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Changes in textural and rheological properties of gels from tilapia muscle proteins induced by high pressure and setting

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Abstract

Tilapia meat pastes were prepared with a combination of hydrostatic pressure (200 MPa) and setting (50 °C) treatments, to investigate the changes in their rheological properties, gel-forming ability, whiteness and protein solubility of their gels. The control, a cooked gel (90 °C/30 min), was elastic and white, with low gel-forming ability. Gels formed by setting were elastic, rigid and mainly consisted of covalent bonds. Gels formed by pressurisation were soft and consisted of hydrogen bonds and hydrophobic interactions. Pressurisation prior to setting reinforced the gel structures, by formation of both covalent and non-covalent bonds. Setting prior to pressurisation did not alter the characteristics of the gel. Setting under pressurisation constructed a viscous gel with non-covalent bonds. Various fish gel products were formed in this study.

Keywords: Pressurisation; Setting; Tilapia; Rheological properties; Gel-forming ability

1. Introduction

Setting is a phenomenon of enhancing the gel-forming ability of surimi, by incubating at low temperature (below 50 °C) for a long period. During setting, partial actomyosin molecules form regular network structures, mainly with ε-(γ-glutamyl)lysine (GL bonds), disulfide bonds and hydrophobic interactions (Cheftel, 1992; Lee & Lanier, 1995; Tsukamasa et al., 1993). The gels obtained through setting are soft and elastic (Chan, Gill, Thompson, & Singer, 1995; Nishimoto, Hashimoto, Seki, & Arai, 1988). After setting, gels are subsequently cooked at 80–90 °C, becoming more rigid and elastic (Roussel & Cheftel, 1990).

Compared with heat-induced gels, pressure-induced gels of fish proteins were softer, more elastic and retained their native colour and flavour (Okamoto, Kawamura, & Hayashi, 1990). Bluefish gels formed by pressure were more translucent, softer, and more digestible than those formed by cooking at 90 °C for 20 min (Sareevoravitkul, Simpson, & Ramaswamy, 1996). At the initial pressurising stage, the hydrophobic interactions of native protein structures were disrupted by the decrease in the volume (Balny & Masson, 1993). During pressurisation, disulfide bonds are formed, due to the decrease in the distances between sulfhydryl groups (Cheftel, 1992). During the period of pressure release, proteins unfolded to induce the formation of hydrogen bonds and hydrophobic interactions. Therefore, pressure-induced fish proteins gelation was mainly due to the formation of disulfide bonds, hydrogen bonds and hydrophobic interactions.

Some authors (Ishikawa, Sakai, Yamaguchi, & Rachi, 1991; Ko, Tanaka, Nagashima, Taguchi, & Amano, 1990b) have reported the advantages of using hydrostatic pressurisation and heating combinations for gelling washed sardine meat pastes, specifically those with low gel-forming ability (Pérez-Mateos & Montero, 1997). The gel strength

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of Alaska pollock and sardine meat paste induced by heat with prior pressurisation (20–100 MPa) was enhanced compared with heat treatment alone (Ko et al., 1990b). When blue whiting surimi were heated under high pressure (200–420 MPa/0–75 °C/10–30 min), the resulting structures were weaker than gels made by heating or pressurised prior to heating (Pérez-Mateos, Lourenço, Montero, & Borderias, 1997). Some researchers reported that in cod surimi treated either by pressure (400 MPa/room temperature/20 min) followed by heat (50 °C/10 min) or heat followed by pressurisation, the hardness of the gels formed was lower than those treated with pressure only, although values were similar to those of heated and pressurised/heated samples (Angsupanich, Eddie, & Ledward, 1999). They demonstrated that myofibrillar proteins were stabilised by hydrogen bonds induced by pressure, and on the other hand, disulfide bonds and hydrophobic interactions induced by heat. Further more, Ko, Tanaka, Nagashima, Mizuno, and Taguchi (1990a) observed that Mg-ATPase activity in sardine actomyosin after pressure treatment is very different from the activity observed after thermal denaturation. Fernández-Martin, Fernández, Carballo, and Jiménez Colmenero (1997) reported that the final denaturing effects in pressure/heat processing of pork meat batters were pressure-temperature interdependent. Pressure, depending on the level, produced major protein denaturation when applied at non-denaturing temperature; conversely, pressure prevented subsequent protein thermal denaturation at denaturing temperatures.

