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Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty

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Abstract

The structures and characteristics of pressure–heat-induced gels of chicken myofibrils and pork patty were investigated. The M-line and Z-line in the chicken myofibril in 0.2 M NaCl were disrupted, and both of the thin and thick filaments were dissociated by pressure treatment. The microstructure of pressure–heat-induced chicken myofibrillar gel was composed of three-dimensional fine strands. Pressurization, at 200 MPa, prior to heating, increased the apparent elasticities of chicken myofibrillar gel and pork patty; however, pressure treatment above 200 MPa decreased it. The apparent elasticity of the pressure-treated (200 MPa) thermal myofibrillar gel was three times higher, and that of pork patty was twice higher than those of the unpressurized ones. The rheological properties of the low salt (1% NaCl) pork sausage can be improved by pressure treatment at 200 MPa prior to heating.

Keywords: Pressure treatment; Muscle; Thermal gelation; Chicken myofibril; Pork patty

1. Introduction

The first report on gelation of proteins by high-pressure treatment dates back to 1914. Bridgman (1914) found that egg white albumen solution was gelled by pressure at 600 MPa. This result implied new gel-like product development through high-pressure treatment. Suzuki and Macfarlane (1984) showed that pressure treatment at 150 MPa enhanced heat-induced gelation of ovine myosin in 0.25 M NaCl, and pressure treatment of myosin at high-salt concentration had no effect on heat-induced gelation. A similar pressure-induced enhancement of heat-induced gelation of rabbit actomyosin at low ionic strength was found by Ikeuchi, Tanji, Kim, and Suzuki (1992). They showed pressure-induced increase of surface hydrophobicity and total SH content and decrease of ATPase activity of actomyosin, and these phenomena were mainly due to actin denaturation below 200 MPa. They concluded that the pressure-induced denaturation of F-actin, including depolymerization to G-actin, resulted in the rheological change of thermal actomyosin gel. A large amount of F-actin exhibits a negative effect on the heat-induced gelation of myosin at high (Yasui, Ishioroshi, & Samejima, 1982) and low (Ishioroshi, Samejima, & Yasui, 1983) salt concentrations. Pressure-induced gelation of myosin occurred above 200 MPa at low ionic strength (Yamamoto, Miura, & Yasui, 1990), while no gelation took place, even at 500 MPa, in high ionic strength solution (Yamamoto, Hayashi, & Yasui, 1993; Yamamoto, Yoshida, Morita, & Yasui, 1994). Macfarlane, Mckenzie, Turner, and Jones (1984) showed that comminuted beef were bound by pressurization at 150 MPa and 0–3 °C,

Abbreviations: BSA, bovine serum albumin; EGTA, O,O′-Bis(2-aminoethyl) ethyleneglycol-*N*,*N*,*N*′,*N*′-tetraacetic acid; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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and the binding strength increased with subsequent cooking at 70 °C for 30 min. The increase in binding strength of beef patty depended on the pressure level and duration of pressurization, NaCl concentration, and pH. When beef patties containing 0–0.5% NaCl were pressurized at 150 MPa prior to heating, the cooking loss increased, but it decreased with addition of 1% or 3% of NaCl (Macfarlane, Mckenzie, & Turner, 1986).

The present study is aimed to clarify the cause of the pressure pretreatment-induced changes in elasticities of thermal gel of chicken myofibrils and pork patty.

2. Materials and methods

2.1. Preparation of chicken myofibrils and glycerinated fibre bundles, and porcine myosin

Myofibrils were prepared from fresh chicken breast muscle (*Musculus pectoralis superficialis*). The minced muscle was homogenized with a Waring blender in 5–10 fold of 0.1 M NaCl containing 1 mM EDTA and 20 mM K phosphate (pH 7.0), and then the homogenate was washed with 0.1 M NaCl and 20 mM K phosphate (pH 7.0) containing 1% Triton X-100. Further washing with 0.1 M NaCl was done five times.

Glycerinated fibre bundles were also prepared from fresh chicken breast muscle. The fibre bundle, fixed onto a wood stick, was immersed in 50% glycerol and 10 mM K phosphate (pH 7.0) containing 5 mM EGTA at 4 °C overnight, and then stored in the same solution at −20 °C.

Frozen pork (Landrace) loin, stored at −30 °C for 3 months, was thawed under running water. The muscle was minced and homogenized in 0.1 M NaCl, 10 mM K phosphate and 1 mM NaN_3 (pH 7.0) with a waring blender. The myofibrils were collected by low-speed centrifugation and washed three times in the same solution. The washed myofibrils were then extracted with three volumes of modified Guba-Straub solution (0.3 M NaCl, 0.15 M K phosphate, 10 mM Na pyrophosphate, and 1 mM $MgCl_2$, pH 6.5) for 10 min. After centrifugation, the supernatant was diluted with a 12-fold volume of 1 mM EDTA, and the precipitated myosin was collected and purified by the method of Offer, Moos, and Starr (1973).

2.2. Preparation of samples for pressure and heat treatment

Chicken myofibrils were suspended in 0.2 M NaCl containing 20 mM Bis–Tris (pH 6.0), and the protein concentrations were adjusted to 5 or 20 mg/ml.

Thawed pork loin, which is free from fat and connective tissue, was coarsely ground, and then comminuted using a food processor to make paste. Forty % (w/w) of water and 1—2% of NaCl were added to the paste and thoroughly mixed.

