institute, Cary, NC, USA) with treatment (MSC1, MSC2, BT) as fixed factors, and day and day x treatment as random factors. Significance was determined at $P < 0.05$.

Results and Discussion

All meat sources exhibited gelation with increased temperature (Figure 1). A peak, decline, and subsequent increase was observed in all 3 treatments in 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 larger ($P < 0.05$) for BT than for MSCs (Table 2). BT's S3 was significantly steeper, indicating greater instability of the solid-like structure in the temperature range of 50–55°C (myosin rod denaturation). For G" (Table 3), BT S2 and S3 were significantly different ($P < 0.05$) from MSC treatments, but there was no significant difference in S4 ($P > 0.05$). Results suggest the lower functionality of MSC can partly be explained by differences in the gelation behavior of MSC myofibrils. Distinct protein band patterns were observed between MSC and BT myofibrillar proteins, corroborating fiber type differences and providing evidence that fragmentation or modification of myosin may also be contributing to overall differences between MSC and BT.

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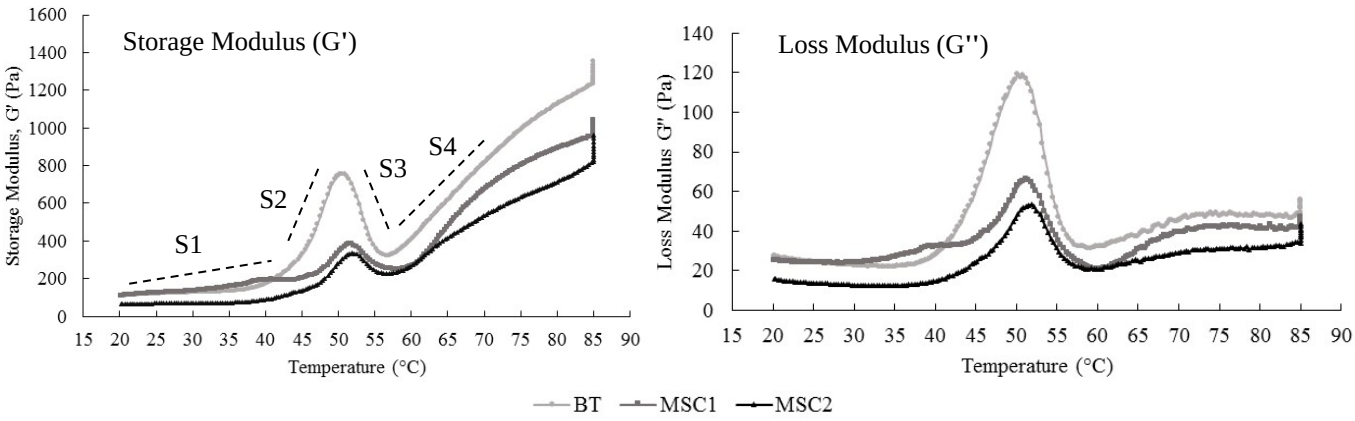

Figure 1. Storage (G'; A) and loss (G"; B) moduli of myofibrillar protein solutions (0.6 M NaCl, 50 mM sodium phosphate, 2.8% (w/v) protein concentration, pH 6) during thermal gelation (20 to 85°C). (Dashed lines on left graph labeled S1–S4 denote calculated slopes 1–4. Slope lines are shown for illustrative purposes only and are not numerically precise)

Table 1. Composition and pH of chicken raw materials.

Source	Moisture (%)	Fat (%)	Protein (%)	pH
BT	74.41[a]	2.40[c]	23.48[a]	5.88[c]
MSC1	68.35[c]	16.17[a]	14.40[b]	6.82[a]
MSC2	71.00[b]	14.83[b]	14.00[b]	6.70[b]
S.E.M.	0.34	0.16	0.14	<0.01

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

Table 2. Storage modulus (G'), temperatures, inflection points and calculated slopes of myofibrillar protein solutions[1] during heating[2].

	Initial		Inflection 1		Peak		Inflection 2		Final		Slopes[3] (Pa °C^{-1})			
	G' (Pa)	T (°C)	G' (Pa)	T (°C)	G' (Pa)	T (°C)	G' (Pa)	T (°C)	G' (Pa)	T (°C)	1	2	3	4
BT	125.19[a]	20.15	181.07[a]	39.90[b]	863.67[a]	50.44[b]	346.16[a]	56.64[b]	1351.57[a]	85.00	3.32[a]	60.16[a]	-81.44[b]	36.36[a]
MSC1	108.03[ab]	20.16	203.19[a]	45.20[b]	371.48[b]	51.74[a]	245.27[ab]	58.03[a]	1041.77[b]	84.99	3.77[a]	28.25[b]	-20.82[a]	29.53[b]
MSC2	64.36[b]	20.17	106.14[b]	44.97[b]	315.62[b]	51.94[a]	216.35[b]	56.74[b]	963.00[b]	85.00	1.78[b]	32.21[b]	-20.26[a]	26.13[b]
S.E.M.	16.80	0.01	16.70	1.36	97.46	0.23	39.02	0.28	58.06	0.01	0.31	5.07	8.24	1.69

[a-b] 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] Slope 1: Initial to Inflection 1; Slope 2: Inflection 1 to Peak; Slope 3: Peak to Inflection 2; Slope 4: Inflection 2 to Final

Table 3. Loss modulus (G''), temperatures, inflection points and calculated slopes of myofibrillar protein solutions[1] during heating[2].

	Initial		Inflection 1		Peak		Inflection 2		Final		Slopes[3] (Pa °C^{-1})			
	G" (Pa)	T (°C)	G" (Pa)	T (°C)	G" (Pa)	T (°C)	G" (Pa)	T (°C)	G" (Pa)	T (°C)	1	2	3	4
BT	36.63[a]	20.15	30.51[a]	39.51	142.52[a]	50.63[b]	36.42	59.42	55.28[a]	85.00	-0.07[b]	9.79[a]	-11.54[b]	0.91
MSC1	25.14[ab]	20.16	30.29[a]	40.81	65.05[b]	51.16[ab]	20.73	59.88	47.05[ab]	84.99	0.22[a]	3.35[b]	-5.08[a]	1.06
MSC2	15.75[b]	20.17	13.64[b]	41.75	50.21[b]	51.72[a]	19.10	59.15	43.50[b]	85.00	-0.08[b]	3.68[b]	-4.22[a]	0.91
S.E.M.	3.98	0.01	4.16	0.97	19.96	0.21	6.42	0.41	3.20	0.01	0.08	1.34	8.24	0.17

[a-b] 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] Slope 1: Initial to Inflection 1; Slope 2: Inflection 1 to Peak; Slope 3: Peak to Inflection 2; Slope 4: Inflection 2 to Final