

Comparison of Different Gelation Methods Using Washed Sardine (*Sardina pilchardus*) Mince: Effects of Temperature and Pressure

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This paper relates the protein solubility and rheological changes occurring in washed sardine mince gels produced by different treatments: heat-induced, pressure-induced, and pressure-assisted gelation. In batter and set gel, there was considerable involvement of low molecular mass proteins (<67 kDa), such as actin, linked chiefly by weak bonds. High molecular mass proteins (200–67 kDa) were implicated in the other samples, but only myosin heavy chain (MHC) appeared in the cases heated at 90 °C. The highest values for work of penetration in the heat-induced gel and the pressure-induced gel were associated with the highest amounts of insoluble proteins. All the gel networks were stable enough to hold water. The highest lightness value was obtained in the pressure-assisted gel as a result of the application of both treatments: pressure and heat. The ultrastructure of the gels induced by heating, with or without pressure treatment, was more spongy than in the case of the gel induced only by pressure, which was more compact.

Keywords: Heat; pressure; gel; solubility; ultrastructure; rheology

INTRODUCTION

Heat-induced gelation of washed sardine mince at atmospheric pressure is normally carried out in two stages (Niwa, 1992): the batter (*sol*) is first held at 37 °C for 30 min or 4 °C for 18 h, losing its characteristic viscosity and turning into an elastic, translucent gel (a phenomenon known as *suwari* or setting); next, that gel is heated to over 80 °C to produce a stronger, more opaque, and more highly elastic gel than that produced by heating in a single step only. Many authors (Foe-geding *et al.*, 1986; Niwa, 1992) have observed that fish gels heated directly to high temperatures without first undergoing setting tend to have a compact, less uniform structure than gels prepared after initial setting. This has been attributed to syneresis of the proteins caused by the high temperatures applied.

Pressure increases solubilization of the myofibrillar proteins subsequent to depolymerization at low salt concentrations or even in the absence of salt (Cheftel and Culioli, 1997). There is no general pattern of pressure-induced denaturation, which depends on the species and the condition of the protein. In general, pressure-induced gelation of fish mince at low temperatures (Okamoto *et al.*, 1990; Chung *et al.*, 1994; Carlez *et al.*, 1995; Pérez-Mateos and Montero, 1997) has yielded gels with a different appearance (smoother, glossier) and different rheological characteristics (less hard, more elastic) than gels induced by heating at atmospheric pressure. Setting at relatively high temperatures (37 °C, 30 min) or at low temperatures (4 °C, 24 h) before pressurization did not produce alterations in pressure-induced threadfin bream gel *surimi* (*Nemipterus tambuloides*) (Carlez *et al.*, 1995). However, Serennes *et al.* (1996) found that setting at 4 °C for 24 h increased texturization in coalfish (*Pollachius virens*) pulp.

Alternatively, heating and pressurization may be combined, being applied either concurrently or successively. The effects of combining heat and pressure will vary according to the processing conditions. Combining pressurization with heating appears to increase the number of bonds in the heavy myosin chain and the number of hydrophobic interactions (Pérez-Mateos *et al.*, 1997), yielding high-quality gels that are more translucent in appearance than gels obtained by heating alone, which tend to be more opaque (Chung *et al.*, 1994). Heating after pressurization (pressure-assisted gelation) stabilizes the gel quality during storage, minimizes the amount of pressure needed for gelation, though it is associated with lower gel transparency (Ishikawa *et al.*, 1991; Shoji *et al.*, 1992), and improves gelation of poor gel-forming muscle, such as squid mantle (Nagashima *et al.*, 1993) and slightly washed fish minced muscle, as compared to the high gel-forming strength of *surimi*.

The objective of the present study was to characterize the effect of different heat and pressurization treatments. In addition, pressurization brings about solubilization of the proteins and in this way may enhance the subsequent pressure-assisted heat gelation process. Since pressure-induced gels are similar in appearance to set gels, another objective was to ascertain whether the traditional heat setting could be replaced by the pressure treatment to produce gels with improved rheological characteristics.

MATERIALS AND METHODS

Materials. Sardine (*Sardina pilchardus* Risso) used in this study was caught off the Mediterranean coast in May and kept at 4 °C about 24–48 h. The mean size was 14.4 ± 0.9 cm and the mean weight 26.7 ± 6.4 g. The fish were deheaded, gutted, and washed. The skin and bones were removed with a deboning machine (Baader Model 694, Lübeck, Germany). The muscle was minced and washed in a solution of 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

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using a screw press (Baader Model 523, Lübeck, Germany). Sorbitol (4%) and sodium tripolyphosphate (0.2%) were added as cryoprotectants. The washed mince was immediately vacuum-packed in bags (Cryovac BB-1, Grace, Barcelona, Spain) and frozen in a plate freezer (Saabroe SMC) to a temperature of -30°C . The bags were stored at -80°C in a freezer cabinet (Revco ULT, Giralt, Revco Scientific, Inc., Asheville, NC) to minimize alterations during frozen storage until preparation of the gels.

