Dynamic Rheological Measurements on Heat-Induced Pressurized Actomyosin Gels

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The heat-induced gelation of actomyosin (or natural actomyosin) treated with high pressure was investigated by dynamic rheological measurements. When actomyosin at 0.6 M KCl and pH 6.0 was subjected to a pressure of 150 MPa for 5 min, the dynamic rheological behavior during heat gelation showed a pattern similar to that of myosin. That is, the rheological transition in the 46–53 °C range induced by the presence of F-actin disappeared. The storage modulus (G') of pressurized actomyosin at 80 °C was almost double that observed in unpressurized actomyosin. In 0.2 M KCl at pH 6.0, where unpressurized actomyosin forms a very weak heat-induced gel, pressurized actomyosin formed a firm heat-induced gel having higher G' value than either pressurized or unpressurized actomyosin at 0.6 M KCl. The gel of pressurized actomyosin at 0.2 M KCl also resembled that of pressurized myosin at 0.2 M KCl in the dynamic rheological behavior. The remarkable increase in the storage modulus of pressurized actomyosin at low and high KCl concentrations seemed to arise from pressure-induced denaturation of actin in actomyosin. These results suggest that high hydrostatic pressure technology is potentially useful for improvement in the functional property (e.g., gel-forming ability) of muscle proteins.

INTRODUCTION

Pressure is well-known to exert a great influence on the properties of proteins by rearrangement and/or destruction of noncovalent bonds such as hydrogen bonds, hydrophobic interactions, and electrostatic bonds of the tertiary structure of proteins. Therefore, the application of high hydrostatic pressure to food processing has lately attracted considerable attention as a new technique instead of heat (Farr, 1990). Efforts are also being made to apply high-pressure techniques to the meat industry in expectations of controlling the toughness of meat and improvement of the gel-forming properties of muscle proteins in processed meat products (Macfarlane, 1985; Suzuki et al., 1990, 1991).

Heat-induced gelation of the salt-soluble myofibrillar proteins leads to the formation of a three-dimensional network which exhibits both viscous and elastic properties. Myosin plays a very important role in this gelation process (Asghar et al., 1985). Actin is also important as a cofactor reinforcing the gel structure of myosin (Yasui et al., 1980). Needless to say, pressure affects the properties of these proteins, depending on the extent of applied pressure, pH, salt concentration, and so on. For example, pressurization of myosin promotes formation of aggregates in high salt solution at pH 6.5 and results in the formation of a gel consisting of a fine network in low salt solution at pH 6.0 (O'Shea et al., 1976; Yamamoto, 1990). F-actin in the absence of ATP undergoes irreversible denaturation at a pressure of above 150 M Pa, whereas ATP shows a significant protective effect against pressure-induced denaturation of actin (Ikkai and Ooi, 1966). In actomyosin, a gel to sol transition is promoted as a result of pressure treatment. In the absence of ATP, an association remains between myosin and actin of actomyosin under pressure, whereas in the presence of ATP, actomyosin dissociates into the individual components (Ikkai and Ooi, 1969).

The changes in the properties of myofibrillar proteins under the influence of pressure as described above may be used in meat processing. From this viewpoint, the effect of high-pressure treatment on the thermal gelation of different kinds of skeletal muscle proteins has recently been investigated, especially in Japan (Ko et al., 1990; Shoji et al., 1990; Suzuki et al., 1991). Shoji et al. (1990) reported that excellent gels could be produced from Alaska pollack by pressure treatment at 200–400 MPa. Also, pressurized pork actomyosin was reported to show higher work done values (breaking energy) than unpressurized actomyosin (Suzuki et al., 1991).

Our study was conducted to clarify the properties of heat-induced gel formation of actomyosin at pH 6.0 with regard to variations in pressure intensity and salt concentration. The investigation bears some resemblance to the study by Japanese workers described above who measured the jelly strength or the work done value. However, the fundamental difference was the rheological technique. We used the dynamic rheological apparatus which permits observation of continuous changes in elastic and viscous elements of actomyosin during the transition of a sol into a gel (nondestructive method). Accordingly, the data reported here gave additional information about the heat-induced gelation of pressurized actomyosin. Here, it should be noted that the terms "myosin" and "actomyosin" in this paper imply myosin A and natural actomyosin (or myosin B) including regulatory proteins and other minor myofibrillar proteins, respectively.