The chicken myofibril suspension and pork paste were put into a plastic tube and sealed with a silicon rubber plug without any trapped air bubbles, then placed in a pressure vessel, having a diameter of 30 mm and a length of 100 mm, filled with water. Hydrostatic pressure was generated by pumping water into the vessel with a hydraulic machine (Yamamoto Suiatsu Co., Ltd., Osaka, Japan). Pressure treatment was done with varying pressure levels (100–400 MPa) for 10–20 min at room temperature. The pressurized samples were then heated at 70 °C for 20 min.

2.3. Solubility measurement and SDS-PAGE analysis

Pressurized chicken myofibrils were centrifuged at 20,000*g* for 30 min. The protein concentration of the supernatant was measured by the biuret method and the protein composition of the supernatant was analyzed by SDS-PAGE.

The ground pork loin was homogenized in 20-fold of 1% NaCl, and then pressurized. After centrifugation (20,000*g* for 30 min), the supernatant was analyzed by SDS-PAGE.

SDS-PAGE was carried out with a minislab gel apparatus (Yamamoto & Moos, 1983). The acrylamide concentration was 7.5%.

2.4. Apparent elasticity measurement

As a rheological parameter, the apparent elasticity of the pressurized or heated sample was measured by penetration of a spherical plunger using a creep meter (RE-33005, Yamaden, Japan). The spherical plunger, with a diameter of 5 mm, was penetrated into a gel in a plastic tube at 0.5 mm/min. The apparent elasticity (G) of the gel was calculated by the following Hertz's equation modified by Lee and Radok (1960),

$$G = F/[(16\sqrt{R}/3) \times h^{\frac{3}{2}}], \quad (1)$$

where R is the radius of the spherical plunger (2.5×10^{-3} m), F is the load (N), and h is the indentation (0.5×10^{-3} m).

2.5. Optical microscopy analysis

The treated pork patty was cut into cubes ($7 \times 7 \times 3$ mm) and fixed with 10% paraformaldehyde in 0.1 M K phosphate (pH 7.4) for 3 days at room temperature, then rinsed with running water overnight. Dehydration was performed with a graded series of ethanol solutions (70%, 80%, 90%, 95% and 100%), then the sample was put into xylene. The sample, embedded in paraffin, was sectioned with a thickness of 3.5 μm and collected

on 0.1% BSA-coated microscope slides. Following azan staining, the sections were observed with an optical microscope (Axiovert S100, Zeiss).

2.6. Scanning electron microscopy

Unpressurized and pressurized chicken myofibril suspensions were dropped on a carbon-coated SEM pore filter (JEOL, Tokyo, Japan). After 10 min, excessive water was removed with a blower, then the myofibrils were fixed with 1% paraformaldehyde-1.25% glutaraldehyde for 1 h. After dehydration in a graded series of ethanol solutions (50%, 70%, 90%, 95% and 100%), the specimens were transferred into 2-methyl-2-propanol and freeze-dried. The specimens were coated with osmium tetroxide to give a thickness of 10 nm, using an osmium plasma coater (NL-OPC60N, Nippon Laser and Electronic Lab). These specimens were observed in a Hitachi S-2460N scanning electron microscope.

Pressure–heat-induced gel of chicken myofibrils was cut into 1–2 mm cubes and fixed with 3% glutaraldehyde in 0.1 M K phosphate (pH 7.4). Dehydration and observation methods were the same as described above. The sample preparation for scanning electron microscopy of pressure–heat-induced gel of the pork meat patty was performed as follows: the paraffin block used in histochemical analysis was immersed in 100% xylene and then 100% ethanol to remove paraffin. The specimens were transferred into 2-methyl-2-propanol and freeze-dried. The specimens were osmium coated with a thickness of 10 nm.

2.7. Transmission electron microscopy

After three weeks of glycerination of the chicken fibre bundles, glycerol was washed out with 5 mM EGTA-PBS, and the fibre bundles were immersed in 0.2 M NaCl containing 20 mM Bis–Tris (pH 6.0) for 10 min (three times). Pressurized chicken fibre bundles were fixed with 3% glutaraldehyde in 0.1 M K phosphate (pH 7.4) for 2 h, and postfixed with 1% OsO_4 for 1 h. After dehydration with graded ethanol solutions, these samples were embedded in Quetol 812 (Nisshin EM Co., Ltd., Tokyo, Japan). Sections of about 100 nm thickness were prepared on a Leica Ultracut ultramicrotome with a diamond knife. The sections were stained with 4% uranyl acetate and lead citrate, then examined under a Hitachi H-800 electron microscope.

2.8. Immunoelectron microscopy

The chicken fibre bundles, in 0.2 M NaCl containing 20 mM Bis–Tris (pH 6.0), were pressurized at 100 or 200 MPa for 10 min, and then fixed with 4% paraformaldehyde for 6 h at 4 °C. The bundles were embedded in Cryo-embedding compound (Microm International GmbH) and put into liquid nitrogen. Frozen semi-thin (20 μm) sections were mounted on a slide glass. The sections were incubated with 1000-fold-diluted monoclonal anti-α-actinin mouse IgG1 (A 7811, Sigma) overnight at 4 °C, followed by 200-fold-diluted gold (1 nm) conjugated anti-mouse IgG goat IgG (F $(ab')^2$) (BB International). The sections were fixed with 1% glutaraldehyde for 10 min, and then silver-enhanced for 10 min at room temperature using a silver enhancing kit (BB International). The silver-enhanced sections were postfixed with 1% OsO_4 for 15 min, dehydrated with graded ethanol, and embedded in Quetol 812. Sectioning, staining and observation were done as described above.