Many of the effects governing pressure-temperature-assisted gelation are not fully understood (Jiménez Colmenero, 2002). The aim of this study was to investigate the changes of rheological properties, gel-forming ability, external colour and protein solubility of tilapia meat paste treated by pressure and setting combination treatments.

2. Materials and methods

2.1. Materials

Live round tilapia weighing approximately 600 g were purchased from a local retail market, killed and then kept on ice. The fish were filleted immediately and minced in a chopper with a hole size of 4 mm. The duration from purchase to mincing was within 30 min, therefore, all samples were at pre-rigor stage, in order to prevent muscle contraction during rigor mortis.

2.2. Meat paste preparation

The moisture content of the mince was adjusted to 80%. The mince weighing about 50 g was manually ground with a pestle and mortar for 1 min, followed by grinding with 2.5% NaCl for 4 min. The temperature was maintained below 4 °C during grinding. The meat paste thus obtained was packed and sealed in stainless steel tubes (diameter 30 mm × height 20 mm).

2.3. High-pressure and setting

The experimental design was shown in Table 1. Preliminary work showed that, the greatest value of gel strength of tilapia meat pastes treated by setting (30–70 °C) was at 50 °C, and that of pastes treated by pressure (50–300 MPa) was at 200 MPa (data not shown). Therefore, we adopted the setting temperature as 50 °C and performed pressurisation at 200 MPa. A high-pressure apparatus (CIP UNIT, Mitsubishi Heavy Industries Ltd., Japan) with an oil-pressure generator and a compressing vessel, in which the internal portion (diameter: 50 mm; height: 120 mm) was a flat-bottomed cylinder, was employed. The vessel temperature during pressure treatments was kept at 4 °C or 50 °C by a circulator. Setting was carried out in a water bath at 50 °C for 60 min, followed by cooling in ice for 15 min. The control sample used was the meat paste cooked at 90 °C for 20 min and subsequently cooled in ice for 15 min.

2.4. Rheological properties

A rheostress RS-100 (HAAKE, Germany) with a parallel plate sensor (diameter 30 mm) was used. The gap between the plates was set at 2.0 mm. Oscillatory measurements (25 °C) at a constant shear stress of 10 Pa over the 0.1–100 Hz frequency range were used to determine the storage modulus (G'), loss modulus (G'') and loss tangent ($\tan\delta = G''/G'$). The frequency was situated in the centre of the linear viscoelastic region, in order to compare different treatments. The linear regions for all of the samples were always in the frequency range of 0.4–2.0 Hz. Therefore, we adopted the frequency of 1 Hz (Dickinson & Pawlowsky, 1996). The maximum shear strain of 0.04 was set to lie within the linear viscoelastic regime. G' and G'' represented the elastic and viscous components, respectively.

2.5. Gel-forming ability

A rheometer (CR-200 D, Sun Scientific Co. Ltd., Japan) with a ball type plunger of 5 mm diameter and a table speed of 200 mm/min was used for gel strength measurement. The gel strength was expressed as the product of breaking force and breaking strain (g mm)(Ko et al., 1990b).