Proximate Analysis. The proximate analysis of the washed mince was performed according to AOAC procedures (1984), and crude fat was determined by the method of Bligh and Dyer (1959). Crude protein was $12.7\% \pm 0.2\%$, moisture $78.7\% \pm 0.2\%$, ash $0.82\% \pm 0.02\%$, and crude fat $4.6\% \pm 0.2\%$ (analyses do not show the added cryoprotectants).

Color. The color was determined using a colorimeter (HunterLab MiniScan MS/S-4000S, Hunter Associates Laboratory Inc., Reston, VA) using the CIE Lab scale ($D65/10^{\circ}$) where L^* is the measure of lightness. The result was the mean of six measurements taken at ambient temperature at different points on the sample.

Gel Preparation. Washed sardine 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 high speed (start II). An amount of 2.5% sodium chloride (w/w), final concentration (Panreac, Montplet & Esteban S.A., Barcelona, Spain), was added and the mixture homogenized with crushed ice for 5 min at slow speed (start I) to give the required final gel moisture content (78%). The resulting batters were stuffed into cases (Krehalon Soplaril, Barcelona, Spain) 40 μm thick and 3.5 cm in diameter.

In a previous study (Pérez-Mateos and Montero, 1997), response surface methodology was used to determine how the interaction of pressure, time, and temperature affected the gelling of sardine muscle. On the basis of that study, one set of pressure-time-temperature (300 MPa, $<10^{\circ}\text{C}$, 10 min) gelling conditions was chosen. The treatments employed were setting (gel S: 4°C , 18 h), heating by immersion in a water bath (gel H: 37°C , 30 min followed by heating at 90°C , 50 min), pressurization (gel P: 300 MPa, $<10^{\circ}\text{C}$, 10 min) in a high-pressure pilot unit (ACB 665, Gec Alsthom, Nantes, France), and pressure-assisted gelation (gel P-H: 300 MPa, $<10^{\circ}\text{C}$, 10 min followed by heating at 90°C , 15 min at atmospheric pressure in a water bath). All the samples were cooled immediately and stored in a cold room at 4°C overnight before analysis; except batter (mince homogenized with salt), which was analyzed immediately following preparation.

Gel-Forming Ability. Gels were removed from their casings, cut, and tempered at 20°C . On gel slices (3 mm high, 3.5-cm diameter), a *folding test* (scoring from 1 to 5) following Suzuki (1981) was applied. Samples (3.5 cm in diameter by 3 cm in height) were placed on the flat plate of the texturometer (Instron 4501, Instron Engineering Corp., Canton, MA). A *puncture test* (Bourne, 1982) was performed using a cylindrical stainless steel spherical probe (5-mm diameter) attached to a 100-N load cell; the gel was penetrated to the breaking point at 10 mm/min and to develop force-deformation curves (breaking force [N], breaking deformation [mm], and work of penetration [N-mm]). A *stress-relaxation test* (Bourne, 1982) was performed using a cylindrical plunger (58-mm diameter) 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 the samples to 60% of the height for 1 min and force exerted recorder. The percent of relaxation was calculated as $Y_T = 100(F_0 - F_1)/F_0$, where F_0 is the force registered at the onset of relaxation (hardness [N]) immediately after sample compression and F_1 is the force registered after 1 min of relaxation. Thus, $100 - Y_T$ is taken as the index of elasticity and is expressed as the percent elasticity of the gel. At least four replications of all determinations were performed.

Protein Solubility of the Gels. In order to ascertain the kinds of bonds, the gels were solubilized successively in four

