Suwari and Kamaboko Sardine Gels: Effect of Heat Treatment on Solubility of Networks

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Suwari (set) and kamaboko (set and cooked) gels made with sardine surimi were prepared at normal heat-setting (35 or 40 °C) and modori (60 °C) temperatures and treated with solutions breaking ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bridges. The lower gel strength of the gels obtained at modori temperature corresponded to a higher solubility of these gels in solutions breaking hydrophobic interactions and disulfide bridges. For suwari gels set at 35 and 40 °C more protein was solubilized when electrostatic interactions and hydrogen bonds were ruptured. SDS–PAGE showed a higher amount of actin in these two fractions. No myosin heavy chain (MHC) bands were observed in any of the fractions obtained from suwari gels heat-set at 35 and 40 °C. However, MHC bands were visible when disulfide bridges were disrupted in gels set at 60 °C and in kamaboko gels set at 35 °C. This means that the structure of the set gels was altered at cooking temperatures when the kamaboko network was formed.

Keywords: *Gels; setting; suwari; kamaboko; modori; surimi; sardine; myosin; actin; solubility*

INTRODUCTION

The texture of kamaboko-type thermostable gels made from surimi is determined by interactions or bonds that occur among myofibrillar proteins after solubilization with salt, to give a firm, elastic network. Gelling involves unfolding of the proteins and the establishment of bonds among them. It is believed that hydrogen bonds (Suzuki, 1981; Beas *et al.*, 1988), hydrophobic interactions (Sano, 1988; Beas and Crupkin, 1990; Niwa *et al.*, 1991), disulfide bridges (Ishioroshi *et al.*, 1981; Sano, 1988; Roussel and Cheftel, 1990), and covalent bonds other than disulfide (Numakura *et al.*, 1985; Seki *et al.*, 1990) intervene differently in the formation of the network depending on the various parameters involved. The importance of the various bonds in gel network formation was recently reviewed by Niwa (1992). The factors determining the number and kind of interactions or bonds include not only the species from which the surimi is derived (Suzuki, 1981; Shimizu, 1985; Nishimoto *et al.*, 1988) but also the heat conditions in which the gel is made (Ishikawa, 1978; Akahane and Shimizu, 1990; Lee *et al.*, 1990; Yamazawa, 1990). It is believed that during setting the skeleton of the network is laid down and cross-linking occurs in varying degrees (Nishimoto *et al.*, 1988).

Most of the work in this field has been done on Alaska pollock (*Theragra chalcogramma*) and other species widely used to obtain surimi or on actomyosin isolates. There are very few references to gels made from sardine surimi (Leinot and Cheftel, 1990; Roussel and Cheftel, 1990).

In suwari gels (set gels without final cooking) and kamaboko gels (cooked gels) made from sardine surimi, some authors have found that texture varies depending on setting temperature, with peak gel strengths recorded at setting temperatures of 35–40 °C (Roussel, 1988; Alvarez, 1993). Minimum gel strengths have been observed at modori temperatures (60 °C), at which the network becomes brittle. These texture changes go together with an altered appearance as viewed with electron microscopy (Alvarez, 1993; Couso, 1994).

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The aim of this work was to establish whether the differences among gels formed at different temperatures are reflected in networks where the proteins form different numbers and/or kinds of interlinking bonds. The gels were therefore treated with a succession of solutions to achieve selective rupture of the bonds involved in network formation.

MATERIALS AND METHODS

Frozen sardine (*Sardina pilchardus*) surimi, prepared in one batch for this study by the firm SCOMA (Lorient, France), was air freighted with solid CO_2 to the laboratory, cut into blocks, vacuum-packed in Cryovac BB-1 bags (80 Torr of pressure), and stored at −20 °C (±1) for 1 month. The cryoprotectants added to the surimi were 4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate. Crude protein was measured in the surimi by Kjeldahl method (AOAC, 1975), crude fat by the Bligh and Dyer (1959) method as modified by Knudsen *et al.* (1985), and moisture and ash by AOAC procedures (1975). The proximate composition of the surimi was as follows: crude protein, 13.13%; crude fat, 3.05%; moisture, 75.82%; ash, 0.63%; pH 6.6.

