Thermal Aggregation of Sardine Muscle Proteins during Processing

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This paper seeks to relate rheological changes occurring in sardine mince homogenized with NaCl (1.5% and 2.5%) during gelation to the formation of different types of chemical bonding and to identify the involvement of myofibrillar proteins in these bonds. Setting and modori occur in sardine muscle to a marked degree. In setting there is heavy involvement of the myosin heavy chain (MHC), which is polymerized chiefly by means of stronger bonds than hydrophobic interactions. In modori, on the other hand, hydrophobic interactions prevail. In the gel made at 90 °C, MHC was the main protein implicated, through disulfide bonds or other covalent bonds. In gelation of sardine muscle, in general there is little involvement of other myofibrillar proteins such as actin, tropomyosin, and troponins and other low MW proteins.

Keywords: *Gelation; suwari; Kamaboko; modori; setting; myofibrillar proteins; sardine mince*

INTRODUCTION

A gel may be defined as a state of matter intermediate between solid and liquid, consisting of a number of "chains" intertwining to make up a three-dimensional network immersed in a liquid medium (Tanaka, 1981). By homogenizing minced muscle with NaCl, myofibrillar protein can be dissolved, thus facilitating polymerization by thermal gelation. Myosin plays an essential role in gelation of muscle, the myosin heavy chain (MHC) being chiefly responsible for the differences from one species to another (Niwa et al., 1980).

The myofibrillar proteins of some fish species remain soluble at very low ionic strength and as a result can gelate with a very low salt concentration, or even without any salt at all (Wu et al., 1991a,b). The process of thermal gelation of proteins comprises a stage of heat denaturation followed by progressive aggregation of the denatured proteins, for which the molecules involved are required to interact at specific points by establishing certain kinds of chemical bonds. When a batter of fish mince homogenized with salt is kept under 40 °C, it loses its characteristic viscosity and becomes an elastic, translucent gel. This phenomenon is known as suwari or setting. When there is a setting stage prior to final cooking, there is a slow, gradual unfolding of proteins, which favors protein−protein interaction and results in a stronger, more elastic gel (Hermansson, 1979; Foegeding et al., 1986). Subsequent research has helped to demonstrate the occurrence of noncovalent protein−protein interaction—chiefly hydrophobic—during setting, as a consequence of unfolding of the MHC α-helix (Sano et al., 1990a,b; Gill and Conway, 1989). More recent studies have pointed to a process of cross-linking during setting, which is catalyzed by enzymes called transglutaminases (TGases) through the formation of ϵ-(γ-glutamyl)lysine (Kim et al., 1993; Kamath et al., 1992; Tsukamasa and Shimizu, 1990).

Modori is partial deterioration of texture during heating at temperatures between 50 and 60 °C. The mechanism whereby this comes about is generally poorly understood. Proteolytic activity of alkaline proteases in the muscle has been suggested as the most probable cause of gel weakening in this temperature range (Lanier et al., 1981; Makinodan et al., 1987). Another possible explanation is based upon thermal coagulation of myofibrillar proteins during heating: It may be that once a homogeneous protein network has been established (setting), a rise in temperature to 50−60 °C causes an excessive increase in hydrophobic interaction, which would cause intense "shrinkage" of the network, resulting in release of water and dispersion of the network in a far more heterogeneous way (Niwa, 1992). At over 60−70 °C, the bulk of protein−protein interaction takes place through the head portions of the myosin molecules, by means of either disulfide bonds or hydrophobic interactions (Sano et al., 1990a,b; Taguchi et al., 1987).

The object of this research was to relate rheological changes occurring in sardine mince, homogenized with two different levels of NaCl (1.5% and 2.5%) and at various gelling temperatures, to the formation of different types of chemical bonding determined by protein solubility and to identify the involvement of myofibrillar proteins in these bonds.