MATERIALS AND METHODS

Materials. Rabbit skeletal muscles were obtained from the carcasses immediately after slaughter and minced with a meat chopper following removal of fat and connective tissue. Myosin and actomyosin were extracted with Guba–Straub and Weber–Edsall solutions, respectively, and then purified according to the procedure of Briskey and Fukazawa (1971). Actomyosin (15 mg/mL) and myosin (10 mg/mL) were dissolved in the solution containing various KCl concentrations (0.2–1.0 M) and 20 mM sodium phosphate buffer (pH 6.0) before pressure application.

Pressurization of Muscle Proteins. Each muscle protein was vacuum-sealed in flexible polyethylene bags and transferred to a large polyethylene bag. The space of the bag was filled with cold water. Each bag was put into the pressure vessel which was filled with cold water (cold isostatic press apparatus, Nippon

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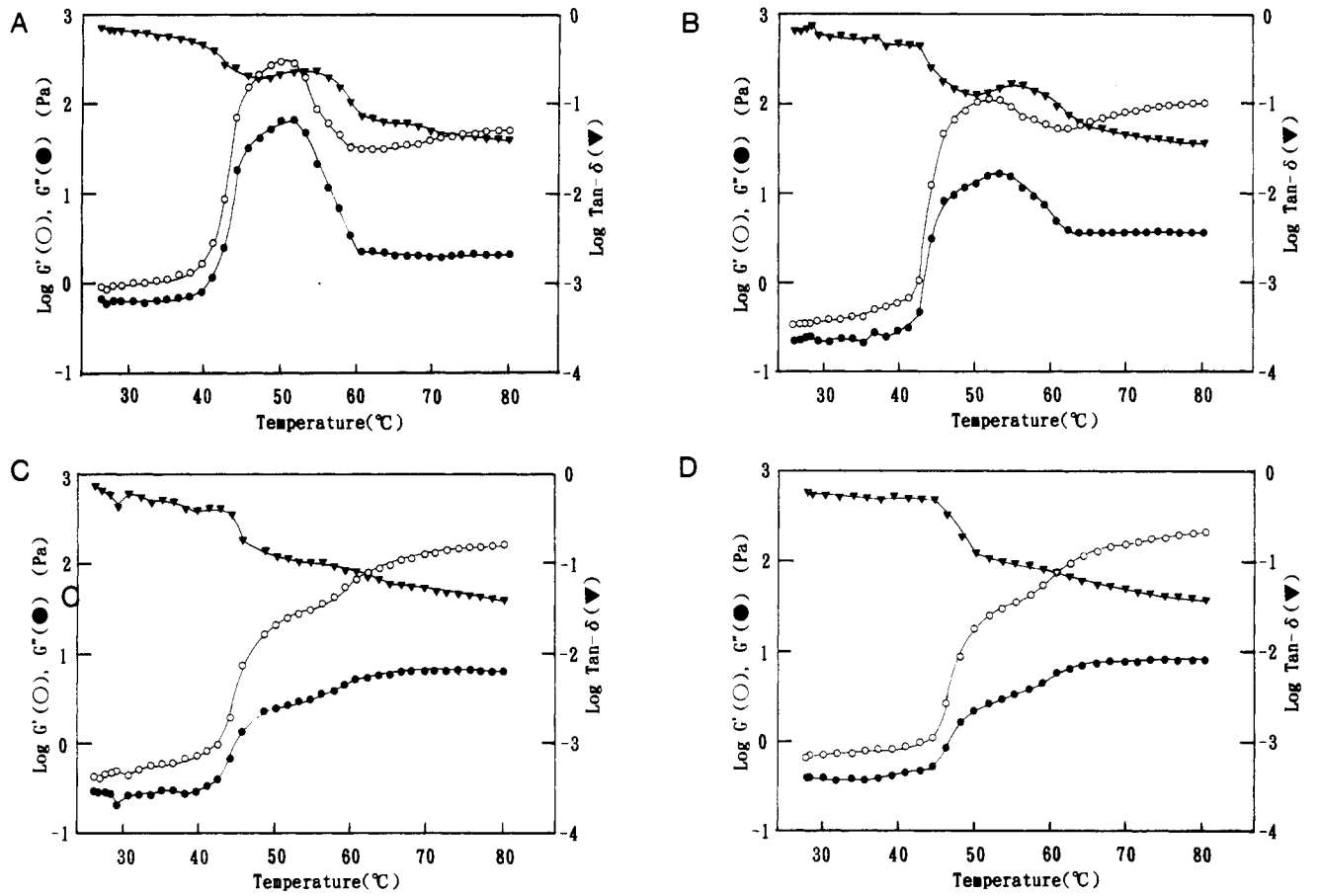

