# **Rheological and Biochemical Characteristics of High-Pressure- and Heat-Induced Gels from Blue Whiting** *(Micromesistius poutassou)* **Muscle Proteins**

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Heat- and high-pressure-induced gels were prepared from blue whiting muscle (*Micromesistius poutassou* R.). Minced muscle was homogenized with salt (1% NaCl), and response surface methodology was used to determine the influence of pressure, time, and temperature on the work of penetration. Two combinations of pressure–time–temperature were chosen to give the highest work of penetration, and samples were compared with a heat-induced gel. The following lots were subjected to chemical and rheological analysis: 200 MPa, 3 °C, 10 min (lot L); 375 MPa, 38 °C, 20 min (lot H); and atmospheric pressure, 37 °C, 30 min/90 °C 50 min (lot T). Breaking force, deformation, and cohesiveness were greater in gels of lot L. Hardness and water holding capacity were greatest in gels of lot T. Elasticity was greater in high-pressure-induced gels. The percentage of hydrophobic interactions, essentially linking actin molecules, was greater in gels of lot H than in gels of lots T and L. There were more cross-linkages in the heat-induced gels (lot T).

**Keywords:** *Gelling; high-presure; blue whiting (Micromesistius poutassou)* 

# INTRODUCTION

A number of studies have been reported in recent years on gelling mince of a variety of fish species using high-pressure and mixed pressure-temperature procedures. Such gels are described as glossier, smoother, softer, and more deformable than heat-induced gels (Hayashi, 1989; Farr, 1990; Okamoto et al., 1989; etc.). Some authors have reported advantages to be derived from the combined effects of pressurizing and heating, either simultaneously or consecutively (Ko et al., 1990; Ishikawa et al., 1991). Gelling of mince or surimi of muscle of fish under high pressure has been attributed by some authors to an increase in hydrophobic interactions and by others to cross-linking among myosin chains (Yamamoto et al., 1990; Ikeuchi et al., 1992; Shoji et al., 1994). Ikeuchi et al. (1992) report pressureinduced denaturation of the main myofibrilar proteins, actin and myosin.

In this paper, response surface methodology was used to select the best pressure-time-temperature conditions to achieve adequate texture (work of penetration) in blue whiting muscle gels. The paper also examines rheological and chemical characteristics in the two sets of conditions selected: high pressure/low temperature (200 MPa/3 °C/10 min) and high pressure/moderate temperature (375 MPa/38 °C).

# MATERIALS AND METHODS

Blue whiting (*Micromesistius poutassou* Risso) used in this study was caught off the Cantabrian coast. Average size was  $23.42 \pm 1.17$  cm, and average weight was  $77.83 \pm 12.27$  g. Fish were headed, gutted, and washed. Skin and bones were removed with a deboning machine (Baader 694, Lübeck, Germany). Muscle was minced and washed in a solution of

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0.2% NaCl at 0–3 °C, proportion 3:1 (solution:minced muscle), first with constant stirring for 10 min and then without stirring for another 10 min. After draining, excess water was removed using a screw press (Baader 523). Sorbitol (4%) and tripolyphosphate (0.2%) were added as cryoprotectants. The mince was immediately vacuum-packed in bags (Cryovac BB-1, Grace, Barcelona, Spain) and frozen in a plate-freezer (Saabroe SMC, Denmark) to a temperature of -30 °C. The bags were stored at -80 °C in a vertical freezer cabinet (Revco ULT, Giralt, Revco Scientific, Inc., Asheville, NC) in order to minimize alteration during frozen storage up to gel preparation.

Proximate analysis of mince was performed according to AOAC procedures (1984) and crude fat by Bligh and Dyer (1959). The proximate composition was crude protein, 12.34  $\pm$  0.3; moisture, 82.88  $\pm$  0.9; crude fat, 0.47  $\pm$  0.1; and ash, 0.63  $\pm$  0.01. Analyses do not show sorbitol (4%) added as a cryoprotectant.

Color of mince and gels was determined on a HunterLab MiniScan MS/S-4000S (Hunter Associates Laboratory Inc., Reston, VA) using the CIE Lab scale (D65/10°) where  $L^*$  is the parameter that measures lightness. The result was the average of six measurements taken at ambient temperature at different points on the sample.