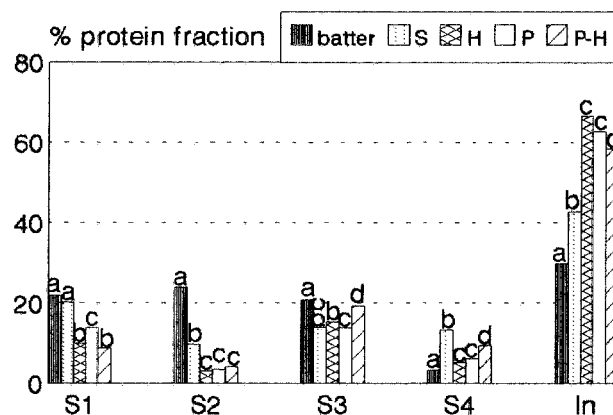


Figure 1. Soluble protein fractions of the batter and gels (S, 4°C , 18 h; H, 37°C , 30 min/ 90°C , 50 min; P, 300 MPa, $<10^{\circ}\text{C}$, 10 min; P-H, 300 MPa, $<10^{\circ}\text{C}$, 10 min/ 90°C , 15 min) extracted using the different solutions: 0.6 M sodium chloride (S1), 1.5 M urea + 0.6 M sodium chloride (S2), 8 M urea + 0.6 M sodium chloride (S3), and 0.5 M $2\text{-}\beta$ -mercaptoethanol + 0.6 M sodium chloride + 8 M urea (S4) and the insoluble fraction (In). Differing letters at the tops of the bars represent significant differences ($p \leq 0.05$) for the respective parameters among the gels.

solutions at pH = 7.0 (Pérez-Mateos *et al.*, 1997): 0.6 M sodium chloride (S1) comprising ionic bonds, 1.5 M urea + 0.6 M sodium chloride (S2) comprising hydrogen bonds, 8 M urea + 0.6 M sodium chloride (S3) comprising hydrophobic interactions, and 0.5 M $2\text{-}\beta$ -mercaptoethanol + 8 M urea + 0.6 M sodium chloride (S4) comprising disulfide bonds and an insoluble fraction comprising other covalent bonds (no disulfide bonds). 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 20 000g in a centrifuge (Beckman J2-MC, Beckman Instruments, Inc., Palo Alto, CA). The pellet obtained was homogenized in solution S2 by the same process, then again in S3 (in this case, twice), and finally in S4. The protein concentration was determined in triplicate: in the supernatants, based on the method of Lowry (Lowry *et al.*, 1951) using a commercial preparation (DC Protein Assay Reagent S No. 500-0116, BIO-RAD Laboratories), and in the insoluble protein by the method of Kjeldhal. In order to prevent interference in protein determination, solutions were dialyzed. Colorimetry was performed in a spectrophotometer at 750 nm (UV-1203, Shimadzu, Kyoto, Japan). The results are the average of two determinations and are expressed as the percentage of solubilized protein with respect to total protein.

Electrophoresis. The supernatants obtained on extracting the gel samples as described above were adjusted to a final mean concentration of 2 mg/mL. Electrophoresis was carried out using a horizontal Phast-System apparatus (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) and 12.5% poly(acrylamide) gels (SDS PAGE, PhastGel, Pharmacia Biotechnology AB, Uppsala, Sweden). The conditions of electrophoresis were 10 mA, 250 V, and 3 W at 15°C . Protein bands were stained with Coomassie brilliant blue (PhastGel Blue R, Pharmacia). A commercial high-molecular-weight protein mix was used as the standard (HMW, Pharmacia Biotech., Piscataway, NJ) containing the following reference molecular masses: ferritin half-unit (220 kDa), albumin (67 kDa), catalase subunit (60 kDa), lactate dehydrogenase subunit (36 kDa), and ferritin subunit (18.5 kDa).

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 dried pipet filters (Gilson Pipetman, Villiers le Bel, France). The water-holding capacity was expressed as the water retained per 100 g of water present in the sample prior to centrifuging (Sorvall RT6000B, DuPont Co., Wilmington, DE) at 4000g for 15 min at ambient temperature. All determinations were carried out in triplicate.