2.9. Cooking loss measurement

The cooking loss was calculated by the following equation:

$$\text{Cooking loss } (\%) = (A - B)/A \times 100, \quad (2)$$

where A and B are the sample weights before and after cooking, respectively.

2.10. Preparation of myosin-added model sausage

The pork paste was prepared as in 2.2. Forty % (w/w) of water, 1% of NaCl, and 1/100–1/1000 (w/w) of purified pork myosin were added to the paste and thoroughly mixed. The myosin-added pork paste was then heated at 70 °C for 20 min.

2.11. Statistical analysis

The data were analyzed using Statistical Analysis System, Stat View 5 for Macintosh (SAS Inc., Cary, NC). Statistical analysis was performed by analysis of variance (ANOVA) and Tukey–Kramer test. The results are expressed as mean ± SD, and the differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Solubilization of chicken myofibrils

Table 1 shows the protein concentration of the supernatant of pressurized chicken myofibrils in 0.2 M NaCl and 20 mM Bis–Tris (pH 6.0) after centrifugation. The concentration of the solubilized protein in the pressurized myofibrils at 100 MPa was slightly higher than that of unpressurized myofibrils, and it increased with elevating pressure up to 200 MPa, while it decreased at 300 MPa.

Table 1
Protein concentrations of the supernatant of pressurized chicken myofibrils

Pressure (MPa)	Protein concentration (mg/ml)
0.1	0.82 ± 0.01^{a}
100	0.94 ± 0.01^{b}
200	1.80 ± 0.07^{c}
300	1.26 ± 0.01^{d}

Values not sharing a common letter differed, $p < 0.05$. SD, standard deviation; $n = 3$.

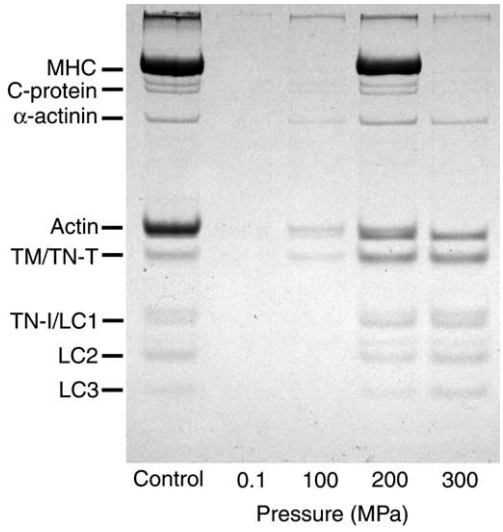

Fig. 1. SDS-PAGE pattern of solubilized fraction of pressurized chicken myofibrils. A sample (10 μl) of solubilized fraction of pressurized myofibrils was analyzed on 7.5% polyacrylamide gel. The control was unpressurized myofibrils. MHC; myosin heavy chain, TM; tropomyosin, TN-T; troponin-T, TN-I; troponin-I, LC; myosin light chains.

The protein composition of the supernatant was analyzed by SDS-PAGE (Fig. 1). In the supernatant of unpressurized myofibrils, a protein band was not observed. The densities of bands of α-actinin, actin and tropomyosin and/or troponin-T bands slightly increased in the myofibrils pressurized at 100 MPa. Solubilization of myosin, C-protein, α-actinin, actin, tropomyosin, and troponins occurred at 200 MPa. This indicates dissociation of both of thin and thick filaments. The densities of the bands of α-actinin, tropomyosin, and troponin-T at 300 MPa were almost the same as those at 200 MPa, while those of myosin heavy chain and actin decreased at 300 MPa.

3.2. Morphological change of pressurized chicken myofibrils and fibre bundle

The morphology of the pressure-treated myofibrils in 0.2 M NaCl (pH 6.0) is shown in Fig. 2. The pressurized myofibrils at 100 MPa (Fig. 2(b)) looked thicker than

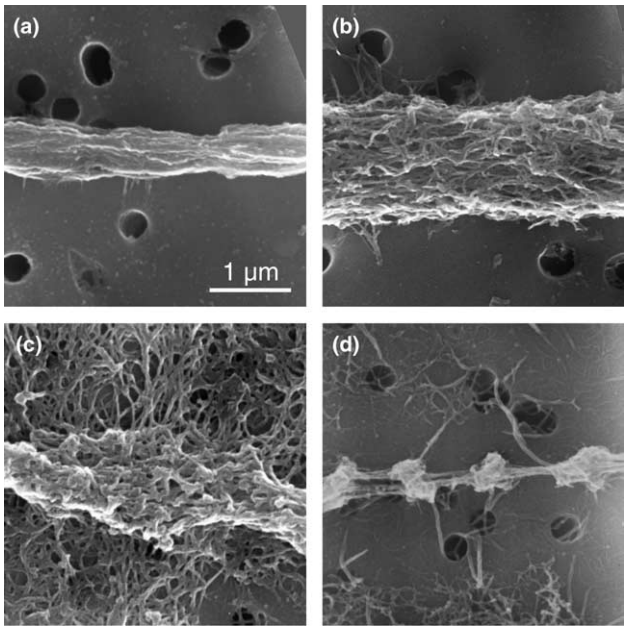

Fig. 2. Pressure-induced morphological change of isolated chicken myofibrils. The morphological observation of the myofibrils was done by scanning electron microscopy with an accelerated voltage of 20 kV: (a) unpressurized myofibril; (b–d) pressurized at 100, 200 and 300 MPa for 10 min, respectively. The magnification of all the photographs is the same.

the unpressurized ones (Fig. 2(a)). The structure of the myofibrils was disrupted at 200 MPa, and the myofilaments were dispersed (Fig. 2(c)). The debris of myofibrils was observed at 300 MPa, and the aggregated structures were observed in the debris periodically. In addition, the dispersed myofilament became short at 300 MPa (Fig. 2(d)).