Gel Preparation. Washed blue whiting mince was semithawed and placed in a refrigerated vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany). The muscle was ground for 1 min at 3000 rpm. Sodium chloride (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added at 1% final concentration (w/w), and the mixture was homogenized for 3 min (the proportion of 1% NaCl has been reported by Pérez-Mateos et al. (1996) as giving the highest values for work of penetration). Next, starch (Clearam CH 20, Laisa, Barcelona, Spain) was added at 5% final value, with crushed ice to give the required final gel moisture (78%). Homogenizing was continued for 6 min at 1500 rpm in vacuum conditions. The resulting batters were stuffed into cases (Krehalon Soplaril, Barcelona, Spain) of 40  $\mu m$  thickness and 3.5 cm diameter. The filled casings were placed in a high-pressure pilot unit (ACB 665, Gec Alsthom, Nantes, France) and subjected to either of two pressure-timetemperature combinations according to the statistical model described further below. This model was used to select the conditions that would give the highest values of work of penetration; the two sets conditions selected were 200 MPa

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**Figure 1.** Pressure-time-temperature effect on work of penetration on gels of blue whiting muscle made with 1% NaCl. Each response surface graph shows the midpoint value of the third constant variable.

at 3 °C for 10 min (lot L) and 375 MPa at 38 °C for 20 min (lot H). For comparison with the conventional gelling procedure, heat-induced gels were made at atmospheric pressure by waterbath immersion: 37 °C for 30 min, followed by 90 °C for 50 min (lot T). All the casings were immediately stored in a cold room at 4 °C for 24 h before analysis.

**Water Holding Capacity.** The method of Montero *et al.* (1996) was used. About 1.5 g of sample was placed in a centrifuge tube, along with two Gilson Pipetman dried pipet filters. A centrifuge (Sorvall RT6000B, DuPont Co., Wilmington, DE) was used at 5000*g* for 15 min at ambient temperature. Water holding capacity (WHC) was expressed as water retained per 100 g of water present in the sample prior to centrifuging. All determinations were carried out in triplicate.

**Puncture Test.** Samples were removed from their casings, cut (3.5 cm diameter, 3 cm height), and tempered at 20 °C. Gels were penetrated to the breaking point using a texturometer (Instron 4501, Instron Engineering Corp., Canton, MA) with a round-ended stainless steel plunger ( $\emptyset = 5$  mm). Crosshead speed was 10 mm/min, and a 100 N load-cell was used. Breaking force (N) and breaking deformation (mm) were determined. All determinations were carried out at least in quadruplicate.

**Texture Profile Analysis.** TPA was performed as described by Bourne (1978). Samples (3.5 cm diameter, 3 cm height) were tempered at 20 °C and placed on the flat plate of the texturometer. Compression was applied by a cylindrical plunger ( $\emptyset = 58$  mm) adapted to a 5 kN load cell at a deformation rate of 50 mm/min. On the basis of previous trials to establish a compression limit that would ensure no cracking and recoverability of most samples, it was decided to compress samples to 50% of height. In the test, each sample was compressed twice running. The parameters determined were hardness (N), elasticity (%), and cohesiveness. All determinations were performed in quadruplicate.

Protein Solubility of Gel. In order to ascertain the kinds of ionic, hydrogen, and hydrophobic interactions and S-S bonds, the gels were solubilized in four solutions (Matsumoto, 1980): 0.6 M sodium chloride (S1), 1.5 M urea + 0.6 M sodium chloride (S2), 8 M urea+ 0.6 M sodium chloride (S3), and 0.5 M 2- $\beta$ -mercaptoethanol + 0.6 M sodium chloride + 8 M urea (S4) at pH = 7.0. Two grams of chopped gel was homogenized with 10 mL of S1 (Ultraturrax TP18/10, Janke & Kunkel, Ika-Werk, Staufen, Germany) for 2 min at maximum speed. The resulting homogenate was stirred at 4 °C for 1 h and then centrifuged for 20 min at 20000g in a centrifuge (Beckman J2-MC, Beckman Instruments, Inc., Palo Alto, CA). The pellet obtained was homogenized in S2 by the same process, and then again in S3 (in this case, twice), and finally in S4. Protein concentration was determined in triplicate: in the supernatants, based on the method of Lowry et al. (1951) using a commercial preparation (DC Protein Assay Reagent S No. 500-0116, Bio-Rad Laboratories, Hercules, CA), and in the insoluble protein by the method of Kjeldhal. To prevent interference in protein determination, solutions were dialyzed. Colorimetry was performed in a spectrophotometer at 750 nm (UV-1203, Shimadzu, Kyoto, Japan). Results are the average of two determinations and are expressed as percentage of solubilized protein with respect to total protein.