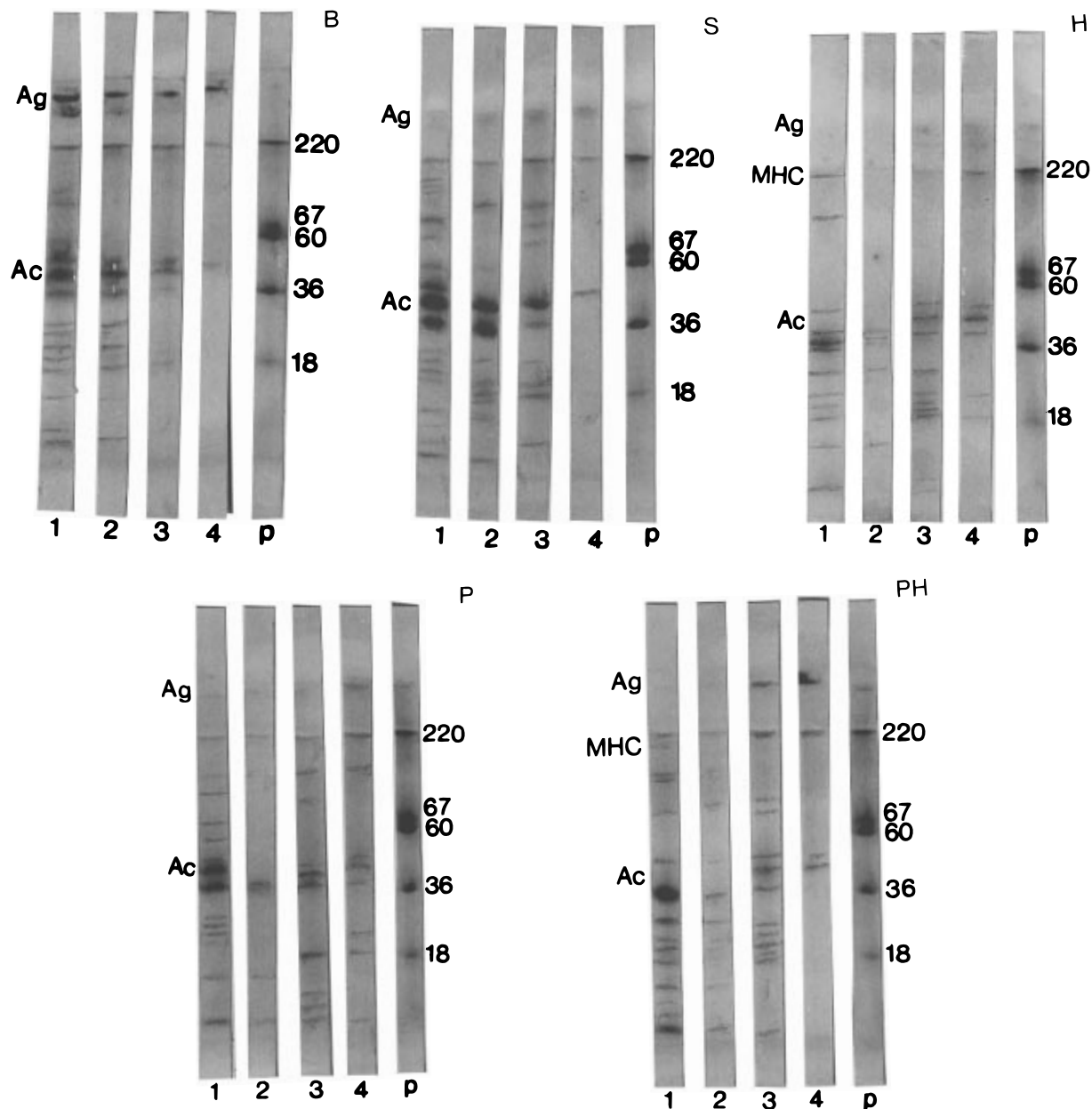


Figure 2. SDS PAGE (12.5%) patterns for the soluble fractions of the batter (B) and gels (S, 4 °C, 18 h; H, 37 °C, 30 min/90 °C, 50 min; P, 300 MPa, <10 °C, 10 min; PH, 300 MPa, <10 °C, 10 min/90 °C, 15 min) extracted using the different solutions: 0.6 M sodium chloride (S1), 1.5 M urea + 0.6 M sodium chloride (S2), 8 M urea + 0.6 M sodium chloride (S3), and 0.5 M 2- β -mercaptoethanol + 0.6 M sodium chloride + 8 M urea (S4) and the insoluble fraction (In). Ag = aggregated; MHC = myosin heavy chain; Ac = actin; p = standard.

Scanning Electron Microscopy (SEM). Cubes measuring 2–3 mm per side were cut from inside the gels for microscopic examination. Samples were fixed in 2% glutaraldehyde in phosphate buffer (pH = 7.3) and dehydrated in increasing concentrations of acetone (from 40% to 100%). They were then critical point dried with CO₂ as the transition fluid in a dryer (Balzer, CPD030, Liechtenstein) and mounted on copper sample holders; this was followed by sputter-coating with gold in a metallizer (Balzer, SCD004). Samples were kept in a dryer until examination by a scanning electron microscope (Jeol, JSC 6400) at 20 kV.