Preparation of Suwari and Kamaboko Gels. For gel-making, the surimi was tempered until it reached −5 °C and ground for 1 min [Stephan UM12 vacuum cutter mixing machine (Stephan u Söhne GmbH & Co, Hameln, Germany), 0.1 bar, coolant temperature −2 °C]. The surimi was chopped for 1 min at high speed (setting 1). Then sodium chloride was added (3 g/100 g of surimi, equivalent to 2.44% in the final gel) with ice flakes (as required to adjust moisture content to 78%), and the mixture was beaten slowly (setting 2) for 5 min. The temperature of the mixture was kept below 8 °C (±1) throughout the process.

Twenty-four grams (±1) of the sol formed was heated in stainless steel cylinders (30 mm diameter by 30 mm height) with screw-on tops and bottoms. These were sealed at both ends, taking special care to ensure that they were well packed and free of bubbles. To produce the various suwari (S) and kamaboko (K) gels, heating was performed for 30 min at 35, 40, and 60 °C (S and K gels) in a water bath (Julabo F10, Labortechnik GmbH, Seelbach, Germany) and cooking by steam saturation for 30 min at 90 °C (K gels) in a Rational Combi-Master CM6 (Grosküchentechnik GmbH, Landsberg Lech, Germany). Each lot was prepared at least in triplicate. After treatment, the samples were cooled under running tap water and stored at 4 °C (±1) for 24 h.

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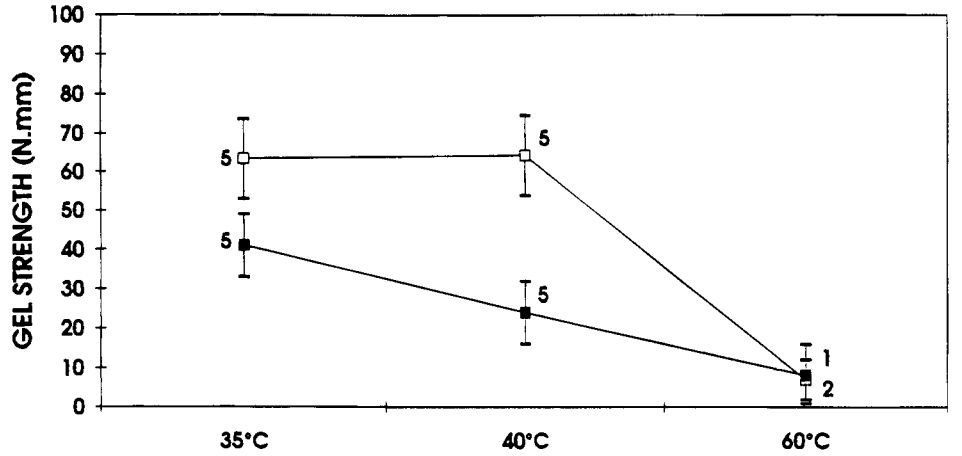

Figure 1. Influence of setting temperatures on gel strength (N·mm) of suwari (■) and kamaboko (□) gels. Vertical bars indicate confidence intervals at 95%. Folding test scores are indicated as a number in the curve. Suwari gels were set for 30 min at 35, 40, or 60 °C; kamaboko gels were heated at 90 °C for 30 min after setting.

Determination of Gel Texture. A penetration test was performed on gel samples (30 mm height by 30 mm diameter, 20 ± 1 °C) using a cylindrical stainless steel round-end plunger (diameter 5 mm) attached to a 100 N load cell connected to an Instron Universal Testing Machine Model 4501 (Instron Engineering Corp., Canton, MA). A Hewlett-Packard Vectra ES/12 computer was used to program the cross-head movement to 10 mm/min and to obtain and analyze the force–deformation curves. Gel strength (GS) (N·mm) was determined as the product of yield strength (YS) (N) and yield deformation (YD) (mm) measured at the point of gel breakage (Roussel, 1988; Hamann and MacDonald, 1992).

Determination of folding test (FT) was performed on gel slices of 3 mm height according to the method of Tanikawa *et al.* (1985). The maximum score (5) is obtained when no cracks are observed when the slice is folded twice without breaking. The minimum score (1) is obtained when the slice breaks into fragments when folded in half. All determinations were performed at least in triplicate.