**Electrophoresis**. Soluble proteins were treated according to the method of Hames (1985). They were adjusted to a final

average concentration of 2 mg/mL. Electrophoresis was carried out on a Phast-System horizontal apparatus (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) using 12.5% polyacrylamide gels (SDS–PAGE, PhastGel, Pharmacia Biotech AB, Uppsala, Sweden). Electrophoresis conditions were 10 mA, 250 V, and 3.0 W, at 15 °C. The protein bands were stained with Coomassie Brilliant Blue (PhastGel Blue R, Pharmacia). As reference for molecular weights, a standard high-molecular-weight protein mix was used (HMW, Pharmacia Biotech., Piscataway, NJ).

Statistical Analysis. One-way analysis of variance was carried out using the Statgraphics computer programme (STSC Inc, Rockville, MD). The difference of means between pairs was resolved by means of confidence intervals using a LSD range test. Level of significance was set for  $p \le 0.05$ . Response surface methodology (RSM) was used to study the simultaneous effect of three independent variables (pressure, time, and temperature) according to a central composite rotatable design (20 points). Assessment of error was based on five replications of the treatment in the central conditions as proposed in the design (Cochran and Cox, 1957) using the Statgraphic statistical program. The levels of each variable were pressure, 200, 245, 310, 375, and 420 MPa; time, 10, 14, 20, 26, and 30 min; and temperature, 0, 15, 38, 60, and 75 °C. A 3-D graph was drawn to show interrelationships among these variables and textural properties and water holding capacity. Minimum level of significance was set for  $p \leq 0.05$ .

### **RESULTS AND DISCUSSION**

In a previous paper (Pérez-Mateos et al., 1996), RSM was used to determine how the interaction of pressure, time, and temperature affects gelling of blue whiting muscle at two different levels of salt addition. The lower level of NaCl (1%) was chosen on the basis of this study. RSM analysis was performed (Figure 1) to determine the conditions at which the highest values can be achieved for work of penetration. This figure fitted a regression model with r = 0.99, and the significant variables ( $p \le 0.01$ ) were time, temperature (linear), pressure-temperature interaction, and pressure-time interaction. The highest values occurred at 200-300 MPa pressure and low temperature (0-15 °C) for a short time or at 350-400 MPa at 25-30 °C (20-30 min). Working with silvery pout (*Pollachius virens*), Serennes (1994) obtained maximum work of penetration at 400 MPa/30 °C/35 min, noting that the minimum pressure conditions under which myosin molecules would aggregate was 200 MPa/20 °C/15 min. Carlez et al. (1995), working on threadfin bream surimi (Nemipterus tambuloides), found maximum firmness in lots processed at 300 MPa/5-10 °C/15 min.

The batters were subjected to high pressure; the conditions chosen were 200 MPa/3 °C/10 min (lot L) and 375 MPa/38 °C/20 min (lot H). Both resulting gels were glossy, highly deformable, and soft to touch. These characteristics agree with the findings of Hayashi (1989) and Okamoto *et al.* (1989) on high-pressure-induced gels



One-Way Analyis of Variance: Different Letters (a, b, c) in the Same Column Indicate Significant Differences ( $p \le 0.05$ ) among Gels

	breaking deformation	breaking force	hardness	elasticity	cohesiveness
Т	а	а	а	а	а
L	b	b	b	b	b
Н	с	а	b	b	а

Figure 2. Rheological characteristics of lots T, L, and H (T, 37 °C, 30 min/90 °C, 50 min; L, 200 MPa, 3 °C, 10 min; H, 375 MPa, 38 °C, 20 min).

made from meat or fish myofibrillar proteins or from soy or egg proteins. Yamamoto *et al.* (1990), examining the behavior of myosin after subjecting it to different pressures, found that it would gel under 210 MPa (at ambient temperature) but not under 140 MPa. They further noted conformational changes in the tail region of myosin molecules as well as aggregation of myosin heads projecting from the surface of the filaments.