Statistical Analysis. One-way analysis of variance was performed using a computer statistical package (Statgraphics, STSC Inc., Rockville, MD). The differences between the means for value pairs were calculated using an LSD range test. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Protein Solubility. The soluble and insoluble fractions (Figure 1) in the samples extracted using the

different solutions selected for their ability to disrupt certain kinds of bonds varied according to the different gelation procedures employed. Batter showed the highest percentage ($p \leq 0.05$) solubility in the extractions using solutions S2 and S3, indicating a predominance of hydrogen and hydrophobic interactions; the lowest solubility values ($p \leq 0.05$) for batter were for the extract from solution S4 and the insoluble fraction due to a smaller proportion of covalent bonds. Set gel (gel S) exhibited an increase in the proportion of covalent bonds ($p \leq 0.05$) with respect to the batter, mainly disulfide bonds in the extract from solution S4, which in this gel had the highest percentage of disulfide bonds ($p \leq 0.05$) of all the samples. Disulfide bond formation at the setting temperature has also been reported by some workers (Niwa *et al.*, 1982). It would thus appear virtually certain that for fish proteins, the low-temperature-setting effect uniquely involves non-disulfide cross-linking of myosin (Gill and Conway, 1989; Sano *et al.*,

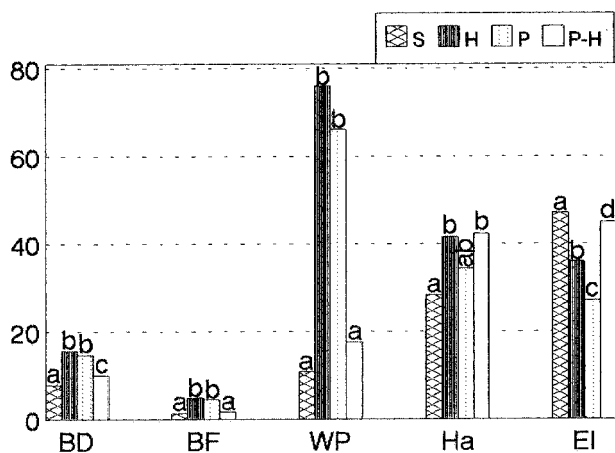


Figure 3. Breaking deformation (BD), breaking force (BF), work of penetration (WP), hardness (Ha), and elasticity (EI) of sardine gels prepared by different gelation treatments (S, 4 °C, 18 h; H, 37 °C, 30 min/90 °C, 50 min; P, 300 MPa, <10 °C, 10 min; P-H, 300 MPa, <10 °C, 10 min/90 °C, 15 min). Differing letters at the tops of the bars represent significant differences ($p \leq 0.05$) for the respective parameters among the gels.

1990). The heat-induced gel had a higher proportion ($p \leq 0.05$) of the insoluble fraction (comprising covalent bonds) than the set gel, because during heating the proteins underwent denaturation and aggregation that lowered their solubility. The action of *transglutaminase* enzymes has been postulated as the mechanism of the cross-linking reactions (Seki *et al.*, 1990; Niwa, 1992). The pressure-induced gel (gel P) exhibited a smaller percentage of extract from solution S3 than the heat-induced gel ($p \leq 0.05$), indicating fewer hydrophobic interactions and no difference in covalent bonds that were not attributable to the degradation of *transglutaminase* under pressure in the region of 300 MPa, as Shoji *et al.* (1994) reported. Moreover, Carlez *et al.* (1995) and Pérez-Mateos *et al.* (1997) found that high pressure applied at moderate temperatures (30–40 °C) induced hydrophobic interactions, compared to lower induction of hydrophobic interactions at chilled temperatures (<10 °C). As in the case of the heat-induced gel, in the pressure-induced gel, the insoluble fraction (comprising non-disulfide covalent bonds) was higher and the fractions extracted from solutions S1 and S2 (comprising ionic and hydrogen bonds) were lower than in the set gel ($p \leq 0.05$). Cheftel and Culioli (1997) reported that the pressure increased the solubilization of myofibrillar proteins, but this effect was not observed for the washed sardine mince. In the pressure-assisted gel (gel P-H), heating after the application of pressure caused greater extractability in solution S3 and, hence, more hydrophobic interactions ($p \leq 0.05$) than in the heat- or pressure-induced gels.

Electrophoretic Study. The electrophoretic profile of the fractions solubilized from the batter using the different solutions (Figure 2B) yielded bands in the sample application zone and in the interphase zone between the stacking and resolving gels, representing polymerized protein in the form of large aggregates that were nonetheless soluble in the solutions, which prevented them from entering the electrophoretic resolving gel. The extracts from solutions S1 and S2 had a large number of bands with molecular masses lower than 67 kDa, chief among them actin, which would indicate that this protein was, at least partially, weakly bonded with linkages formed by nonspecific associations, ionic link-

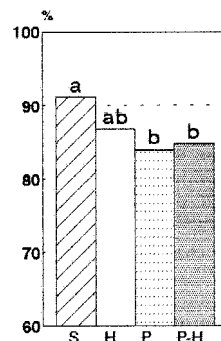


Figure 4. Water-holding capacity of the batter and gels (S, 4 °C, 18 h; H, 37 °C, 30 min/90 °C, 50 min; P, 300 MPa, <10 °C, 10 min; P-H, 300 MPa, <10 °C, 10 min/90 °C, 15 min). Differing letters at the tops of the bars represent significant differences ($p \leq 0.05$) for the respective parameters among the gels.