MATERIALS AND METHODS

The fish used in these experiments were sardines of the species *Sardina pilchardus* (Walbaum), caught off the Cantabric Sea in June. Preparation of frozen minced sardine meat: Sardines were headed, gutted, and washed. Skin and bones were removed with a Baader model 694 deboning machine. Muscle was minced and held for 10 min at 0−3 °C in an aqueous solution of 0.5% bicarbonate, proportion 3:1 (solution:minced muscle, pH 7), stirring constantly. Solution was left for 10 min. Excess water was then removed using a screw press. As cryoprotectants, 4% sorbitol and 0.2% tripolyphosphate were added. The mince was immediately vacuum-packed in Cryovac BB-1 bags and frozen in a plate freezer (−40 °C setting) until the thermal center reached −20 °C. The various lots were stored at −80 °C in a Revco vertical freezer cabinet in order to minimize alteration during frozen storage for the duration of the experiment.

Proximate Analyses. To characterize the washed sardine mince, moisture, ashes, crude fat, and crude protein were determined according to AOAC (1975). Results were averages of three determinations and are expressed as percent of minced muscle (moisture, 80.42 ± 0.01%, fat, 1.59 ± 0.08%, protein, 14.04 ± 0.44%, ash, 0.71 ± 0.02%).

Homogenization of Muscle with NaCl. The washed sardine mince was semithawed and placed in a refrigerated vacuum homogenizer (Stephan model UM5, Stephan u. Söhne GmbH & Co., Germany). The muscle was ground for 1 min

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at high speed (rotor angular velocity 3000 rpm). Sodium chloride (1.5% or 2.5%) (Panreac, Barcelona, Spain) was then added with crushed ice to give the required final gel moisture (76%), and the mixture was homogenized for 5 min at low speed (1500 rpm) in vacuum conditions.

Heat Treatment. The resulting batters was stuffed into stainless steel cylinders (inner diameter 3 cm, height 3 cm) with screw-on lids and rubber gaskets to provide a hermetic seal. At no time during this part of the process did sample temperature exceed 10 °C. Samples were heated at 35, 50, 60, and 90 °C by immersion in a water bath for 50 min. Immediately after heating, the cylinders were placed in recipients containing ice water for rapid cooling of the gel. They were then stored in a cold room at 4 °C for 24 h before analysis.

Rheology. *Puncture Test.* Cylindrical samples (3 cm diameter × 3 cm height) were removed from the molds and tempered to about 20 °C. Gels were pierced to breaking point using a texturometer (Instron model 4501, Instron Engineering Corp., Canton, MA) with a 5 mm diameter, round-ended metal probe. Cross-head speed was 10 mm/min and a 100 N load cell was used. Gel strength was determined by multiplying maximum breaking force (N), by breaking deformation (mm). All determinations were carried out at least in quadruplicate.

Folding Test. The test piece was a 3 mm slice cut from the cylinders. The evaluation was performed in accordance with a 5-point grade system as follows: grade 5, no crack when folded into quadrants; grade 4, no crack when folded in half; grade 3, crack develops gradually when folded in half; grade 2, crack develops immediately when folded in half; grade 1, crumbles when pressed by finger.

Scanning Electron Microscopy (SEM). Small (2−3 mm) cubes of gel were taken from the center of the cylindrical gel specimen for microscopic examination. Samples were fixed with 2% glutaraldehyde in a phosphate buffer (pH 7.3), dehydrated in increasing series of acetone (from 40% to 100%), and critical-point-dried (Balzer model CPD030) using CO_2 as the transition fluid. The prepared samples were mounted on copper specimen holders, sputter-coated with gold (Balzer model SCD004), and examined on a Jeol JSM 6400 scanning electron microscope up to 20 kV.