Gel Solubilization. The solubilization of the proteins in the network was conducted using various agents which differ from each other according to their ability to cleave intermolecular bonds: electrostatic and hydrogen (0.6 M NaCl), hydrogen (1.5 M urea), hydrogen and hydrophobic (8 M urea), and S–S bonds (β-mercaptoethanol) (Kauzmann, 1959; Lapange, 1978; Tsuchiya *et al.*, 1980; Wall and Huebner, 1981). The gels were treated successively with 0.6 M NaCl (solution 1), 0.6 M NaCl plus 1.5 M urea (solution 2), 0.6 M NaCl plus 8 M urea (solution 3), and 0.6 M NaCl and 8 M urea plus 5% β-mercaptoethanol (ME) (solution 4). The protocol followed for each kind of gel was as follows : 1 g of gel was mashed for 5 min at ambient temperature in a mortar with 10 mL of solution 1 and centrifuged (Sorvall RT6000B, DuPont Co., Wilmington, Delaware) for 30 min at 1950*g*. Once the supernatants were removed, 10 mL of solution 1 was added to the precipitate, which was stirred (Ika Vibrax VXR stir tubes from Janke & Kunkel, Germany) for 24 h at room temperature. The samples were then centrifuged for 30 min at 1950*g* and the two supernatants (S1) were mixed. Ten milliliters of solution 2 was then added to the precipitate, which was mechanically stirred for 24 h and then centrifuged, and the precipitate washed again with solution 2 in the same conditions as previously but with 30 min of mixing. The procedure was repeated successively up to separation of the precipitate and supernatant with solution 4, to give fractions S1, S2, S3, and S4, corresponding to protein solubilized with solutions 1, 2, 3, and 4, respectively, and a precipitate (PP) which was insoluble in any of these solutions.

Determination of Protein Solubility. Protein concentration in the S fractions (1–4) was determined by the microbiuret method (Itzaki and Gill, 1964). Because of interference of ME in the method, 1 mL of each S4 fraction was precipitated with 1 mL of 24% trichloroacetic acid (TCA), left to stand for an hour in an ice and water bath, and then centrifuged for 10 min at 1950*g*. The precipitates were treated with 5 mL of 12% TCA and the last two steps repeated. The resulting precipitate was solubilized with 0.6 M NaCl plus 8 M urea (solution 3), following which protein was determined as in the foregoing cases, using specific standard curves for each solution. The results are expressed as milligrams of solubilized protein per gram of gel.

Electrophoresis. The samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in a Phast-System horizontal apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden) using 12.5% and 4–15% polyacrylamide gels. The samples were treated according to the method of Hames (1985) (2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue) and then heated for 5 min in a boiling water bath. Samples were centrifuged (Sorvall Microspin 24S, DuPont) for 1 min at 12 000*g*. One microliter aliquots of the different fractions containing known amounts of protein (ranging from 0.05 to 1.6 mg/mL) were applied to the gels.

Electrophoresis conditions were 4 mA/gel, 250 V, and 3 W. The protein bands were stained with coomassie brilliant blue (PhastGel Blue R, Pharmacia LKB Biotechnology). The molecular mass (MM) of the main protein bands in the samples was estimated by comparing their mobility with that of a standard high-MM protein mix (Pharmacia LKB Biotechnology).

Statistical Analysis. Two-way analyses of variance were performed using an *F* test. The differences of means between pairs were resolved by lsd test to obtain the confidence intervals. Level of significance was set for $P < 0.05$.

RESULTS AND DISCUSSION

Texture. GS values of suwari and kamaboko gels are shown in Figure 1. GS was much greater in kamaboko gels prepared at 35 and 40 °C than in those prepared at 60 °C or in suwari gels. There were no significant differences between the first two kamaboko gels. In the suwari gels, maximum GS occurred at the lowest setting temperature, decreasing as setting temperature increased. This was due to a decline in YD and YS, the decline being more pronounced in YD. The suwari and kamaboko gels prepared at 35 and 40 °C settings gave the highest folding test scores (FT = 5). Both type of gels prepared at 60 °C scored poorly, indicating either that the initial suwari gel was of poor quality (FT = 2) or that it was destroyed on further heating (FT = 1). This would confirm that the modori phenomenon occurred when the gels, cooked or not, were set at temperatures in the region of 60 °C, showing that sardine is a species particularly prone to modori (Tsukamasa and Shimizu, 1989).

Solubility. Protein solubility (milligrams of protein per gram of gel) in solutions 1–4 in suwari and kamaboko gels are shown in Figure 2.