Table 1
Experimental design in this study

Symbol	Treatment
Control	0.1 MPa/90 °C/20 min
S	0.1 MPa/50 °C/60 min
P	200 MPa/4 °C/60 min
S–P	0.1 MPa, 50 °C, 60 min/200 MPa, 4 °C, 60 min
P–S	200 MPa, 4 °C, 60 min/0.1 MPa, 50 °C, 60 min
P/S	200 MPa/50 °C/60 min

2.6. Whiteness

The colour of pastes and gels was determined on a Color Meter ZE-2000 (Nippon Denshoku Co., Japan) in reflection mode with the standard plate ($Y = 94.01$, $X = 92.02$, $Z = 110.59$). Whiteness value was calculated as described by (Park, 1994):

$$\text{Whiteness} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

2.7. Protein solubility

In order to ascertain the kinds of ionic, hydrogen, and hydrophobic interactions, disulfide bonds and other covalent bonds (such as GL bonds), the gels were solubilised in four solutions (Pérez-Mateos et al., 1997): 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), 0.5 M β-mercaptoethanol + 0.6 M sodium chloride + 8 M urea (S4) at pH 7.0, and insoluble fraction (In). Two grams of chopped gel were homogenised at 5000 rpm with 10 ml of S1 for 2 min. The resulting homogenate was stirred at 4 °C for 1 h and then centrifuged at 20,000*g* for 20 min. The pellet obtained was homogenised with 10 ml of S2 by the same process, and then again with 10 ml of S3 (in this case, twice), and finally with 10 ml of S4. Protein concentration was determined in triplicate: in the supernatants, based on the method of Lowry, Rosebrough, Farr, and Randall (1951) using a commercial preparation (DC Protein Assay Reagent S No. 500-0116, Bio-Rad Laboratories, Hercules, CA), and in the insoluble proteins by the Kjeldahl method. To prevent interference in protein determination, supernatants were treated with trichloroacetic acid, and the pellet was recovered and dissolved in 0.5 ml of 1 N NaOH. Colorimetry was performed at 750 nm (Hitachi U-2000, Tokyo, Japan). All Experiments were conducted in triplicate.

2.8. Statistical analysis

The Statistical Analysis System Version 8.2 (SAS Institute Inc., Cary, N.C.,) was used to perform analysis of variance and Duncan's test. The differences between treatments were verified by their least significant difference.

3. Results and discussion

3.1. Gel-forming ability

Table 1 shows the specifications of the prepared tilapia gels. The breaking force, strain and gel strength values of the P–S gel with 658 g, 7.5 mm and 4935 g mm, respectively, were the greatest in this study ($p \leqslant 0.05$)(Fig. 1). All the parameters of gel-forming ability of S and S–P gels were not significantly different, however, the values were all greater than the control ($p \leqslant 0.05$). The breaking force of P gel was only 118 g, although the breaking strain was not significantly different from that of S and S–P gels. The gel strength of P gel was the lowest compared with the others and almost one-thirds of the control. The breaking strain of S/P gel was only 3.3 mm and about two-third of the control. Moreover, due to the small breaking force and strain of P/S gel, the gel strength was 716 g mm, similar to that of P gel ($p > 0.05$).