The ultrastructure of the pressure-treated fibre bundle in 0.2 M NaCl (pH 6.0) is shown in Fig. 3. In the fibre bundle pressurized at 100 MPa, particles appeared on both sides of the Z-line (Fig. 3(b); closed triangles). These morphological changes became obvious by pressurization at 200 MPa, and disorder of the I-band and the M-line occurred. The additional particles appeared in the A-band (Fig. 3(c); open triangles). In the fibre bundle pressurized at 300 MPa, the structural continuity of sarcomere was completely lost, while the deposit of dense materials remained and grew concomitant with the loss of Z-line as well as the I and A-bands (Fig. 3(d); closed triangles).

It is probable that the dense materials appearing on both sides of the Z-line of the pressurized fibre bundle at 100 MPa is α-actinin. Immunoelectron microscopy, using anti-α-actinin antibody, was done to elucidate this possibility (Fig. 4). α-Actinin was located in the Z-line region in the unpressurized fibre bundles (Fig. 4(a)), and α-actinin was also observed in the Z-line in the pressurized fibre bundle at 100 MPa (Fig.

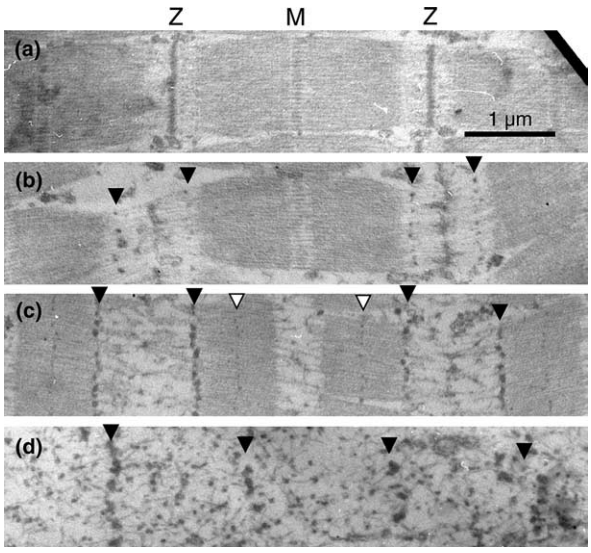

Fig. 3. Ultrastructure of myofibrils in the pressurized chicken muscle fibre. The pressurized chicken muscle fibres were fixed with 3% glutaraldehyde and post-fixed with 1% OsO_4, embedded in Quetol 812, and stained with 4% uranyl acetate–lead citrate: (a) unpressurized fibre; (b–d) pressurized at 100, 200 and 300 MPa for 10 min. M, M-line; Z, Z-line. Closed and open triangles indicate pressure-induced protein aggregates. Pressure-induced aggregates showing closed triangles appeared on both sides of the Z line, and these aggregates remained at 300 MPa. Another aggregate shown as open triangles appeared in the A-band.

4(b)). The gold particles were not observed in the dense materials on the both sides of the Z-line in the pressurized fibre bundle at 100 MPa (Fig. 4(b)). The number of binding of colloidal golds in the collapsed Z-line decreased at 200 MPa (Fig. 4(c)). We examined whether anti-α-actinin antibody was bound to pressure-denatured α-actinin or not. It was confirmed that the antibody was bound to α-actinin pressurized at 100–400 MPa for 10 min in the immunodiffusion test. These results suggested that the Z-line was destroyed by pressure above 200 MPa and α-actinins dispersed into A and I bands, and the dense materials on both sides of the Z-line were not α-actinin.

3.3. Rheology and microstructure of pressure–heat induced myofibrillar gel

Apparent elasticity of heat-induced gel of pressurized and unpressurized myofibrils in 0.2 M NaCl and 20 mM Bis–Tris (pH 6.0) was measured (Fig. 5). The apparent elasticity of unpressurized heat-induced myofibrillar gel was 1152 ± 125 Pa ($n = 3$), while that of the sample pressurized at 100 MPa, prior to heating, was 2582 ± 85.6 Pa ($n = 3$) and that at 200 MPa was 3455 ± 224 Pa ($n = 3$). The apparent elasticity of the gel pressurized at 300 MPa was 2987 ± 213 Pa ($n = 3$), and it was lower than that at 200 MPa.

Fig. 6 shows the microstructure of heat-induced and pressure–heat-induced myofibrillar gels. The heat-induced gel was formed with strands which consisted of the bundles of myofibrils (Fig. 6(a)), while the gel pressurized at 200 MPa before heating consisted of a fine strand network (Fig. 6(b)).