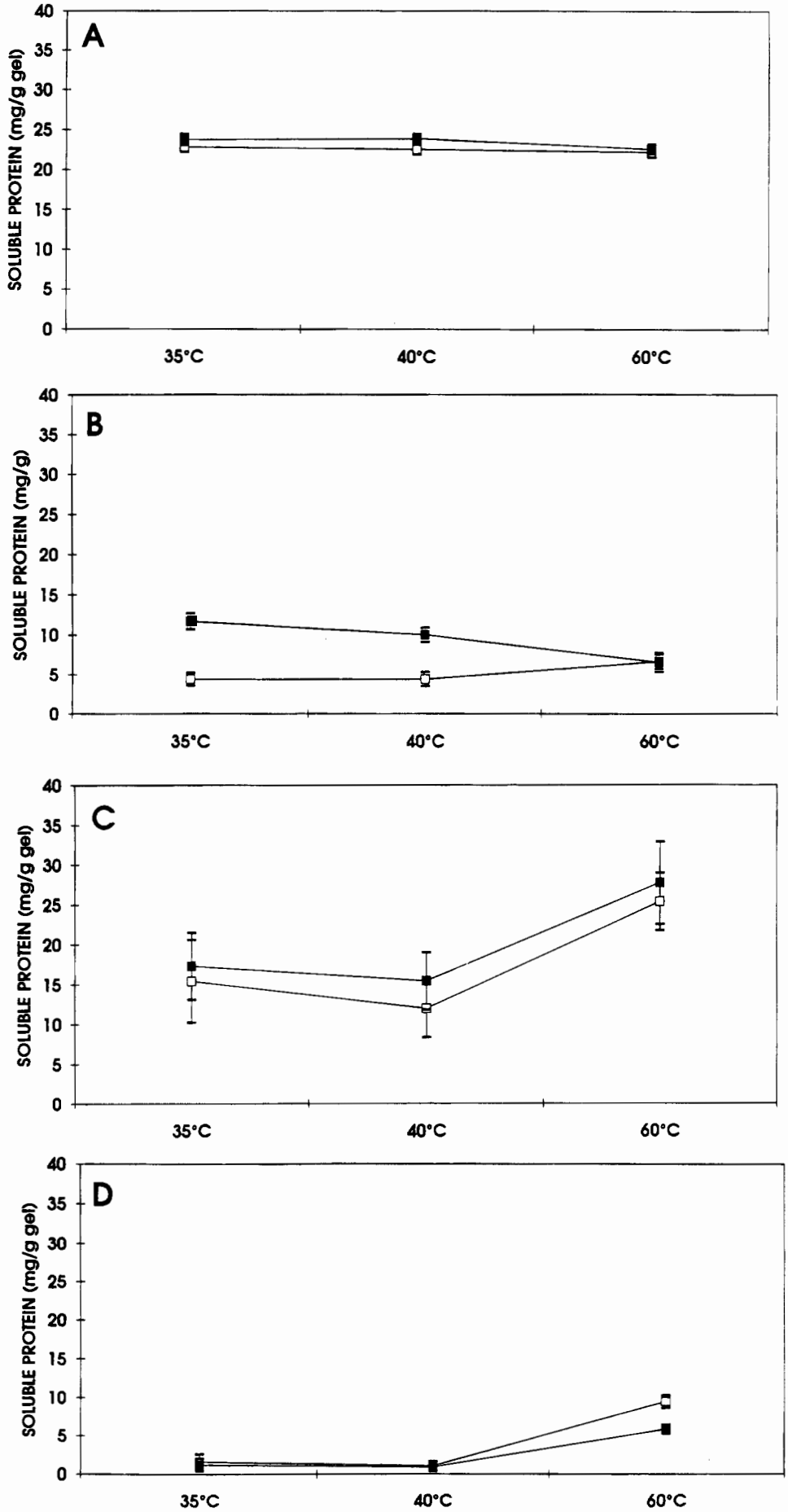

Figure 2. Soluble protein (mg/g of gel) of suwari (■) and kamaboko (□) gels solubilized with 0.6 M NaCl (A), 0.6 M NaCl plus 1.5 M urea (B), 0.6 M NaCl plus 8 M urea (C), or 0.6 M NaCl and 8 M urea plus 5% ME (D). Vertical bars indicate confidence intervals at 95%. The amount of protein in the gels was 106.9 mg/g of gel. Suwari and kamaboko gels conditions were as in Figure 1.

The gels treated with solution 1 (0.6 M NaCl) gave protein solubility values of around 25 mg of protein/g of gel. The suwari gels prepared at 35 and 40 °C scored slightly higher, but no statistically significant differences were found (Figure 2A). No significant differences were found among S and K gels prepared at 60 °C. Most myofibrillar proteins are likely to solubilize at 0.6 M NaCl in undenatured conditions. Therefore, the fractions soluble in 0.6 M NaCl could be related to proteins bound to the network through electrostatic interactions or hydrogen bonds or to proteins that were not involved in the formation of the network and had not undergone insolubilization of another kind (Jiménez-Colmenero *et al.*, 1994).