Determination of Gel Solubility. Studies commenced immediately when the newly made gels were sufficiently cool. According to Matsumoto (1980) and Careche et al. (1995), gels were treated with chemicals selected for their capacity to cleave certain kinds of bonds: 0.05 M NaCl (S1), 0.6 M NaCl (S2), 0.6 M NaCl + 1.5 M urea (S3), 0.6 M NaCl + 8 M urea (S4), and 0.6 M NaCl + 8 M urea + 0.5 M 2-β-mercaptoethanol (S5). Proteins were partially solubilized with these solutions in order to determine the existence of nonspecific associations (percent protein solubilized in S1), ionic bonds (difference between percent protein solubilized in S2 and percent protein solubilized in S1), hydrogen bonds (difference between percent protein solubilized in S3 and percent protein solubilized in S2), hydrophobic interactions (difference between percent protein solubilized in S4 and percent protein solubilized in S3), and disulfide bonds (difference between percent protein solubilized in S5 and percent protein solubilized in S4). Two grams of chopped gel was homogenized with 10 mL of each solution in an Omni-Mixer (model 17106) homogenizer (Omni International, Waterbury) for 2 min at setting 5. The resulting homogenates were stirred at room temperature (18−20 °C) for 1 h and then centrifuged for 15 min at 20000*g* in a Cryofuge 20-3 centrifuge (Heraeus Christ GmbH, Germany). Protein concentration in supernatants was determined according to Lowry et al. (1951), using a commercial preparation, DC Protein Assay Reagent S no. 500-0116 (Bio-Rad Laboratories, CA). Where neccessary the solutions were dialyzed previously. Results are averages of two determinations and are expressed as percent protein solubilized with respect to total protein in the gel, which was previuosly determined by the Kjehldahl method.

Electrophoresis (SDS−PAGE). Supernatants obtained with S1 and S4 solutions were treated according to the method of Hames (1985) with a solution composed of 5% 2-β-mercaptoethanol, 2.5% SDS, 10 mM Tris-HCl, 1 mM EDTA, and 0.002% bromophenol blue. They were adjusted to a final average concentration of 2 mg/mL and then heated at 100 °C for 5 min. Electrophoresis was carried out on a Phast-System horizontal apparatus (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) using 12.5% polyacrylamide gels (PhastGel, Pharmacia LKB Biotechnology). 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 LKB Biotechnology). As reference for molecular weights, a standard high molecular weight reference kit (Pharmacia LKB Biotechnology) was used, ferritin half-unit 220 kDa, albumin 67 kDa, catalase subunit 60 kDa, lactate dehydrogenase subunit 36 kDa, and ferritin subunit 18.5 kDa.

Statistical Analysis of Data. Two-way analysis of variance was carried out for the different samples. The computer program used was Statgraphics (STSC Inc.). The difference of means between pairs was resolved by means of confidence intervals using a LSD range test. Level of significance was set for $p \leq 0.05$.

Table 1. Rheological Parameters of Gels[a]

treatment	gel strength (N × mm)	breaking force (N)	breaking deformation (mm)
2.5% NaCl			
35 °C	19.02_a	1.58_a	12.01_a
90 °C	4.08_b	0.65_b	6.24_b
35/90 °C	60.25_c	4.85_c	12.43_a
1.5% NaCl			
35 °C	13.12_d	1.24_d	10.58_c
90 °C			
35/90 °C	55.94_c	4.83_c	11.51_d

[a] Different letters in the same column indicate significant differences ($p \leq 0.05$).