Breaking deformation values (Figure 2) were significantly higher ( $p \le 0.05$ ) in the high-pressure-induced gels (lots L and H) than in the heat-induced gels (lot T), particularly in the gel made at 200 MPa. Also, Pérez-Mateos et al. (1996) reported that gels made from minced blue whiting muscle (with 1% added NaCl) at pressures ranging from 200 to 250 MPa (3 °C, short time) exhibited greater deformation and work of penetration (product of breaking force and deformation) than those made under higher pressure and temperature conditions. Serennes (1994), working with silver pout muscle (Pollachius virens), reported maximum gel strength at 400 MPa and 30 °C. Carlez et al. (1995), working with threadfin bream surimi (Nemipterus tam*buloides*), found maximum deformation and gel strength at 300 MPa and 4 °C. In this last study, however, breaking force was significantly greater in the gel made at 90 °C under atmospheric pressure than in highpressure-induced gels. This contrasts with the present work, in which no significant differences were found between lots T and H. Nagashima et al. (1993), working with squid muscle (Loligo bleekeri), found no direct correlation between the pressure at which gels were made and the resulting breaking force. Again, Chung et al. (1994) reported that, in gels made from Pacific whiting (Merluccius productus) surimi without protease inhibitors, strain and strength were tripled when gels were made under high pressure instead of atmospheric pressure. The same authors, working with Alaska pollack (Theragra chalcogramma) surimi, further noted that, in gels made at 170 and 240 MPa and below 50 °C, shear stress and shear strain were greater than in heat induced gels.



**Figure 3.** Water holding capacity of lots T, L, and H (T, 37 °C, 30 min/90 °C, 50 min; L, 200 MPa, 3 °C, 10 min; H, 375 MPa, 38 °C, 20 min). Different letters at the top of the bars mean significant differences ( $p \le 0.05$ ).

Breaking force values (Figure 2) were significantly higher ( $p \le 0.05$ ) in gels made at 200 MPa/3 °C/10 min (lot L).

Hardness as measured by TPA is shown in Figure 2. In heat-induced gel (lot T), this was more or less double what it was in the high-pressure-induced gels (lots L and H). Both Hayashi (1989) and Carlez *et al.* (1995) have also reported that gels made using high-pressure methods are softer than heat-induced gels; nonetheless, Yoshioka *et al.* (1992) have reported the contrary. Gels of lot H were found to be not significantly harder than gels of lot L. However, Okamoto *et al.* (1990) reported greater hardness in carp crude actomyosin and rabbit paste samples pressurized at 25 °C, increasing as pressure rose.

Elasticity (Figure 2) as measured by TPA was significantly greater ( $p \le 0.05$ ) in pressure-induced gels. Yoshioka *et al.* (1992), working with carp and Pacific mackerel *surimi*, reported that elasticity was greater in high-pressure- than in heat-induced gels. Ishikawa *et al.* (1991) drew attention to the importance of heating subsequent to pressurizing, reporting greater elasticity in sardine *surimi* high-pressure-induced gels at 100 MPa then heated at 55 °C than in high-pressureinduced gels at 400 MPa. In the present case, however, heating at 38 °C during pressurizing did not significantly increase elasticity as compared to the sample pressurized at 3 °C.

Cohesiveness (Figure 2) as determined by TPA differed significantly in pressurized lots according to temperature, being greater ( $p \le 0.05$ ) in lot L (200 MPa/3 °C/10 min). Pérez-Mateos *et al.* (1996) found that temperature and pressure have a linear effect on cohesiveness ( $p \le 0.01$ ) in gels made in the same way and with the same ingredients as in the present experiment, their highest cohesiveness values occurring at low temperatures and low pressures. Heating could, perhaps, be the factor that caused cohesiveness to be significantly similar in gels of lots H and T.

In water holding capacity (Figure 3), there were no significant differences ( $p \le 0.05$ ) between gels of lots H and L, but these did differ from lot T. Okazaki (1991) reported that the occurrence or otherwise of variations in WHC of muscles gelled under pressures between 200 and 500 MPa was dependent on species and declined



**Figure 4.** Lightness of lots T, L, and H (T, 37 °C 30 min/90 °C, 50 min; L, 200 MPa, 3 °C, 10 min; H, 375 MPa, 38 °C, 20 min). Different letters at the top of the bars mean significant differences ( $p \le 0.05$ ).