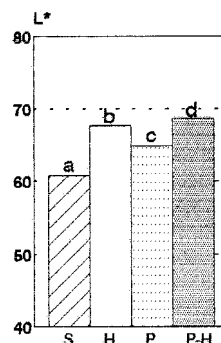


Figure 5. Lightness of the batter and gels (S, 4 °C, 18 h; H, 37 °C, 30 min/90 °C, 50 min; P, 300 MPa, <10 °C, 10 min; P-H, 300 MPa, <10 °C, 10 min/90 °C, 15 min). Differing letters at the tops of the bars represent significant differences ($p \leq 0.05$) for the respective parameters among the gels.

ages, and hydrogen bonds. The band for actin was also present in the extract from solution S3 (comprising hydrophobic interactions) and to a small extent also in the extract from solution S4 (comprising disulfide bonds). The electrophoretic profile of the fractions solubilized from the set gel (Figure 2S) was quite similar to that of the batter, but there were more high-molecular-mass bands (100–67 kDa) in the extract from solution S4. These higher molecular mass bands could represent polypeptides produced by proteolysis (Niwa *et al.*, 1993). Montero and Gómez-Guillén (1996) did not observe as many bands in sardine set gel prepared at 35 °C. In the heat-induced gels (Figure 2H), noncovalent bonds reduced the number of bands, especially in the zone between 200 and 67 kDa, but a faint band of myosin heavy chain (MHC) was present in the extract from solution S1, indicative of linkages formed by weak bonds. The extract from solution S4 contained a few bands of protein (containing disulfide bonds). The electrophoretic profile of the soluble fractions from the pressure-induced gel (Figure 2P) differed from that of the heat-induced gel, mainly because MHC was absent in some bands with weights between 200 and 60 kDa, which could have been due to the degradation of myosin protein, and were detected in the extracts from solutions S1, S3, and S4. Conversely, Montero and Gómez-Guillén (1996) reported fewer bands for linkages consisting of weak bonds in heat-induced sardine gels, a result they attributed to the smaller proportion of soluble fraction obtained. The pressure-assisted gel (Figure 2PH) exhibited an electrophoretic pattern with