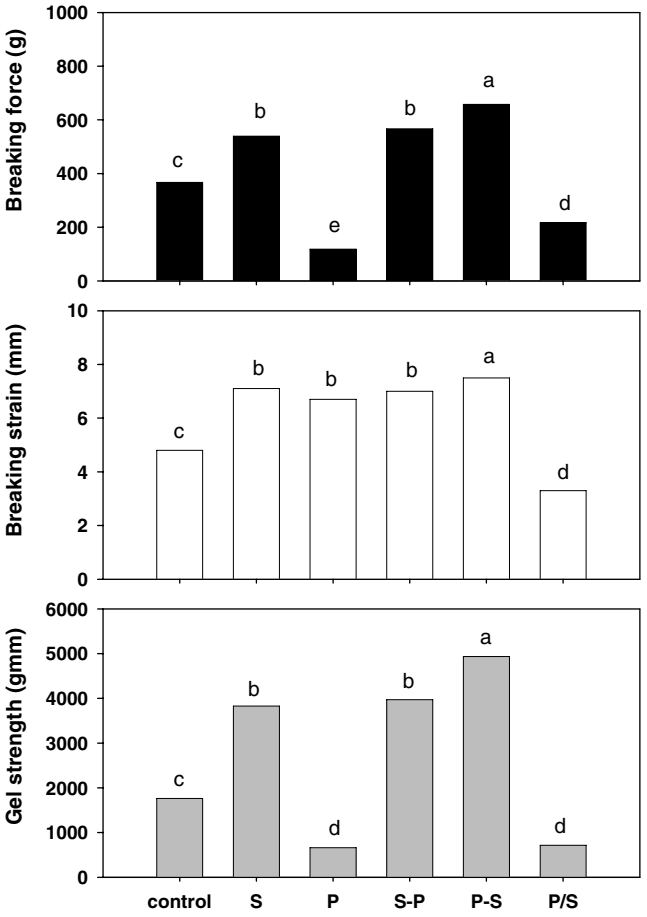

Fig. 1. Changes in gel-forming abilities of tilapia muscle proteins induced by pressure and setting. Different letters at the tops of the bars represent significant differences ($p \leqslant 0.05$) for the respective parameters between the gels.

The gel-forming ability of the gel induced by setting (S) was better than that by cooking (control), due to the formation of well network textures mainly contributed by the action of endogenous transglutaminase (TGase)(Gilleland, Lanier, & Hamann, 1997). The breaking force of S gel was greater than that of P gel by 7 times, but there were no significant differences between the two gels in breaking strain ($p > 0.05$). Moreover, breaking strain of S and P gels was 40–48% greater than that of control. Confirming previous reports (Ashie & Lanier, 1999; Okamoto et al., 1990), S gel and control were much stronger than P gel. Based on infra-red spectral analysis and rheological studies, Heremans, Van Camp, and Huyghebaert (1997) attributed this difference to more extensive protein unfolding in setting gels, resulting in a more stable network with increased

interactions. Carlez, Borderias, Dumay, and Cheftel (1995) found that breaking force was significantly greater in gels made at 90 °C under atmospheric pressure than in high-pressure-induced gels from threadfin bream surimi (*Nemipterus tambuloides*) and possessed a maximum deformation and gel strength at 300 MPa and 4 °C. However, Chung, Gebrehiwot, Farkas, and Morrissey (1994), working with Alaska pollack surimi (*Theragra chalcogramma*), noted that in gels made at 170 and 240 MPa and below 50 °C, shear stress and shear strain were greater than in gels heated at 90 °C. The different results between present and previous works were probably attributed to the different species of a materials, moisture contents, and concentrations of sodium chloride that were used. Pérez-Mateos et al. (1997) found a greater presence of ionic and hydrogen bonds in a gel induced by pressurisation at 200 MPa and 3 °C (lot L) than in a gel prepared by setting followed by cooking (lot T). Moreover, there were more disulfide and other covalent bonds in gels of lot T, which largely explain the high breaking force and water-holding capacity of the gel. It has been noted that when elastic gels became stronger due to increased formation of intermolecular covalent bonds, very little additional deformability was imparted (Lee & Lanier, 1995). Therefore, the reason for no significant differences in breaking strain between S and P gels may be that the S gel had well network textures with good water-holding capacity, due to strong covalent bonds. In this study, pressurisation at 200 MPa and 4 °C for 60 min prior to setting at 50 °C for 60 min (P–S) enhanced the gel-forming ability of tilapia muscle proteins, compared with setting without prior pressurisation. Similar results were reported for the gel strength of chum salmon meat pressurised at 500 MPa and 0 °C for 10 min, followed by heating (30–90 °C/30 min) was reinforced (Okazaki et al., 1997).