3.4. Effect of pressure treatment on cooking loss and rheological properties of pork sausage

The changes in the cooking loss of the pressure–heat-induced sausage are shown in Table 2. The cooking loss of the pressure–heat-induced sausage without NaCl was almost double those of the unpressurized sausages, while those of the sausages containing 1–2% NaCl were lower than that of the unpressurized sausage, except in the case of 400 MPa with 2% NaCl. No significant difference in cooking loss of the sausage with addition of 1–2% NaCl was observed, regardless of the applied pressure.

The changes of the apparent elasticity of the pressure–heat-treated sausage containing 1–2% NaCl are shown in Fig. 7. The apparent elasticities of pressure–heat-treated sausages were higher than that of unpressurized sausage. In particular, the sausage containing 1% NaCl, which was pressurized at 200 MPa before heating, showed the highest apparent elasticity of 28,646 Pa. The apparent elasticity of the pressure–heat-treated pork sausage decreased with increasing pressure before heating.

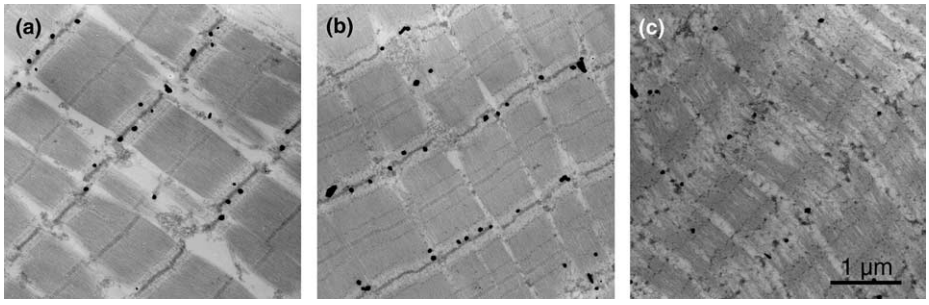

Fig. 4. Immunoelectron micrographs showing localization of α-actinin: (a) unpressurized fibre bundle; (b) pressurized at 100 MPa for 10 min; (c) pressurized at 200 MPa.

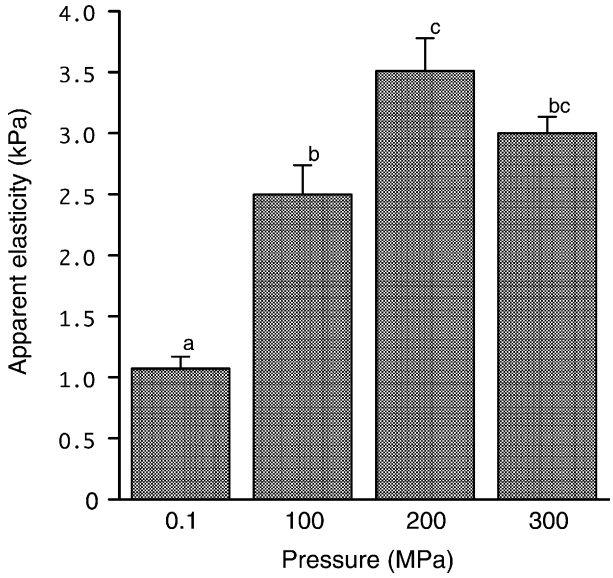

Fig. 5. Changes in the apparent elasticity of pressure–heat-induced gel of chicken myofibrils. Myofibrils (20 mg/ml) in 0.2 M NaCl and 10 mM Bis–Tris (pH 6.0) were pressurized at 0.1–300 MPa for 15 min, then heated at 70 °C for 20 min. The apparent elasticity was measured at room temperature. Error bars represent standard deviations ($n = 3$). Means without common superscript letters differ ($P < 0.05$).

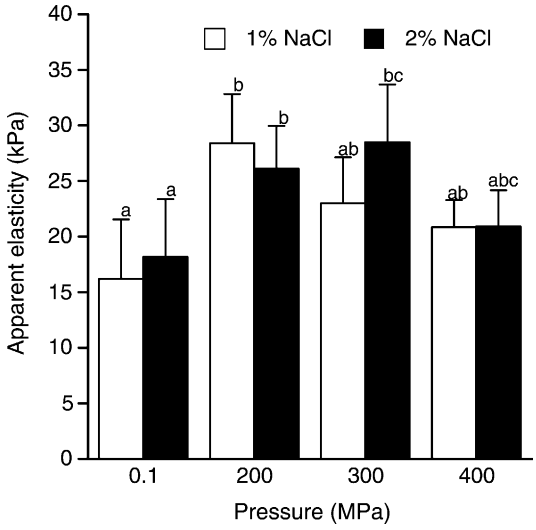

Fig. 7. Effect of NaCl concentration and pressurization on the apparent elasticity of the heat-induced gel of pork patty. The meat patty was pressurized at 200–400 MPa for 20 min, and then heated at 70 °C for 20 min. The apparent elasticities were calculated from the load vs. indentation curve of the pressure–heat-induced gel. Error bars represent standard deviations ($n = 7$). Means without common superscript letters differ ($P < 0.05$).