RESULTS AND DISCUSSION

Rheology. Table 1 shows gel strength, breaking force, and breaking deformation of sardine mince gels made with 2.5% and 1.5% NaCl, at 35, 90, and 35/90 °C (preset gels). The gel made at 35 °C (suwari gel) scored maximum in the folding test (5) and exhibited greater gel strength than those made at any other temperature, with either salt concentration. The low-salt suwari gel exhibited significantly lower gel strength, breaking force, and breaking deformation ($p \leq 0.05$) than the high-salt gel. This temperature (35 °C) falls within the range 32−43 °C established for the high-temperature setting process traditionally used by Japanese kamaboko producers to improve the textural properties of the final product (Matsumoto and Noguchi, 1992). Moreover, gels made at 35 °C have certain characteristics which set them apart from gels cooked at high temperatures (80−90 °C), being very elastic and translucent (Montejano et al., 1984). It was not possible to perform the puncture test on gels made at 50 and 60 °C, with either salt concentration, as the samples tended to fall apart, scoring 1 in the folding test. This deterioration of texture is attributed to the modori phenomenon (Matsumoto and Noguchi, 1992). Gels made at 90 °C with 2.5% NaCl scored 2 in the folding test, with gel strength, breaking force, and breaking deformation very inferior to those of the suwari gels. With 1.5% NaCl, it was not even possible to perform the puncture test as samples tended to fall apart, and the folding test score was 1. In either case, direct heating at high temperature (90 °C) caused extensive protein syneresis (Lee and Chung, 1990), and hence the matrix that formed was more unstable (Foegeding et al., 1986).

Figure 1 shows scanning electron microscope pictures of gels made at 35 and 90 °C, with 2.5% and 1.5% NaCl. At 35 °C the gel exhibited an irregular mesh structure

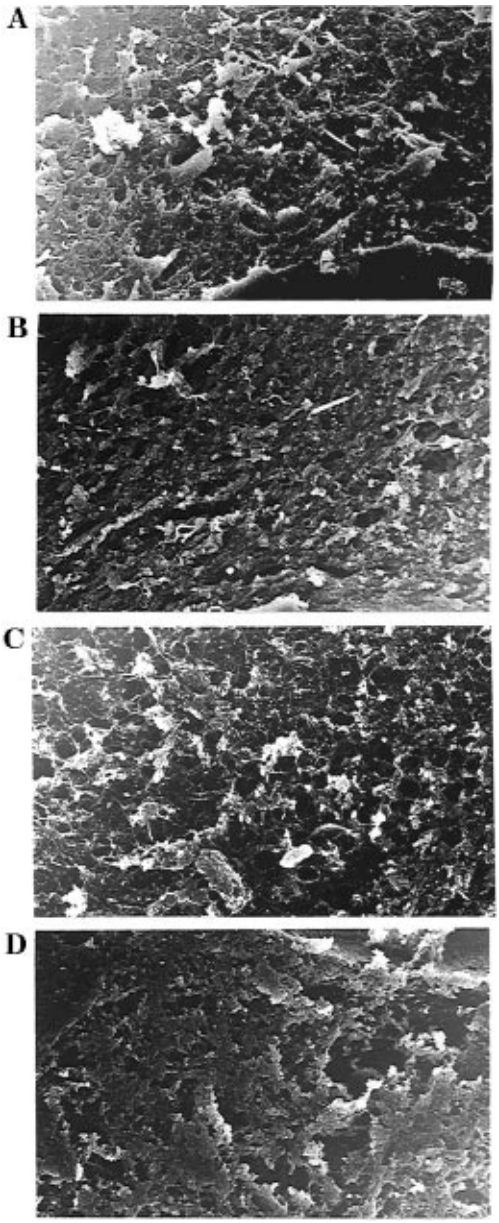

Figure 1. Scanning electron microscope image, at 500× magnification (reproduced at 75% of original size) of gels made at 35 and 90 °C, with 2.5% and 1.5% NaCl: (A) 35 °C, 2.5% NaCl; (B) 35 °C, 1.5% NaCl; (C) 90 °C, 2.5% NaCl; (D) 90 °C, 1.5% NaCl.

with numerous pores of heterogeneous size. The matrix was less porous with 1.5% than with 2.5% NaCl. Although at both salt concentrations suwari gels were obtained scoring maximum points in the folding test, microscopic examination revealed no formation of a true network. Couso (1994) reported similar results in sardine surimi gels made at 35 and 40 °C. The high-salt gel cooked at 90 °C exhibited a more open matrix than the gel made at 35 °C because of muscle protein aggregation (Hermansson, 1986). The result was a not-very-homogeneous reticular structure combined with more compact zones. In the low-salt gel, the matrix exhibited extensive highly-agglomerated zones with profound heterogeneous cavities, giving a highly disorganized appearance.