Many researchers have demonstrated that the increase of gel strength of pressure-set gel, compared with the setting gel, was attributed to the endogenous TGase, which remained active after pressurisation at 250–300 MPa (Ashie & Lanier, 1999; Gilleland et al., 1997). Moreover, pressure-induced accessibility of substrate for TGase catalysis and the increase of disulfide bond formation under pressure may enhance the gel-forming ability of S gel (Ashie & Lanier, 1999; Montero, Pérez-Mateos, & Solas, 1997). In S–P gel, we revealed that pressurisation with prior setting had no effect on the gel. Angsupanich et al. (1999) reported that with pressurisation (400 MPa/room temperature/20 min) after setting (50 °C/10 min), cod muscle hardness was similar to that of samples. Some researchers demonstrated that the structures of protein aggregates induced by setting were strongly stabilised by covalent bonds. Therefore, setting prior to pressurisation limited the pressure effect on gel-forming ability of meat batters (Jiménez Colmenero, 2002; Macfarlane, Mckenzie, & Turner, 1986). P/S gel performed as a weaker and less elastic gel, compared with the control. Two possible explanations have been suggested for the limitation found in the gelation process in pressure-under-setting treatments on muscle proteins (Jiménez Colmenero, 2002). The first is that the pressurisation process partially preserves the protein from thermal denaturation (Balny & Masson, 1993; Ko et al., 1990b). The second is that proteolytic activity increases, causing some myofibrillar protein breakdown and the formation of various molecular fragments (Jiménez Colmenero, Cofrades, Carballo, Fernández, & Fernández-Martin, 1998; Macfarlane et al., 1986). Ashie and Lanier (1999) concluded that effects of pressurisation at setting temperatures of 25 and 40 °C on the activities of TGase in Alaska pollack (*Theragra chalcogramma*) surimi gels were not significantly different. Therefore, we eliminated the effects of TGase action on the gel-forming abilities of P and P/S gels. Similar results were reported in chum salmon, Alaska pollack surimi (500 MPa/60 °C/10 min) (Okazaki et al., 1997), Pacific whiting surimi (0.1–240 MPa/50 °C/60 min) (Chung et al., 1994), and blue whiting (200–400 MPa/0–75 °C/10–30 min) (Pérez-Mateos et al., 1997).

3.2. Rheological properties

The rheological properties of gels prepared by all the treatments were determined (Fig. 2). G′ and tan δ values

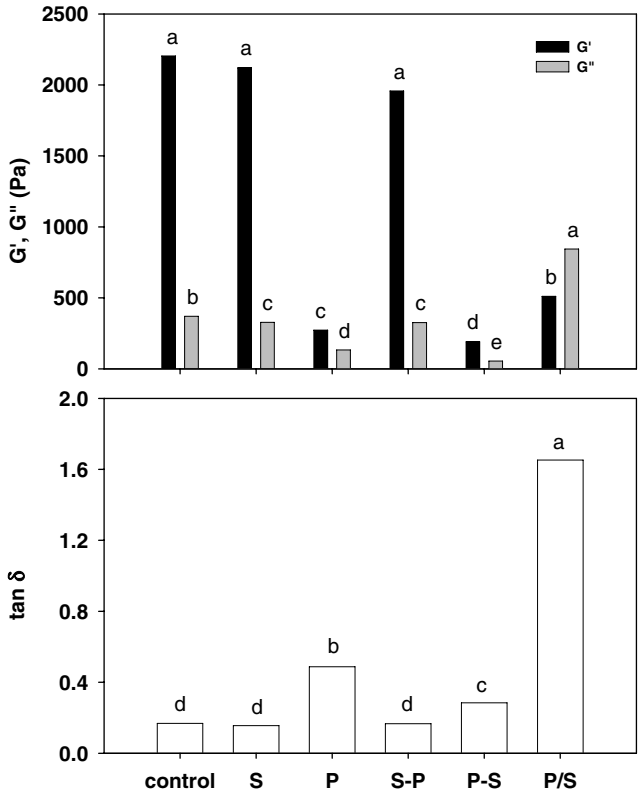

Fig. 2. Changes in rheological properties of tilapia muscle proteins induced by pressure and setting. Rheological properties were measured by frequency sweep from 0.1 to 100 Hz at 25 °C. Different letters at the tops of the bars represent significant differences ($p \leqslant 0.05$) for the respective parameters between the gels.