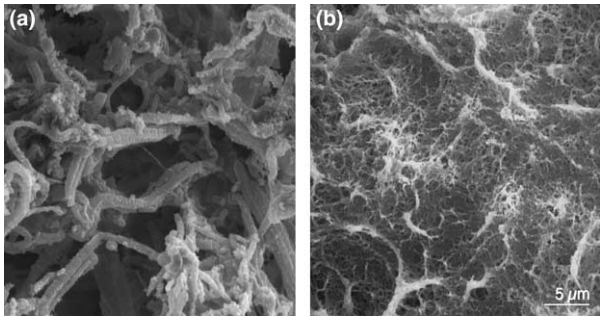

Fig. 6. Scanning electron micrographs of pressure–heat-induced gel of chicken myofibrils. The protein concentration was adjusted to 20 mg/ml in 0.2 M NaCl (pH 6.0): (a) heat-induced gel of unpressurized myofibrils; (b) pressurized at 200 MPa for 20 min prior to heating.

Table 2
Effect of NaCl concentration and pressurization on the cooking loss (%; mean ± SD) of heat-induced gel of pork meat patty

Pressure (MPa)	NaCl (w/o)	NaCl (1%)	NaCl (2%)
0.1	13.73 ± 0.99^{a}	12.97 ± 1.17^{ab}	8.61 ± 0.98^{c}
200	26.17 ± 1.03^{d}	10.85 ± 1.04^{bc}	9.17 ± 0.49^{c}
300	24.72 ± 2.07^{df}	10.75 ± 0.98^{bc}	5.71 ± 1.09^{e}
400	23.59 ± 3.22^{f}	8.46 ± 1.53^{bc}	10.54 ± 0.69^{bc}

Values not sharing a common letter differed, $p < 0.05$. SD, standard deviation; $n = 7$.

3.5. Morphological observation of pork sausage

Optical microscopy of pressure–heat-treated pork sausage is shown in Fig. 8. The gaps among muscle fibres in pressure–heat-induced sausage gel became narrow in comparison with those of unpressurized gel; however, no other significant morphological differences among pressurized sausages were observed.

We observed the microstructure of the paraffin-embedded sausage, which was used for optical microscopy, by SEM (Fig. 9). The observed areas are indicated as the circles and the arrows in Fig. 8. The circled area and the arrow indicate muscle fibres (Fig. 9(a)–(d)) and aggregates (Fig. 9(e)–(h)), respectively. The myofibrillar structure was clearly observed in the gel which was pressurized at 200 MPa before heating (Fig. 9(b)), while the sarcomere structure was not observed in the unpressurized heat-induced gel (Fig. 9(a)). The integrity of myofibril was lost with increasing pressure before heating (Fig. 9(c) and (d)). The arrows in Fig. 8 show the area where the myofibrillar structure was not observed and the gel structure consisted of the fragmented myofibrils (Fig. 9(e)–(h)). The fragmented myofibrils became aggregate with elevating pressure before heating.

3.6. Solubilization of pork patty

Pressure-induced solubilization of proteins in pork patty was analyzed by SDS-PAGE (Fig. 10). The bands of myosin heavy chain and actin appeared in solubilized fraction pressurized at 100 MPa. The densities of the actin and myosin heavy chain bands were highest in the supernatant of pressurized homogenate of 100 and 200 MPa, respectively. The solubilized muscle proteins did not form large aggregates at 200 MPa, though they

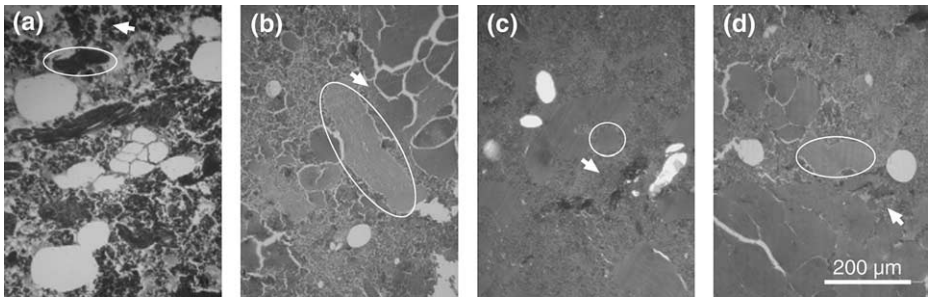

Fig. 8. Optical microscopy of the pressure–heat-induced gel of pork patty. Azan stained paraffin sections of the pressure–heat-induced gel of pork meat patty were observed: (a) unpressurized; (b) Pressurized at 200 MPa for 20 min; (c) Pressurized at 300 MPa; (d) Pressurized at 400 MPa. Circles indicate remaining fibre bundles, and arrows indicate fragmented fibre in the meat patty. These same areas were also observed using the scanning electron microscope, and the results are shown in Fig. 9.