Figure 1. Dynamic rheological behavior of actomyosin (15 mg/mL) and myosin (10 mg/mL) at 0.6 M KCl and pH 6.0 (20 mM sodium phosphate buffer) before and after pressure application: (A) unpressurized actomyosin, (B) actomyosin pressurized at 100 MPa for 5 min, (C) actomyosin pressurized at 150 MPa for 5 min, (D) myosin pressured at 150 MPa for 5 min (note: unpressurized myosin gave almost the same pattern as pressurized one); (○) storage modulus, G', (●) loss modulus, G'', (▼) tangent δ. The detailed conditions are described in Materials and Methods.

Kokan Co., Ltd.). Then, the sample solution was exposed to the appropriate pressure (100–300 MPa for 5 min). After pressurization, the sample was taken from the vessel and immediately cooled in an ice box.

Dynamic Viscoelasticity Measurements. Dynamic viscoelasticity measurements were carried out with a MR-300 (Rheology Co., Japan) using a cone and plate type measuring system, equipped with a thermocontrol unit. The measuring unit consists of a cone 5.295 in degree and 3.196 cm in diameter and a lower plate. The gap between a cone plate and a lower plate was controlled at 1.2 mm. The sample solution was covered with a thin layer of silicone oil to prevent evaporation of water. Storage (G') and loss (G'') moduli were monitored continuously at a fixed frequency of 0.2 Hz and a strain amplitude of 0.087 (i.e., angular displacement 0.0087 rad) as a function of temperature from 25 to 80 °C (heating rate 2.7 °C/min).

Analyses were performed in duplicate or triplicate and average values used in figures. Duncan's multiple range test was used to compare means when effects were evaluated.

Determination of Protein Concentration. Protein concentration was determined by the biuret method using bovine serum albumin as a standard (Gornall et al., 1949).

RESULTS AND DISCUSSION

The dynamic rheological behavior of myosin and actomyosin pressurized under various conditions was measured at temperatures from 25 to 80 °C. Figures 1 and 2 show changes in storage modulus (G'), loss modulus (G''), and loss tangent (tan δ = G''/G') as a function of temperature.

Figure 1A is a typical dynamic rheological pattern of unpressurized actomyosin at 0.6 M KCl and pH 6.0. In this case, the G', which represents the elastic component, increased after the first transition at 38–48 °C, reached a peak, decreased on further rise in temperature to the third transition around 52–60 °C, and then increased again until 80 °C. The G'', which represents the viscous component, showed almost the same pattern as the G', except that it did not increase around 60–80 °C. This result was consistent with that for fish myosin in the presence of F-actin observed by Sano et al. (1988, 1989). Sano et al. (1989) proposed that F-actin contributed to the viscous property of actomyosin sol and caused a decrease in G^* (complex modulus) between the second and third transitions. When actomyosin was subjected to a pressure of 100 MPa for 5 min, the decrease in G' in the 52–60 °C range became apparently smaller (Figure 1B), and it almost disappeared under applied pressure of 150 MPa (Figure 1C). The pattern shown in Figure 1C was quite similar to that of unpressurized or pressurized myosin (Figure 1D), suggesting that the greater part of actin in actomyosin was denatured or depolymerized into G-actin which did not contribute to the heat-induced gel formation of myosin. Yasui et al. (1980) showed that a small amount of actin increases the rigidity of myosin gels formed in buffers containing 0.6 M KCl. Therefore, the pressure-associated increase in G' may be due to a decrease in F-actin.

Figure 2A–D shows the effect of low ionic strength (0.2 M KCl) on the dynamic rheological behavior of myosin and actomyosin when subjected to a pressure of 150 MPa for 5 min. All three rheological parameters gave thermograms that were strongly dependent on the ionic strength as can be seen by comparing them with Figure