**Figure 5.** Protein fraction (%) of lots T, L, and H (T, 37 °C, 30 min/90 °C, 50 min; L, 200 MPa, 3 °C, 10 min; H, 375 MPa, 38 °C, 20 min) in different solutions: 0.6 M sodium chloride (S1), 1.5 M urea + 0.6 M sodium chloride (S2), 8 M urea + 0.6 M sodium chloride (S3), and 0.5 M 2- $\beta$ -mercaptoethanol + 0.6 M sodium chloride + 8 M urea (S4) at pH = 7.0 and insoluble fraction (In). Different letters at the top of the bars mean significant differences ( $p \le 0.05$ ) for each solution.

in all species beyond 400 MPa. Pérez-Mateos *et al.* (1996) reported that, in blue whiting muscle gels, the significant factor ( $p \le 0.01$ ) affecting WHC was temperature and not pressure. Thus, given that the pressures used in the present experiment were too low for starch to gelatinize (Hayashi, 1989), the reason why lot T had greater ( $p \le 0.05$ ) WHC may lie in gelatinization of the starch in lot T (90 °C).

Luminosity (Figure 4) was greater ( $p \le 0.05$ ) in gels of lot H, owing equally to higher pressure and temperature. This is consistent with the findings of Wada and Ide (1991), Nagashima *et al.* (1993), and Pérez-Mateos *et al.* (1996). However, in the case of the heat induced gels, the  $L^*$  value was lower ( $p \le 0.05$ ) than in lot H, probably because it was naturally less bright. Yoshioka *et al.* (1992) also reported differences in luminosity between heat and high pressure induced gels.

Figure 5 shows protein solubility in different solutions as a measurement of the types of bonds present in the three gels. Solubility of lot L in S1 and S2 differed significantly from that of the other two lots, which was reflected in a greater ( $p \le 0.05$ ) presence of ionic and hydrogen bonds at 200 MPa. Carlez *et al.* (1995) also found that ionic bonds (calcium) played an important part in gel texture. The greater ( $p \le 0.05$ ) presence of hydrogen bonds in gels of lot L may have been due to low pressure (200 MPa) or low temperature (3 °C). Approximately half of the protein was recovered in S3, which was used to break hydrophobic interactions. Gels of lot H exhibited the greatest solubility in S3, indicating that it contained significantly more hydrophobic interactions (or that these bonds were more susceptible to rupture, for example because they were less completely enveloped by cross-linkages) than gels of lots T and L. The reason for these differences may be that 200 MPa was insufficient pressure to assist the formation of hydrophobic interactions in lot L, or else that the combination of moderate heating temperature (38 °C) and a higher pressure (375 MPa) favored the formation of hydrophobic interactions in lot H. The weaking of hydrophobic bonds in lot T could have been due to heating at temperatures over 58 °C (Niwa, 1992). Ikeuchi et al. (1992), working on isolated actomyosin gels, and Carlez et al. (1995), working on threadfin bream (Nemipterus tambuloides) surimi, found that high pressure favored hydrophobic interactions, although it is also true that a temperature of 38 °C can also favor such bonding. Niwa (1992) reported that these interactions are weakened by higher temperature, as destabilization of hydrogen bonds among water molecules presented an obstacle to hydrophobic hydration. Formation of these hydrophobic interactions may be connected with changes occurring in the conformation of the myosin tail (Yamamoto et al., 1990). The proportion of insoluble protein, the proportion of protein linked by S-S bonds, and likewise breaking strength and deformation were higher ( $p \le 0.05$ ) in lot L than in lot H. This was consistent with the findings of Shoji et al. (1992), who reported increased breaking strength and changes in myofibrillar protein accompanied by a decrease in myosin heavy chain (HC) and an increase in HC polymers in high-pressure-induced walleye pollack gels stored at 5 °C.