for the control, S and S–P gels were not significantly different, while G″ values of S and S–P gels were slightly lower than those of the control ($p \leqslant 0.05$). G′ and G″ values of the gels formed by pressurisation or pre-pressurisation, except for P/S gels, were much lower than those of the gels formed by heat or pre-heat treatments, and the opposite results occurred with tan δ. Although the G′ value of S/P gels was about one-fourth of the S and S–P gels, it was about twice those of P and P–S gels. The G″ value of P/S gel was more than twice those of the control, S and S–P gels and four to five times the G″ value of P and P–S gels. Due to the high value of G″, the tan δ value of S/P gel was over 1.6, and the gel contained more viscous components than elastic components. G′ values of all the pressure-induced gels except for S–P gel were all below 500 Pa, whereas, those of setting-induced gels and the control were around 2000 Pa. The textures of gels induced mainly by pressure were irregular and much weaker compared with those induced by setting (Pérez-Mateos et al., 1997).

The control, S and S–P gels contained much more elastic components than viscous components, due to the ratios of G″ and G′, and the result indicated that the gels induced by heating or heating prior to pressurising treatments were rigid and elastic (Autio, Kiesvaara, & Polvinen, 1989). Gels made under pressure at low and constant temperature were generally softer than heat-induced gels, due to different kinds of chemical interactions (Gilleland et al., 1997; Jiménez Colmenero, 2002). The P–S gel showed the highest gel strength (Fig. 1) but the lowest G′ and G″ values in this study. A possible interpretation of this phenomenon is as follows (Ikeuchi, Tanji, Kim, & Suzuki, 1992): increasing the surface hydrophobicity, due to pressure-induced structural changes of myosin, can compensate for the decrease in the heat-induced gel strength of myosin denatured by pressure treatment, and that would cause the decreases in G′ and G″ values (Ko, Jao, & Hsu, 2003). Tan δ value of P–S gel was 58% of that of P gel, and this result showed that pressurisation followed by setting caused the gel to be more elastic. We demonstrated that more covalent bonds could be induced in pressurized gels by setting. Carlez et al. (1995) also revealed that pressure processing (300 MPa/5 °C/15 min) followed by cooking (90 °C/30 min) resulted in higher yield force, gel strength and rigidity when compared to pressure processing alone. A tan δ value greater than one showed that the P/S gel was a soft and viscous gel, resulting in low values for the breaking force and strain of P/S gel (Fig. 1).

3.3. Whiteness

The whiteness of tilapia meat paste and the control were 38 and 63 which were the lowest and highest of all the treatments (Fig. 3). The whiteness of S, S–P and P–S gels were not significantly different ($p > 0.05$) but were higher than those of P and P/S gels. Although the whiteness values of P and P/S gels were not significantly different, P/S gel was quite different from P gel in *a* value (data not shown).

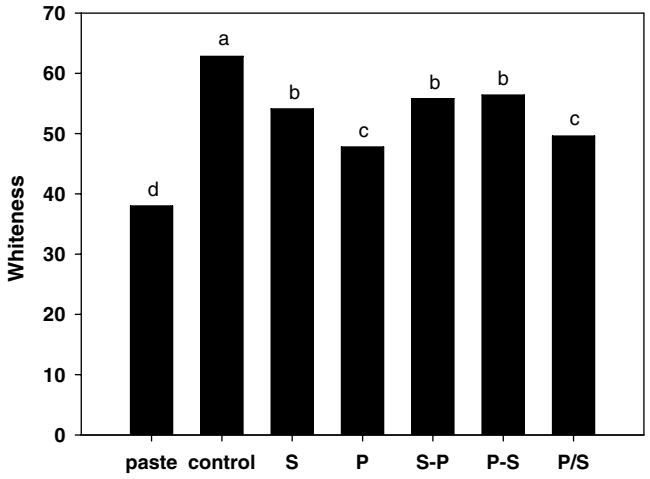

Fig. 3. Change in whiteness of tilapia muscle proteins induced by pressure and setting. Different letters at the tops of the bars represent significant differences ($p \leqslant 0.05$) between the gels.