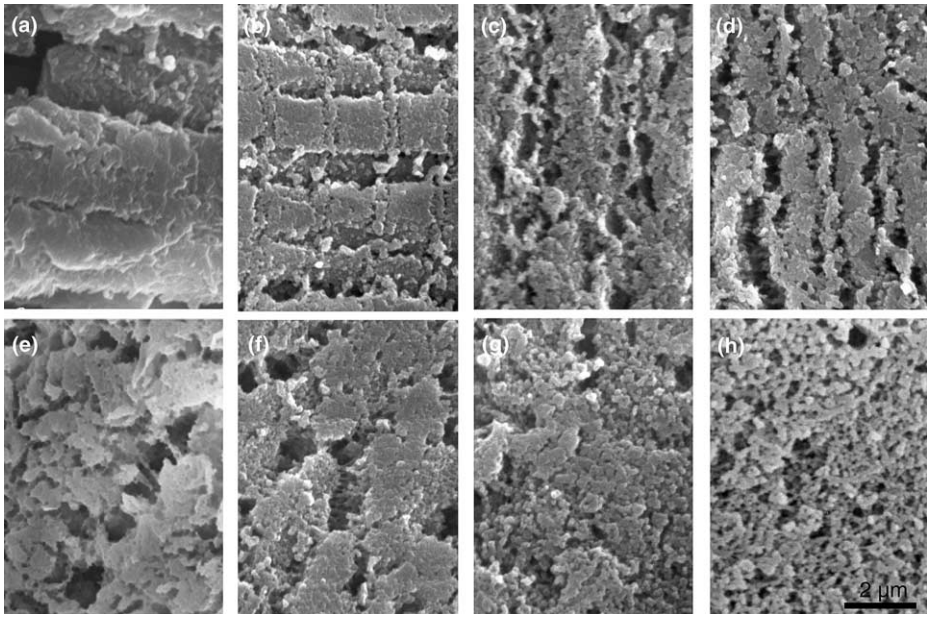

Fig. 9. Scanning electron micrographs of the pressure–heat-treated pork patty. These specimens were the same as those used in the histochemical analysis: (a–d) and (e–f) indicate the circled area and the arrowed area in Fig. 8, respectively; (a) and (e), unpressurized; (b) and (f), pressurized at 200 MPa; (c) and (g), pressurized at 300 MPa; (d) and (h), pressurized at 400 MPa.

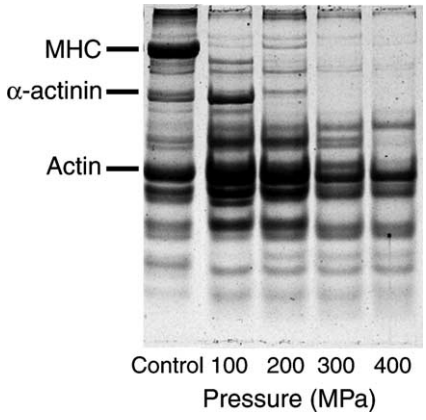

Fig. 10. SDS-PAGE pattern of the solubilized fraction of pork patty. A sample (20 μl) of each of the supernatants after centrifugation was analyzed by SDS-PAGE on 7.5% polyacrylamide. Control was unpressurized pork meat patty.

formed large ones above 200 MPa. Therefore, the amount of myosin in supernatant increased up to 200 MPa. The solubilization of myosin due to the collapse of the myofibrillar structure may be one of the reasons why the apparent elasticity of the gel pressurized at 200 MPa before heating was high.

3.7. Apparent elasticity of myosin-added model sausage

In order to clarify the influence of the destruction of myofibril and the solubilization of myosin on the rheological properties of the sausage, the model sausage was prepared with addition of myosin, and the apparent elasticity was measured (Fig. 11). The apparent elasticities of myosin-added sausages, with the ratios of 1/100 and 1/1000 (w/w), were not significantly different, and large cooking losses were observed in those sausages, especially in the case of 1/1000 (w/w) addition. When

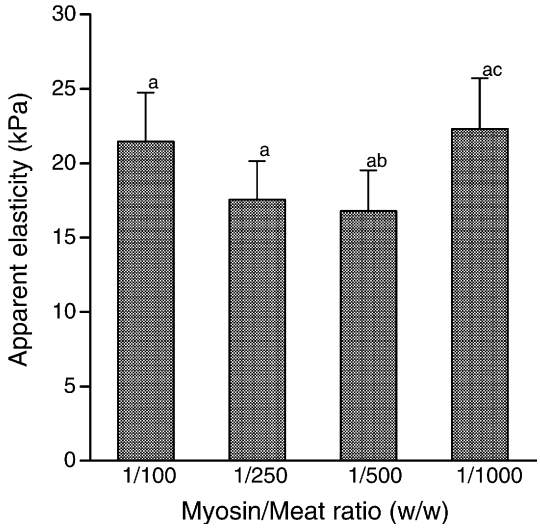

Fig. 11. Effects of the addition of purified myosin on the heat-induced gel of pork patty at 70 °C in 1% NaCl. Myosin was added to pork patty with weight ratios of 1/100, 1/250, 1/500, and 1/1000. The other conditions were the same as in Fig. 8. Error bars represent standard deviations ($n = 5$). Means without common superscript letters differ ($P < 0.05$)

the apparent elasticities of pressure–heat-treated sausage containing 1% NaCl and a myosin-added sausage were compared, the apparent elasticity of the sausage pressurized at 200 MPa before heating was the highest (Fig. 7).