The pressure-induced gels possessed a smaller percentage of disulfide bonds, as indicated by lower ( $p \leq$ 0.05) protein solubility in S4. In rabbit natural actomyosin, Ikeuchi et al. (1992) reported smaller numbers of S-S bonds on pressurizing. The smaller proportion of protein in the insoluble fraction in gels of lots L and H would indicate that high-pressure treatment induced a smaller percentage of covalent bonds (other than S-S bonds) than did heating. One explanation for this could be that, in heat-induced gels at atmospheric pressure, one of the mechanisms of cross-link formation is transglutaminase (Seki et al., 1990), which according to Shoji et al. (1994) becomes degraded under pressures in the region of 300 MPa. Yamamoto et al. (1990) and Shoji et al. (1994) reported cross-linking strands between lateral associations of myosin when pressures in the region of 200 MPa were applied. This suggests conformation changes in the tail region of myosin molecules as well as aggregation of myosin heads. The fact that there were more disulfide and other covalent bonds in gels of lot T largely explains the hardness and lower (p  $\leq$  0.05) elasticity of the gel. The high deformability of gels of lots L and H reflects a lower ( $p \le 0.05$ ) proportion of covalent bonds, due in gels of lot L mainly to the weakness of bonding (ionic and hydrogen bonds) and in gels of lot H to hydrophobic interactions.

The electrophoretic profiles (Figure 6) of proteins solubilized in S1 and S2 for lots L and H showed a larger number of bands in the zone of less than 36 kDa molecular mass. This was probably because these bands consist of sarcoplasmic proteins, or it could have been due to degradation of larger proteins which were formed during postharvest storage (Lin and Park, 1996)



**Figure 6.** (a) Electrophoresis profile of the soluble fractions S1 (0.6 M sodium chloride), S2 (1.5 M urea + 0.6 M sodium chloride), S3 (8 M urea + 0.6 M sodium chloride), and S4 (0.5 M 2- $\beta$ -mercaptoethanol + 0.6 M sodium chloride + 8 M urea) of lots T, L, and H (T, 37 °C, 30 min/90 °C, 50 min; L, 200 MPa, 3 °C, 10 min; H, 375 MPa, 38 °C, 20 min). Ag = aggregated, M = myosin heavy chain, A = actin. (b) SDS-PAGE (12.5%) patterns of the soluble fractions S1–S4.

and during preanalysis chilled storage (24 h at 4 °C), which would indicate that more interactions occur among low-molecular weight proteins through ionic and hydrogen bonds in pressure-induced gels than in heatinduced gels (lot T). This conflicts with the findings of Ohshima et al. (1992), who reported that, at 400 MPa pressure, the sarcoplasmic proteins tended to aggregate and thus disappeared from this profile zone. The same profiles (S1 and S2) showed greater optical density in the G-actin band for gels of lot L. Ikeuchi et al. (1992) reported that F-actin depolymerized into G-actin under 150 MPa pressure. If we consider that high temperatures weaken hydrogen bonds (Niwa, 1992), then these hydrogen bonds would appear to have come into being after the gel was formed, over the 24 h of preanalysis chilled storage, since, according to Niwa and Hamada (1981) and Niwa et al. (1985), a portion of helical structure of myosin heavy chains is recoverable upon

cooling. In gels of lot H, there were bands of 200–150 kDa, which could have been produced by degradation of myosin or by aggregation of lower-molecular-weight proteins. The actin band, on the other hand, was larger in gels of lot H, which could indicate that, under 375 MPa, the actin aggregated by means of hydrophobic interactions. The electrophoretic profile of the protein solubilized in S4 showed that the broken disulfide bonds had bonded mainly myosin in gels of lots H and L, and actin in lots T and H.

# GENERAL CONCLUSIONS

Breaking deformation, breaking force, cohesiveness and elasticity were higher in blue whiting high-pressure-induced gels than in heat-induced gels. Except for elasticity, rheological characteristics presented higher values in lot L than in lot H. Hardness and water holding capacity were higher in lot T. The high deformability and lower hardness of high-pressure-induced gels are consistent with a lower proportion of covalent bonds and a greater presence of weaker bonds such as ionic, hydrogen and hydrophobic. In heatinduced gels, ionic and hydrogen bonds play a more important role in linking the meromyosin chains than they do in the high-pressure-induced gels. In the pressure-induced gel elaborated at 375 MPa, actin was aggregated to a large extent by hydrophobic interactions.

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