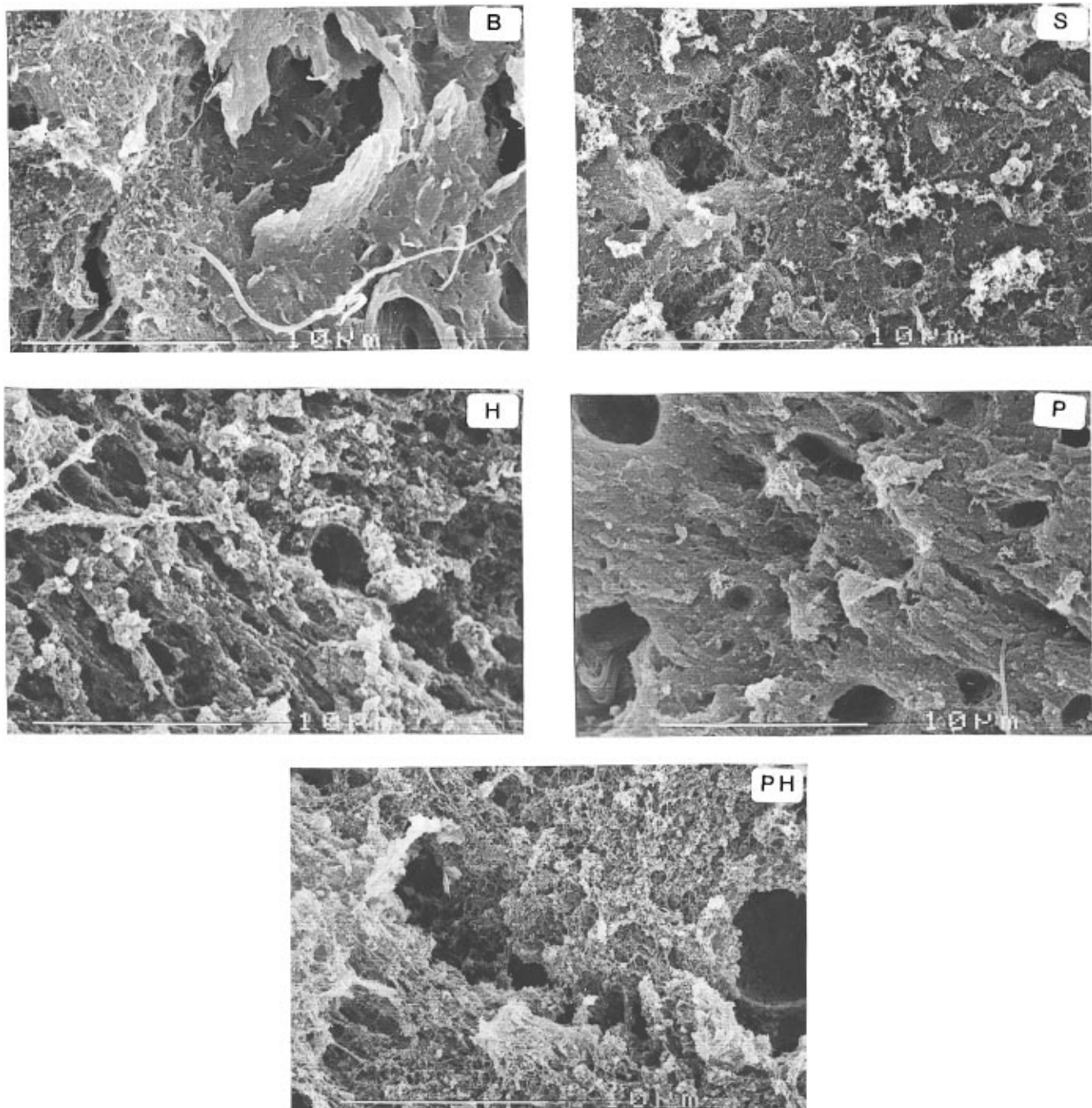


Figure 6. Scanning electron micrographs (3000 \times magnifications, bar 10 μm) for the batter (B) and gels (S, 4 $^{\circ}\text{C}$, 18 h; H, 37 $^{\circ}\text{C}$, 30 min/90 $^{\circ}\text{C}$, 50 min; P, 300 MPa, <10 $^{\circ}\text{C}$, 10 min; PH, 300 MPa, <10 $^{\circ}\text{C}$, 10 min/90 $^{\circ}\text{C}$, 15 min).

the features of both pressure-induced and heat-induced gels, a finding in agreement with the analyses discussed below.

Gel-Forming Ability. All the samples attained the maximum score for the folding test, with the obvious exception of the sample (batter) that did not undergo gelation, which exhibited the consistency of a sol, that is, an intermediate state that precedes gelling; hence, rheological analysis was not possible for that sample. The set gel (gel S) had lower breaking deformation and breaking force values than the heat-induced and pressure-induced gels, though the elasticity values were higher (Figure 3). A number of authors have raised the possibility of differences in evolution between penetration tests and the compression test, since they are different rheological techniques (Burgarella *et al.*, 1985; Lee and Chung, 1989). Lee and Chung (1989) noted

that while the penetration test measures the degree of compactness or density of actomyosin, the compression test measures the overall binding property of the gel material. There were no significant differences in the rheological parameter values between the heat-induced gel and the pressure-induced gels, except for the higher elasticity of the heat-induced gel ($p \leq 0.05$). Pressure-induced gels have been reported to be generally more deformable and elastic than heat-induced gels (Chung *et al.*, 1994; Pérez-Mateos *et al.*, 1997). A number of authors have attributed these differences in behavior to species specificity, the state of the protein, and the gel-forming ability of the mince.

The pattern of denaturation due to the effect of pressure is not generalized (Cheftel and Culioli, 1997) and differs according to the species (Ko *et al.*, 1990). The process and the pressure–time–temperature combina-

tion used (Yamamoto *et al.*, 1990; Okazaki, 1991; Pérez-Mateos *et al.*, 1997) are also factors. In this same sense, Pérez-Mateos and Montero (1997) reported that the effect of high pressure on the gelation of washed sardine mince depended on mince quality; accordingly, application of pressure improved the rheological properties of washed sardine minces that exhibited poor gel-forming ability in response to heat treatment but did not exert any sizable influence on minces that exhibited high gel-forming ability. Yoshioka *et al.* (1992) compared the changes in texture taking place in carp and mackerel surimi and found that pressure-induced gels had higher hardness, elasticity, and viscosity values than heat-induced gels.

The higher values for work of penetration in the heat-induced gels and the pressure-induced gels have been associated with minimal amounts of extractable proteins taken up into the gel structure (Barbut *et al.*, 1996), as shown by the extraction analysis discussed above.