Table 1 compares the rheological behavior of gels cooked at 90 °C with and without prior setting at 35 °C, in terms of gel strength, breaking force, and breaking deformation. Gels set at 35 °C before cooking at 90 °C, with either salt concentration, scored 5 in the folding

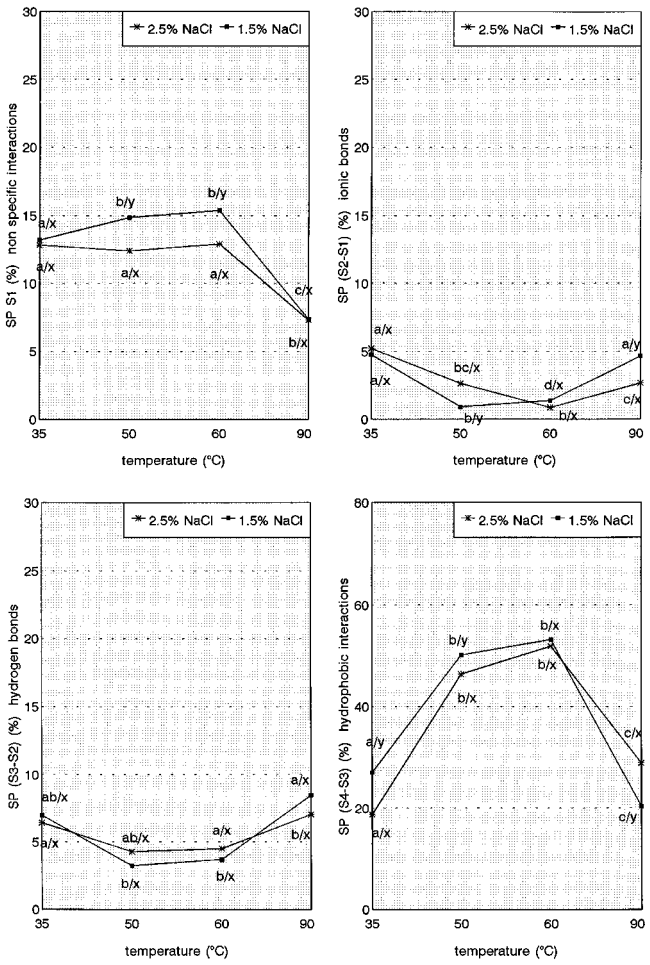

Figure 2. Soluble protein with respect to total protein of gels made at 35, 50, 60, and 90 °C, solubilized with 0.05 M NaCl (S1), 0.6 M NaCl (S2), 1.5 M urea + 0.6 M NaCl (S3), and 8 M urea + 0.6 M NaCl (S4).

test and exhibited considerably greater gel strength, breaking force, and breaking deformation than gels cooked without prior setting and suwari gels made at 35 °C. Where gels are subjected to setting (35 °C) prior to final cooking (90 °C), heating is slower and more gradual, giving the proteins more time to unfold and interact and hence producing a stronger matrix. The resulting network is more orderly, and the gel tends to be more elastic (Hermansson, 1979). No significant differences ($p \leq 0.05$) were found between high-salt and low-salt gels in breaking force, breaking deformation, and gel strength.