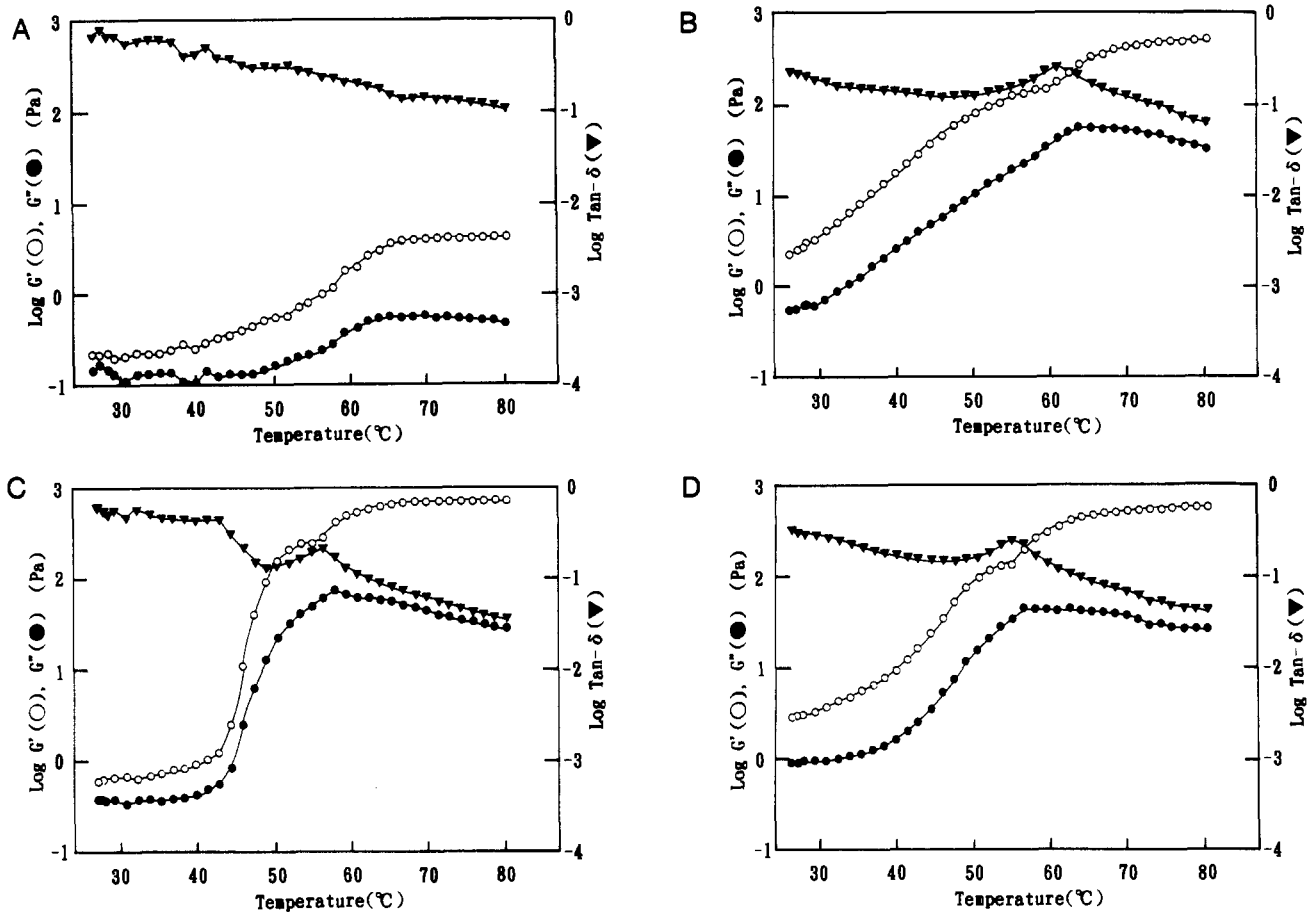

Figure 2. Dynamic rheological behavior of actomyosin (15 mg/mL) and myosin (10 mg/mL) at 0.2 M KCl and pH 6.0 (20 mM sodium phosphate buffer) before and after pressure application: (A) unpressurized actomyosin, (B) actomyosin pressurized at 150 MPa for 5 min, (C) unpressurized myosin, (D) myosin pressurized at 150 MPa for 5 min.