4. Discussion

4.1. The effect of the pressurization on chicken myofibril

Macfarlane and Mckenzie (1976) reported that pressure-induced solubilization of ovine myofibrils occurred above pH 6.3 and 0.2 M KCl. The present study, using chicken myofibrils in 0.2 M NaCl (pH 6.0), is consistent with their result (Table 1). The amount of actin in the solubilized fraction increased with pressurization at 100 MPa, and that of myosin markedly increased at 200 MPa. However, the amount of solubilized myosin and actin decreased at 300 MPa (Fig. 3(b)). The thickness of myofibril increased by pressurization at 100 MPa, and the dissociation of myofilaments occurred with elevating pressure (Fig. 2). Disorder of the Z-line, disappearance of the M-line, and the depolymerization of thin and thick filaments were observed in the pressurized myofibrils (Fig. 3). Suzuki, Suzuki, Ikeuchi, and Saito (1991) investigated the solubilization of myofibrils in 0.1 M KCl by pressure without using a buffer, and found myosin extraction from myofibrils at 300 MPa. The present results show that myosin is solubilized from myofibrils in 0.2 M NaCl at 200 MPa. They indicate that solubilization of thick filaments in myofibril depends on both ionic strength and pressure. The myosin filaments dissociate by pressure treatment at neutral or slightly basic pH (Davis, 1981; Tumminia, Koretz, & Landau, 1989; Tumminia, Koretz, & Landau, 1990); however, the myosin filaments do not dissociate by pressure treatment at acidic pH, such as pH 6.0, and low ionic strength, such as 0.1 M KCl (data not shown). These results indicate that the dissociation of the myosin filaments under high pressure is influenced by pH and salt concentration.

Pressure-induced destruction of the Z-line is possibly due to release of α-actinin (Fig. 4) together with phospholipid, which is a component of the Z-line (Takahashi, Shimada, Ahn, & Ji, 2001). The disruption of chicken myofibril in 0.2 M NaCl by pressurization at 200 MPa is accompanied by the following three phenomena: (1) disappearance of the M-line, (2) dissociation of each of the thin and thick filaments and (3) destruction of the Z-line.

The apparent elasticity of the thermal gel of myofibrils reached its maximum value when the pressure-treatment was done at 200 MPa before heating (Fig. 5). The structure of the gel was markedly different from that of unpressurized gel. The myofibrillar structure remained in the unpressurized gel. In contrast, the myofibrillar structure was not observed in the gel pressurized at 200 MPa prior to heating; instead, the gel had a three-dimensional structure (Fig. 6) known as the strand type (Hermansson, Harbitz, & Langton, 1986). Myosin filament in 0.2 M NaCl and pH 6.0 was dissociated under hydrostatic pressure at 200 MPa, and dispersed myosin was reassociated to myosin filament after pressure release. The strand-type gel is formed by heat-induced interaction among rearranged myosin filaments. Ishioroshi et al. (1983) reported that the strength of the thermal gel of the myosin filaments decreased by the addition of F-actin and concluded that the F-actin inhibited the essential interaction of myosin heads to form heat-induced gelation of the myosin filaments. The depolymerization of the actin filaments was observed at about 100 MPa (Ivanov, Berg, & Lebedeva, 1960; O'Shea & Tume, 1979). Ikeuchi et al. (1992) reported that Mg^{2+}-enhanced ATPase activity was readily decreased at pressures ranging from 0 to 200 MPa, whereas Ca^{2+}-enhanced ATPase activity only decreased at pressures above 200 MPa. They concluded that actin in actomyosin is denatured above 100 MPa, and myosin in actomyosin is denatured above 200 MPa. These previous results and the present study suggest that the cause of the high apparent elasticity of the myofibrillar heat-induced gel after pressurization of 200 MPa is depolymerization of thin filament. When the myosin filament is heated after pressurization, decrease of gel strength is observed, extending duration of pressurization (Yamamoto et al., 1990). Yamamoto, Samejima, and Yasui (1988) also reported that the increase of gel strength was proportional to the length of the fila-

ments. Pressure-induced short myosin filament (shown in Fig. 2(d)) might be considered as one of the causes of the decreasing apparent elasticity of myofibrillar gel at 300 MPa.

4.2. Improvement of the rheological property of the model sausage by pressurization

The cooking loss of pressurized pork patty with addition of 1% NaCl was less than that of the unpressurized one (Table 2) and this is consistent with the result of Macfarlane et al. (1984). On the other hand, Jung and Ghoul (2000) reported that the cooking loss of meat without salt was increased by pressure treatment before heating. The present results indicate that the increase of cooking loss induced by pressurization can be controlled by addition of salt. The elasticity of pork patty at 2% (0.34 M) NaCl-300 MPa was almost the same as that at 1% (0.17 M) NaCl-200 MPa. The apparent elasticity of chicken myofibrillar gel formed at 200 MPa showed the maximal value (Fig. 5). These results indicate that the sufficient elasticity of meat gel at low salt concentration is achieved using hydrostatic pressure at 200 MPa. The disruption of myofibrillar structure, which was accompanied with depolymerization of the thin filament, occurred in the remaining muscle tissue in the gel by pressure–heat treatment (Fig. 9(a)–(d)). The fragmented myofibrils were observed in the pressure–heat-induced gel, and the destruction of myofiblrils increased with elevating pressure (Fig. 9(e)–(h)). In SDS-PAGE analysis of the supernatant of meat homogenate after pressurization, the staining intensities of actin and myosin bands were increased (Fig. 10). The apparent elasticity of myosin-added model sausage was lower than that of control sausage (Fig. 11). The improvement of the rheological properties of heat-induced gel by pressurization may be due to depolymerization of thin filaments, which inhibit head-to-head interaction among myosin filaments.

5. Conclusion

The rheological properties of heat-induced gel of chicken myofibrils and pork patties were improved by pressurization before thermal treatment. Disruption of M-line and Z-line and dissociation of thick and thin filaments took place in the pressurized myofibrils. Pressure treatment at 200 MPa prior to heating improved the rheological properties of the pork sausage through the dissociation of thin filament. The results of this research suggest the possibility of developing low-salt sausage using relatively low-pressure treatment (200 MPa).

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