Pressure-assisted gelation (a pressure stage followed by a heating stage) led to functional changes discernible as a loss in both breaking deformation and breaking force. However, elasticity was higher, though it did not reach the level of the characteristically high values of the set gel; this increase was accompanied by an increase in the percentage of soluble protein in the extracts from solution S3 (comprising hydrophobic interactions). This may be because the second-stage heating step interferes with bond formation, lowering the levels of denaturation and aggregation achieved by pressurization, and hence did not improve the stabilization of the gel.

Ishikawa *et al.* (1991) recorded an increase in the elasticity value in sardine surimi gels after the second-stage heat treatment to levels higher than in gels produced by heat or by pressure alone. Nagashima *et al.* (1993) reported lower breaking deformation values in squid (*Loligo bleekeri*) mantle gels prepared by applying pressure followed by heat than in pressure-induced gels or heat-induced gels. Carballo *et al.* (1996) found that pressurization followed by heating did not enhance the heat-induced gelation ability of meat batters; however, only pressurization at 300 MPa produced an increase in the elasticity of the matrix. Ko *et al.* (1990) observed an increase in breaking force values after heating pressure-induced gels made from sardine mince. Finally, Gilleland *et al.* (1995) reported an increase in the work of penetration values in surimi gels made from Alaska pollack (*Theragra chalcogramma*) prepared by pressurization followed by heating but with a setting stage (25 °C, 3 h) before heating, whereas when the intermediate setting stage did not take place, the work of penetration was lower. This improvement was attributed to the action of transglutaminase during setting. Chung *et al.* (1994), working on the preparation of Alaska pollack and Pacific whiting gels subject to varying combinations of pressure and temperature in a single treatment, found that pressure-releasing enzymes and increasing proteolytic activity were the cause of the weakening of the gel.

Water-Holding Capacity (Figure 4). All the gels exhibited high WHC values, since the gel networks were stable enough to hold water. Conversely, Álvarez and Tejada (1997) found lower WHC values in set gel (set) than in kamaboko (set and cooked).

Lightness (Figure 5). Set gel (S) had the lowest values ($p \leq 0.05$) for the parameter L^* , probably because it was subjected to low-temperature gelation. In con-

trast, gel P-H had the highest values ($p \leq 0.05$), as a result of the two-stage treatment consisting of pressurization followed by heating, with most of the increase attributable to heating, since gel H had higher lightness values than gel P ($p \leq 0.05$). Although lightness has been reported to increase with pressure generally (Okamoto *et al.*, 1990), it is nonetheless dependent on the conditions of treatment. Pérez-Mateos and Montero (1997) studied the effect of high-pressure processing on washed sardine mince at different temperatures, obtaining the highest lightness values at 200 MPa at chilled temperatures (<10 °C) and at 400 MPa with moderate heating (about 40 °C).

SEM Microscopy. The micrograph of the batter (Figure 6B) showed that the microstructure of myofibrils obviously disappear and a dense matrix was observed. In the set gel (Figure 6S), the matrix seems to reveal a fine but dense reticular structure. The heat-induced gel (Figure 6H) seems to display evenly a more open, relatively uniform structure and arrangement of length-wise fibers with a tendency to group together and form small agglomeration. However, the pressure-induced gel (Figure 6P) appears to have a compact, less evenly porous matrix. In the pressure-assisted gel (Figure 6PH), protein strands were visible over the spongier matrix, more similar to the heat-induced gel in appearance than to the pressure-induced gel. Working with sardine gels, Montero and Gómez-Guillén (1996) also observed a higher degree of agglomeration in the heat-induced gel than in the set gel. The differing appearance of the pressure-induced and heat-induced gels is in line with the findings of Montero *et al.* (1997) for gels made from washed blue whiting mince.

CONCLUSIONS

Temperature, pressurizing, and combinations of the two induced various different characteristics in the gels, thus offering a greater variety of technological possibilities.

Although the pressure-induced samples had the appearance of set gels, their ultrastructure, chemical, and physical characteristics were different. Thus, pressure treatment in the given conditions was not an adequate substitute for the precooking setting stage, since the rheological parameters were considerably lower than in the heat-induced gel and hardness was the same.

On comparing a gel made by double heat treatment (H gel) and a pressure-assisted gel (P-H gel), pressurizing followed by heating was found to be the more effective treatment for achieving high elasticity; for deformability, breaking force, and work of penetration, however, the first gave better results. Hardness was similar in either case. What this amounts to is that pressurizing is no substitute for setting, as this affects the final gel, but rather it produces alternative properties. Further research would be desirable to determine the effects of pressurization on denaturation–aggregation and on enzyme action.

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