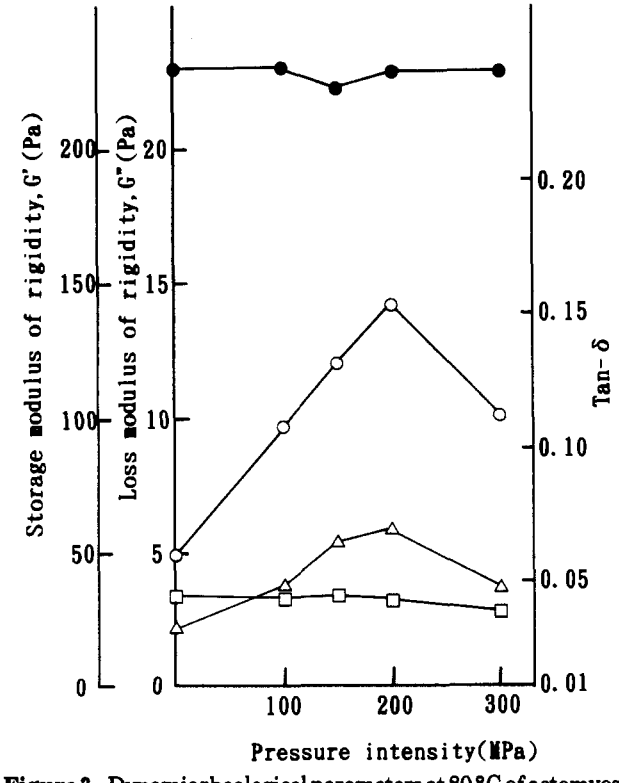

Figure 3. Dynamic rheological parameters at 80 °C of actomyosin (open symbols) and myosin (filled symbols) at 0.6 M KCl and pH 6.0 (20 mM sodium phosphate buffer) as a function of pressure intensity: (○, ●) storage modulus, G'; (△) loss modulus, G''; (□) tangent δ. The protein concentrations of actomyosin and myosin were 15 and 10 mg/mL, respectively.

1. In Figure 2A, the tangent δ at 80 °C was less than that at 25 °C, indicating liquid-like characteristics, and as the temperature was increased the G' increased to a greater degree than did G''. These suggested that a weak gel of actomyosin was formed rather than no gel. On the contrary, pressurized actomyosin formed a firm heat-induced gel having characteristics of high G' and G'' values (Figure 2B). Moreover, the area of the peak/shoulder below 60 °C was small, which was essentially in agreement with the result of pressurized myosin at low ionic strength as shown in Figure 2D. This demonstrates that the remarkable increase in viscoelasticity of actomyosin gels at low ionic strength may also arise from pressure-induced denaturation of actin in actomyosin, an assumption which appears to be supported by the data from the accompanying paper (Ikeuchi et al., 1992).

The representative rheological parameters, G', G'', and tangent δ, which were observed at 80 °C, versus pressure intensity are illustrated in Figure 3. The G' and G'' values of actomyosin linearly increased with increasing pressure in the range of 100–200 MPa, but that further increase in pressure led to a decrease in the gel strength. A fall in the G' at 300 MPa is probably related to the denaturation of both myosin and actin in actomyosin. There were no significant differences in tangent δ observed in the pressure-treated samples. The G' of myosin alone (filled circle) scarcely changed over a wide pressure range from 0 to 300 MPa. If myosin is denatured at 300 MPa as described above, it is in conflict with the result of Figure 3 that there was no decrease in G' of the myosin at 300 MPa. However, a possible interpretation of this phenomenon is as follows: increasing the surface hydropho-

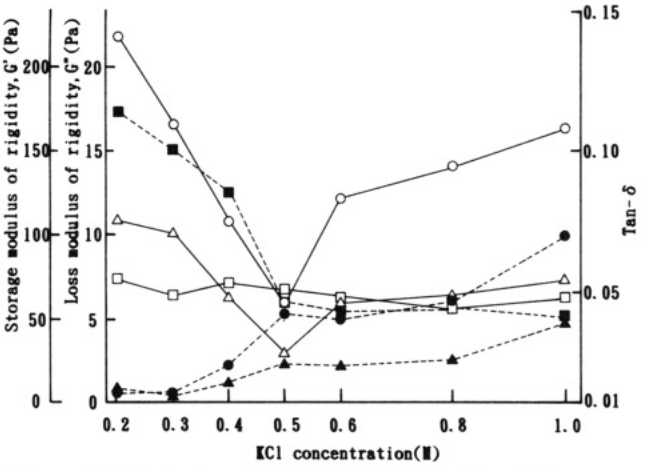

Figure 4. Effect of pressure treatment at various KCl concentrations and pH 6.0 (20 mM sodium phosphate buffer) on the dynamic rheological parameters at 80 °C of actomyosin. Dotted line (filled symbols) and solid line (open symbols) represent unpressurized actomyosin and actomyosin pressurized at 150 MPa for 5 min, respectively: (○, ●) storage modulus, G'; (△, ▲) loss modulus, G''; (□, ■) tangent δ.

bicity due to pressure-induced structural changes of myosin (i.e., denaturation) can compensate for the decrease in the heat-induced gel strength of myosin denatured by pressure treatment. The details will be mentioned in the accompanying paper.