The whiteness, which is related to the degree of protein denaturation, was greatest in the gel formed by cooking (control). According to preliminary tests (data not shown), the whiteness of tilapia meat pastes incubated at 40 °C or below for 60 min slightly but insignificantly increased compared with untreated meat paste. Moreover, those subjected to pressurisation below 150 MPa also insignificantly increased. Whiteness changes of tilapia meat pastes due to heat or pressure treatments were attributed to myoglobin denaturation or water-holding capacity of gels (Shie & Park, 1999). P–S gel had a higher whiteness value than P gel ($p \leqslant 0.05$), as a result of the two-stage treatment consisting of pressurisation followed by heating, with the increase attributable to heating, since S gel had higher whiteness than P gel ($p \leqslant 0.05$) (Montero et al., 1997). However, the lack of difference in whiteness of S and S–P gels indicated that pressure did not enhance the protein denaturation of heat-induced gels. Although P/S gel performed differently in gel-forming ability compared to the others, the whiteness of P/S gel was close to those of S, S–P and P–S gels and apparently greater than that of the meat paste.

Several contrary results in the whiteness of P/S gels have been reported (Pérez-Mateos et al., 1997; Pérez-Mateos & Montero, 1997). The authors demonstrated that the lightness of the gels from blue whiting (*Micromesistius poutassou*) muscle proteins prepared at 375 MPa/38 °C/20 min was higher than those prepared at 200 MPa/3 °C/10 min or by setting at 37 °C for 30 min followed by cooking at 90 °C for 50 min. However, they also found that the lightness of washed sardine (*Sardina pilchardus*) mince prepared at 400 MPa and 40 °C was generally similar to that prepared at 200 MPa and low temperature (<10 °C). Pressures high enough to induce fish protein denaturation would prevent subsequent protein denaturation at setting temperatures (Fernández-Martin et al., 1997).

3.4. Protein solubility

Twelve percent of tilapia meat paste proteins were composed of ionic bonds, 17% hydrogen bonds, 10% hydrophobic interactions, 4% disulfide bonds and 57% other covalent bonds (such as GL bonds) or insoluble proteins, such as sarcoplasmic proteins (Table 2). After cooking at 90 °C for 20 min, the ionic and hydrogen bonds of the gel were largely disrupted ($p \leqslant 0.05$) accompanied by extensive formation of disulfide bonds. The greater ($p \leqslant 0.05$) presence of covalent bonds, including disulfide bonds in the gels induced by setting or pressurisation prior to setting was observed. P and P/S gels both contained over 30% hydrogen bonds and hydrophobic interactions, but much fewer covalent bonds, compared with the other gels. The P–S gel included 12% hydrogen bonds, 12% hydrophobic interactions, and 12% disulfide bonds, and 60% covalent bonds.