Protein Solubility: Disruption of Bonds. Figure 2 shows the solubility of sardine gels in a number of solutions selected for their capacity to disrupt certain kind of bonds. The soluble protein in 0.05 M NaCl (as an index of nonspecific associations) remained practically the same in gels made at 35, 50, and 60 °C, dropping significantly in those cooked at 90 °C. During heating, proteins undergo denaturation and aggregation which reduce their solubility (Li-Chan et al., 1985). Again, as the gel forms, interaction of certain proteins limits their extractability (Camou et al., 1989). The soluble fraction in such conditions (0.05 M NaCl) therefore corresponds to proteins of relatively high thermal stability which have no direct part in gel formation. With 1.5% NaCl, nonspecific associations in gels made at 50 and 60 °C were more frequent ($p \leq 0.05$) than in the sample with 2.5% NaCl.

The solubility in 0.6 M NaCl (ionic bonds) of the gels made at 35 °C was similar at both salt concentrations and less in gels made at higher temperatures (50 and 60 °C). In gels cooked at 90 °C, however, it was slightly higher than in gels made at 60 °C, the difference being somewhat more pronounced in the gel containing 1.5% NaCl.

Gels treated with S3 (0.6 M NaCl + 1.5 M urea) exhibited very low solubility after deducting the percent of soluble protein in S2 (0.6 M NaCl). In terms of hydrogen bond existence, there was no significant difference between gels set at 35, 50, and 60 °C, but it was slightly higher in gels cooked at 90 °C, irrespective of salt concentration. This may be because gelation gives rise to the formation of a three-dimensional network which stabilizes water in the structure physically and chemically, thus enabling it to continue interacting with the protein in the matrix by attractive forces such as hydrogen bonds or electrostatic forces (Acton et al., 1983).

In general, gels made at the different temperatures exhibited greater solubility in S4 (0.6 M NaCl + 8 M urea) after deducting the percentage of soluble protein in S3 (0.6 M NaCl + 1.5 M urea) than with the other solutions, which suggested predominance of hydrophobic interactions over other bonds among those studied. Numerous authors have drawn attention to the role of hydrophobic interactions in setting of gels made with surimi from a variety of species (Samejima et al., 1981; Niwa, 1983; Gill and Conway, 1989). A number of studies have reported that hydrophobic interactions between adjacent regions of the myosin tail, which occur during setting between 30 and 40 °C, constitute the basis for formation of an initial structure at these temperatures (Sano et al., 1990a; Gill and Conway, 1989; Niwa et al., 1986; Numakura et al., 1987). In gels made between 35 and 50 °C, there was a significant increase in the occurrence of hydrophobic interactions. These remained more or less constant in gels made at 60 °C. This temperature range (50−60 °C) coincides with the onset of modori, which is appreciable in rheological terms as textural degradation. This could in part explain the high percent of solubility in S4 at these temperatures. Niwa (1992) suggests that one of the possible mechanisms producing modori could be an excess of hydrophobic interactions; once the network is established by setting, this could cause the structure to "shrink", releasing water and giving rise to a more heterogeneously dispersed network. Finally, the occurrence of these hydrophobic interactions decreased considerably in gels made at 90 °C. This drop was more pronounced in the low-salt gel, which also exhibited appreciable disulfide bond existence (4.88% solubility) in the solubilization conditions specified in Materials and Methods. According to Sano et al. (1990a,b), the participation of hydrophobic amino acid residues in these interactions is favored by greater ionic strength, which in this case corresponds to higher salt concentration gels. Unlike hydrogen bonds, hydrophobic interactions are strengthened by increasing cooking temperature to at most 58 °C; beyond this temperature these interactions may be weakened as hydrophobic hydration is hindered by possible destabilization of the hydrogen bonds linking water molecules (Miyajima, 1974). This, together with the formation of disulfide bonds or other kinds of more stable covalent bond, may be the reason for the small number of hydrophobic interactions detected on gelation at 90 °C with either salt concentration. Numerous authors have stressed the importance of the formation of disulfide bonds through oxidation of sulfhydryl groups during heat gelation of proteins (Shimada and Matsushita, 1981; Itoh et al., 1979). Some authors have even found disulfide bonds to predominate at setting temperatures (Niwa et al., 1982). In this work the lack of a clear detection of disulfide bond formation does not mean necesarily its absence; it could be that the experimental conditions employed for measuring S−S bonding were not sensitive enough, registering appreciable data only when these bonds are present in a large quantity.