Figure 4 depicts the viscoelastic profile of pressurized actomyosin at various KCl concentrations, as compared to that of unpressurized actomyosin. Pressure treatment, on the whole, resulted in the increase in the gel strength (G' and G'') of actomyosin heated at 80 °C. As can be seen in Figure 4, the viscoelastic profile of pressurized actomyosin was quite distinct from that of unpressurized actomyosin. For example, the G' and G'' of pressurized actomyosin constantly decreased with an increase in KCl concentration, reached their minimum values in 0.5 M KCl, and increased again, whereas those values of unpressurized actomyosin remained unchanged with a low value up to 0.4 M KCl and then increased with increasing KCl concentration. The salt concentration of 0.5 M seems to be a turning point of the pressure effect on the heat-induced gelation of actomyosin. Tangent δ values of actomyosin at 0.2–0.4 M KCl were decreased by pressure treatment. This suggests that a considerable decrease in F-actin in actomyosin, which gives the viscous elements of the heat-induced actomyosin gel, occurred by pressure treatment (Sano et al., 1989). No significant difference in tangent δ values was found in actomyosin samples at different KCl concentrations (0.2–1.0 M KCl) after pressurization. This indicates that the heat-induced gels of pressurized actomyosin at low salt concentration such as 0.2 M KCl are not different from those at high salt concentration such as 1.0 M KCl in the rheological gel property; that is, the tangent δ revealing the relative viscous:elastic properties of a gel. However, the gel of pressurized actomyosin at 0.2 M KCl was a sponge-like gel showing apparently less elastic and less translucent nature than that formed at 0.6 M KCl as have already been reported by Suzuki et al. (1991). Probably, the pressurized actomyosin at low salt concentration forms a gel similar to myosin filamentous gel because actin in actomyosin was mostly denatured under such a pressure (Ishioroshi et al., 1983, Figures 2 and 5 in the following paper). The reason for the difference between the properties of gels judged by tangent δ and by an appearance remains unclear. One obvious future avenue will be indicated by comparing the scanning electron micrographs of heat-induced gels of actomyosin pressurized at low and high KCl concentrations, or the difference may be illustrated more clearly by measuring frequency spectra. In connection with Figure 4, the photographs of heat-induced gels of unpressurized and pressurized actomyosins at various KCl concentrations are shown in Figure 5.

In conclusion, it is interesting to report that excellent heat-induced gels of actomyosin at low and high salt concentrations could be produced by pressure treatment. Judging from the data of dynamic rheological measurements, the acquisition of satisfactory gel-forming ability at low salt concentration such as 0.2 M KCl and the increased gel strength at high salt concentration of pressurized actomyosin are probably attributable to pressure-induced denaturation of actin in actomyosin. The accompanying paper will go into details about the mechanism of heat-induced gelation of pressurized actomyosin (Ikeuchi et al., 1992).

Finally, we also emphasize that a fact of the desired gelation of pressurized actomyosin at low salt concentration (0.2 M KCl) opens up the possibility for exploitation of new meat products.

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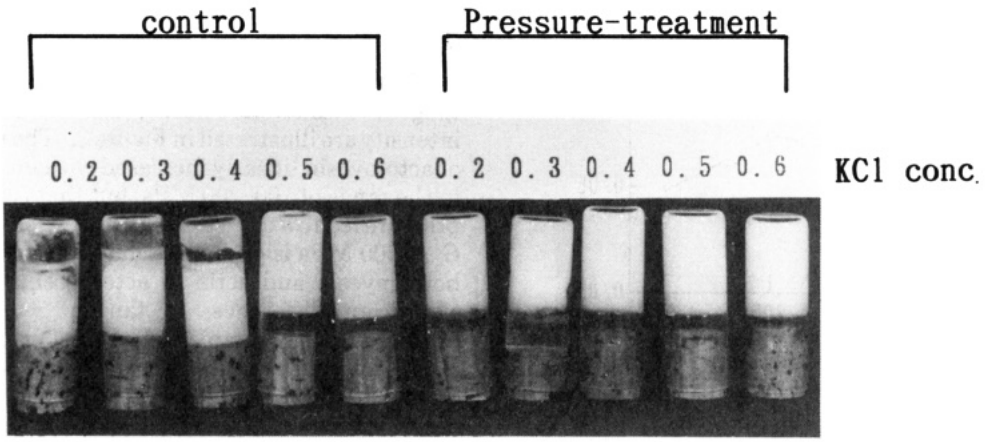