During grinding with salts, myofibrillar proteins would solubilise and hydrate with water in the system, and several chemical bonds would form. However, sarcoplasmic proteins would not be soluble in the same system. Tilapia meat pastes showed the high percentage solubility in the S1 and S2 extracts, indicating a predominance of ionic and hydrogen bonds, and the lowest solubility value in the pastes was for the S4 extract due to the small proportion of disulfide bonds. Similar results were reported by Montero et al. (1997). The high breaking force of the gels via setting at atmosphere pressure (including S, S–P and P–S gels) was mainly due to disulfide and other covalent bonds. The suggestion that the gels with great hardness and low elasticity contained disulfide and other covalent bonds was reported by Pérez-Mateos et al. (1997). Disulfide bond formation at the setting temperature has also been reported by some workers (Niwa, Matusbara, & Hamada, 1982). S and S–P gels had higher proportions of the insoluble fraction (comprising covalent bonds) than P and P–S gels, because during setting the proteins underwent rapid denaturation and aggregation that lowered their solubility (Montero et al., 1997). Also, the action of TGase at setting temperature has been postulated as the mechanism of the cross-linking reactions (Niwa, 1992). Surprisingly, the proportions of the insoluble fraction of S and S–P gels were higher than those of the control. We suggested that setting at 50 °C could induce tilapia meat pastes to form a more elastic gel containing more covalent bonds than the control, and this suggestion was proved by Montero et al. (1997). The result may also explain why the gel-forming abilities of S and S–P gels were greater than those of the control (Fig. 1). The ionic bonds, which play important part in gel textures, were largely retained in P gel (Carlez et al., 1995). The greater presence of hydrogen bonds in P gel may be due to their favourable formation by low pressure treatment (200 MPa) or low temperature (3 °C) (Pérez-Mateos et al., 1997). In P/S gel, hydrogen bonds were more difficult to form than in P gel, due to the higher temperature (Angsupanich et al., 1999). Another surprising result showed that the proportions of the insoluble fraction of P and P/S gels were lower than that of paste. Cheftel and Culioli (1997) reported that a large increase in the solubilisation of myofibrillar proteins was observed when the applied pressures were between 150 and 300 MPa. Suzuki, Suzuki, Ikeuchi, and Saito (1991) found that proteins from thin filaments, such as actin, tropomyosin, troponin C, as well as M-protein were solubilised at 100 MPa, whereas solubilisation of myosin heavy chains required higher pressure (300 MPa). Setting with prior pressurisation (P–S) caused the breakdown of hydrogen bonds and formation of covalent bonds (including disulfide and non-disulfide bonds) (Niwa et al., 1982). It would appear virtually certain that for fish proteins, the setting effect uniquely involves non-disulfide cross-linking of myosin (Gill & Conway, 1989; Sano, Noguchi, Matsumoto, & Tsuchiya, 1990). According to the insoluble fractions, endogenous transglutaminase was inhibited under pressure and re-activated with setting after releasing pressure. Carlez et al. (1995) and Pérez-Mateos et al. (1997) found that high pressure applied at moderate temperatures (30–40 °C) induced more hydrophobic interactions, than at chilled temperature (<10 °C). The breaking strain of P/S gel, which is composed of hydrogen bonds, hydrophobic interactions but small amounts of disulfide bonds, was the lowest in all treatments. The results were different from those of Pérez-Mateos et al. (1997) and Montero et al. (1997), probably due to the differences in fish species and measuring methods. We demonstrated that the breaking strain of P gel was greater than that of P/S gel, due to the greater formation of ionic and disulfide bonds.

Table 2
Protein fractions (%) of gels in different solutions[A]

	S1	S2	S3	S4	In
Paste	12[aB]	17[a]	10[c]	4[c]	57[b]
Control	9[b]	8[c]	10[c]	14[a]	59[b]
S	6[c]	8[c]	10[c]	12[a]	64[a]
P	9[b]	18[a]	14[b]	7[b]	52[c]
S–P	6[c]	9[c]	10[c]	13[a]	62[a]
P–S	6[c]	12[bc]	12[bc]	12[a]	58[b]
P/S	6[c]	14[b]	21[a]	5[c]	54[c]

[A] 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; S4, 0.5 M β-mercaptoethanol+0.6 M sodium chloride + 8 M urea; In, insoluble fraction.

[B] Different letters in each column mean significant differences ($p \leqslant 0.05$) for each solution.

4. Conclusion

The differences in textural, chemical and physical characteristics of protein gels induced by heat and pressure were comprehensively studied. Combinations of setting and pressure treatments produced various products. Pressurisation with prior setting could not change the gel characteristics, due to the limitation of the protein conformation. However, setting with prior pressurisation reinforced the gel-forming ability and rheological properties. A viscous gel was formed

by pressurisation under setting because the pressurisation process preserved the protein from thermal denaturation. Different effects of combinations of heat and pressure treatments on protein gelation have been reported by many researchers due to the distinctive temperature, pressure levels and materials (species) studied. We believe that the application of pressure and heat offers a great variety of technological possibilities.

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