Table 2. Protein Solubility (Percent), Expressing Different Types of Bonds, of Gels Made with or without Prior Setting, at 2.5% and 1.5% NaCl[a]

	90 °C		35/90 °C	
type of bond	2.5% NaCl	1.5% NaCl	2.5% NaCl	1.5% NaCl
nonspecific interactions	7.28_a	7.33_a	5.94_b	4.98_c
ionic bonds	2.67_a	4.66_b	0.69_c	1.48_d
hydrogen bonds	7.03_a	8.46_b	3.44_c	3.61_c
hydrophobic interactions	28.29_a	20.35_b	10.46_c	12.03_c

[a] Different letters in the same row indicate significant differences ($p \leq 0.05$).

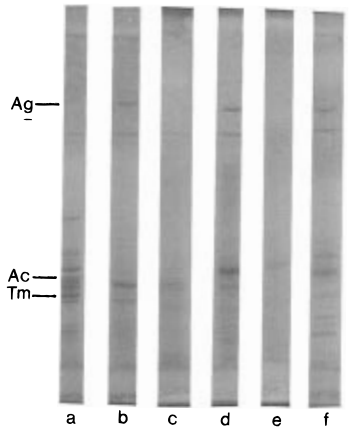

Figure 3. SDS−PAGE of proteins soluble in S1 (0.05 M NaCl) and S4 (8 M urea + 0.6 M NaCl), from gels with 2.5% NaCl, made at 35, 90, and 35/90 °C: (a) soluble fraction in S1 of gel made at 35 °C (suwari), (b) soluble fraction in S4 of gel made at 35 °C (suwari), (c) soluble fraction in S1 of gel made at 90 °C, (d) soluble fraction in S4 of gel made at 90 °C, (e) soluble fraction in S1 of gel made at 35/90 °C, and (f) soluble fraction in S4 of gel made at 35/90 °C.

Where setting took place at 35 °C prior to cooking at 90 °C, gels exhibited a smaller proportion of nonspecific associations, ionic bonds, hydrogen bonds, and hydrophobic interactions than unset gels (Table 2), probably because setting caused a firmer, more orderly network to form, so that there was more involvement of all proteins in gelation through stronger bonds (disulfide bonds or other covalent bonds). Moreover, the proteins not involved would tend to be held more firmly in the gel and hence less extractable. It is worth noting that in preset gels, particularly those containing 2.5% salt, there were much fewer hydrophobic interactions than in corresponding unset gels. In this case no disulfide bonds could be detected, which suggests that a larger number of other covalent bonds were formed in preset gels.

Electrophoretic Study. In the electrophoretic profile of the fraction solubilized with 0.05 M NaCl (S1) (Figure 3a) from gels made at 35 °C (suwari) with 2.5% NaCl, there were a number of bands corresponding by

molecular weight to actin, tropomyosin, troponins, myosin light chains, and sarcoplasmic proteins. Matsumoto (1980) noted that 0.15 M NaCl was the right concentration for extraction of sarcoplasmic proteins; however, it has been found more recently that some actin, tropomyosin, and troponins can be solubilized even at ionic strengths inferior to 0.15 (Jiménez-Colmenero et al., 1994; Samejima et al., 1985). The presence of actin in the fraction solubilized with 0.05 M NaCl would indicate that this protein, at least partially, is weakly bonded to the gel network (nonspecifically associated) and, hence, is easy to extract. Kamath et al. (1992) found that actin was scarcely modified during setting in gels made from surimi of both Alaska pollack (*Theragra chalcogramma*) and Atlantic croaker (*Micropogen undulatus*) and attributed the main changes to the myosin heavy chains. Actin would not therefore appear to play a decisive role in laying down the protein network during setting. Similar conclusions have been reached by Gill and Conway (1989) and Sano et al. (1989). In the given solubilization conditions, there were also other bands of higher molecular weight (89 and 62 kDa) which could represent polypeptides produced by some proteolytic activity (Niwa et al., 1993). Above the actin band referred to, there was another representing a polypeptide of around 50 kDa molecular weight, of unknown origin. Jiménez-Colmenero et al. (1994) have described a similar band in hake actomyosin gels heated at 45 °C, appearing more diffuse at 70 °C.