Figure 5. Heat-induced gels of unpressurized actomyosin and pressurized actomyosin (150 MPa, 5 min) at KCl concentrations of 0.2–0.6 M and pH 6.0 (20 mM sodium phosphate buffer). Samples were heated at 80 °C for 30 min. The protein concentration was 15 mg/mL.

LITERATURE CITED

Asghar, A.; Samejima, K.; Yasui, T. Functionality of muscle proteins in gelation mechanisms of structured meat products. *CRC Crit. Rev. Food Sci. Nutr.* **1985**, *22*, 27–106.

Briskey, E. J.; Fukazawa, F. Myofibrillar proteins of skeletal muscle. *Adv. Food Res.* **1971**, *19*, 279–360.

Farr, D. High pressure technology in the food industry. *Trends Food Sci. Technol.* **1990**, *1*, 14–16.

Gornall, A. G.; Bardawill, C. T.; David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751–766.

Ikeuchi, Y.; Tanji, H.; Kim, K.; Suzuki, A. Mechanism of heat-induced gelation of pressurized actomyosin: Pressure-induced changes in actin and myosin in actomyosin. *J. Agric. Food Chem.* **1992**, following paper in this issue.

Ikkai, T.; Ooi, T. The effect of pressure on F-G transformation of actin. *Biochemistry* **1966**, *5*, 1551–1560.

Ikkai, T.; Ooi, T. The effect of pressure on actomyosin systems. *Biochemistry* **1969**, *8*, 2615–2622.

Ishioroshi, M.; Samejima, M.; Yasui, T. Heat-induced gelation of myosin filaments at a low salt concentration. *Agric. Biol. Chem.* **1983**, *47*, 2809–2816.

Ko, W.-C.; Tanaka, M.; Nagashima, Y.; Taguchi, T.; Amano, K. Effect of high pressure treatment on the thermal gelation of Sardine and Alaska Pollack meat and myosin pastes. *Nippon Shokuhin Kogyo Gakkaishi* **1990**, *37*, 637–642.

Macfarlane, J. J. High pressure technology and meat quality. In *Developments in Meat Science-3*; Lawrie, R., Ed.; Elsevier Applied Science Publishers: London, 1985; pp 155–184.

O'Shea, J. M.; Horgan, D. J.; Macfarlane, J. J. Some effects of pressure treatment on actomyosin systems. *Aust. J. Biol. Sci.* **1976**, *29*, 197–207.

Sano, T.; Noguchi, S.; Tsuchiya, T.; Matsumoto, J. Dynamic viscoelastic behavior of natural actomyosin and myosin during thermal gelation. *J. Food Sci.* **1988**, *53*, 924–928.

Sano, T.; Noguchi, S.; Matsumoto, J.; Tsuchiya, T. Role of F-actin in thermal gelation of fish actomyosin. *J. Food Sci.* **1989**, *54*, 800–804.

Shoji, T.; Saeki, H.; Wakameda, A.; Nakamura, M.; Nonaka, M. Gelation of salted paste of Alaska pollack by high hydrostatic pressure and change in myofibrillar protein in it. *Bull. Jpn. Soc. Sci. Fish.* **1990**, *56*, 2069–2076.

Suzuki, A.; Watanabe, M.; Iwamura, K.; Ikeuchi, Y.; Saito, M. Effect of high pressure treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. *Agric. Biol. Chem.* **1990**, *54*, 3085–3091.

Suzuki, T.; Uehara, T.; Kamoi, I. Effect of pressure treatment on the heat-induced gelation of actomyosin. *Anim. Sci. Technol. (Jpn.)* **1991**, *62*, 154–160.

Yamamoto, K.; Miura, T.; Yasui, T. Gelation of myosin filament under high hydrostatic pressure. *Food Struct.* **1990**, *9*, 269–277.

Yasui, T.; Ishioroshi, M.; Samejima, K. Heat-induced gelation of myosin in the presence of actin. *J. Food. Biochem.* **1980**, *4*, 61–78.

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