On solubilization with S4 (8 M urea + 0.6 M NaCl) (Figure 3b), a distinct band was detected in the sample application zone and in the interphase zone between the stacking and resolving gel, representing a polymerized protein in the form of large aggregates which prevented it from entering the electrophoretic resolving gel. The absence of a band for myosin heavy chain (MHC) suggests that MHC had aggregated via bonds stronger than hydrophobic interactions or hydrogen bonds. Numerous authors have attributed such MHC polymerization during setting to an enzymatic reaction, catalyzed by a transglutaminase, which produces cross-linking in myosin through covalent bonds (Kim et al., 1993; Kamath et al., 1992; Tsukamasa and Shimizu, 1990). There is a positive correlation between cross-linking of the MHC and gel-forming ability (Nishimoto et al., 1987).

In gels heated at 90 °C, the molecular weight band corresponding to actin was visible in the fraction solubilized with S4 (Figure 3d) but not with S1 (Figure 3c). This means that actin aggregates at high temperatures, chiefly by means of hydrogen bonds and hydrophobic interactions. According to Sano et al. (1989a), during heating actin dissociates from myosin, so that at high temperatures there is further aggregation of unprotected actin filaments. As in the gels made at 35 °C, MHC was polymerized by stronger bonds than the hydrophobic interactions, forming large insoluble aggregates. In the fraction solubilized with S1 (Figure 3c), bands corresponding to tropomyosin, troponins, and lower molecular weight proteins were visible, although less distinct than in the suwari gel. These proteins were partially nonspecifically associated to the network of the gel made by direct cooking at 90 °C, but they were also partly involved in hydrophobic interactions and hydrogen bonds, since they have been detected most clearly in the fraction solubilized with S4. Samejima et al. (1985) found that when veal actomyosin was heated, tropomyosin, troponins, and myosin light chains remained soluble in a solution of 0.1 M NaCl. Similar results have been reported by Jiménez-Colmenero et al. (1994) in hake actomyosin gels heated up to 70 °C. Hence tropomyosin is only slightly involved in gel formation (Shiga et al., 1988), possibly due to its high heat resistance (Samejima et al., 1982).

The electrophoretic profile for the S4-solubilized fraction (Figure 3f) of gels preset at 35 °C and then cooked at 90 °C exhibited not only absence of MHC but also an aggregate, both in the application zone and in the interphase zone, which was less well-defined than in gels cooked directly at 90 °C. This means that as a consequence of setting more covalent bonds could be formed, so that the aggregated MHC is retained to a greater extent in the insoluble fraction. The band corresponding to actin in the S4 soluble fraction was less intense in this case than in gels without prior setting suggesting that part of the actin interacted through covalent bonding as a result of setting. It is not possible to elucidate whether such aggregation occurs through the action of transglutaminase or is a direct consequence of heating (Sano et al., 1989a). The band associated with tropomyosin appeared clearly visible in the fraction solubilized with S1 (Figure 3e), which would indicate that in the preset gels tropomyosin, at least in part, was not directly involved in gel formation. Lee et al. (1990) also identified tropomyosin as the chief soluble protein in hoki (*Macruronus novaezelandiae*) gels when set and then cooked at 90 °C. In a study of the contribution of tropomyosin to the characteristics of fish gels, Sano et al. (1989b) reported that tropomyosin negatively affected gel formation, even in gels subjected to double heat treatment.

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