When the precipitate was treated with solution 2 (0.6 M NaCl plus 1.5 M urea), less protein was solubilized than in S1 (Figure 2B). The suwari gels set at 35 and 40 °C did not differ significantly, while solubilization decreased in gels set at 60 °C. Kamaboko gels set at 35 and 40 °C exhibited less solubilization than did the suwari gels. No significant difference was found between these kamaboko gels and those set at 60 °C.

When the above precipitate was treated with solution 3 (0.6 M NaCl plus 8 M urea), both types of gel exhibited significant differences according to setting temperature, with solubilization greater at 60 °C than at 35 or 40 °C (Figure 2C). There were no significant differences among suwari and kamaboko gels set at the same temperature, although the kamaboko gels did display less solubility in general. The similarity of the behavior of suwari and kamaboko gels would suggest that changes in hydrophobicity occurred chiefly during setting and the network underwent little alteration when the gel was heated to produce a kamaboko gel. In the literature, however, it is reported that exposure to hydrophobic groups increases as temperature rises (Niwa, 1992); in the present case this was only found to be true for the two gels set at 60 °C, which did present greater solubility. The answer to this apparent contradiction may be that at cooking temperatures, besides the hydrophobic bonds, other bonds can also be formed that are not cleaved with solution 3.

With solution 4 (0.6 M NaCl and 8 M urea plus 5% ME), there was hardly any protein solubilization in suwari and kamaboko gels set at 35 or 40 °C (Figure 2D). In both gel types, protein solubilization was greater with setting at 60 °C, a characteristic more marked in the kamaboko gel. The results suggest that the network forming at modori temperatures involved hydrophobic interactions and disulfide bridges that were more exposed in the given solubilization conditions. This type of network produces a more brittle texture in suwari and kamaboko gels set at 60 °C (Figure 1). Observing sardine surimi gels prepared at modori temperature (60 °C) under scanning electron microscopy, Couso (1994) detected the formation of networks with disorderly aggregation of globules resembling those produced by direct cooking.

The suwari and kamaboko gels set at 60 °C left smaller insoluble precipitates. The kamaboko gels set at 35 and 40 °C were the least soluble, differing significantly from the suwari gels and from the kamaboko gels set at 60 °C. The highest solubility found in gels set at 60 °C is consistent with the results reported by Roussel and Cheftel (1990) for sardine surimi gels solubilized in different buffers. However, they reported the highest insolubility as occurring in suwari gels set for 30 min at 37 °C, and they found practically no difference between kamaboko gels set at 37 and 60 °C. In gels made with sardine surimi, we have found that when the gels are obtained by setting at temperatures ≤40 °C for 30 min, prolonging the cooking from 30 to 60 min produces gels with lower GS and more brittle networks (Alvarez, 1993; Couso, 1994; Alvarez *et al.*, 1994). As Roussel and Cheftel (1990) obtained kamaboko gels by heating for 50 min, this may be the reason why they found kamaboko gels to be more soluble. Our results were concurrent with the gel strength and folding test scores, which were lower in gels set at 60 °C.

The difference in degree of solubilization of gels depending on setting temperature was due mainly to the fact that more protein was solubilized in gels set at 60 °C when treated with solutions 3 and 4, which rupture mainly hydrophobic interactions and disulfide bridges, respectively. The differences between suwari and kamaboko gels set at the same temperature, on the other hand, were caused by the fact that more protein was solubilized in suwari gels set at 35 and 40 °C when treated with solutions 1–3, while with a 60 °C setting, kamaboko gels presented greater solubility when disulfide bridges were ruptured (solution 4).

The high proportion of insoluble protein remaining may indicate that, in the formation of suwari and kamaboko gels, non-disulfide covalent bonds play an important and as yet unquantified part. The existence of covalent bonds of a kind other than such S–S bonds as lysino–alanine or glutamyl–lysine has been described for other species by Numakura *et al.* (1985), Seki *et al.* (1990), and Jiménez-Colmenero *et al.* (1994) and for sardine by Roussel and Cheftel (1990).

The fact that a gel exhibits less solubility does not necessarily imply that a larger number of bonds are involved in network formation but rather that the proteins may be linked by non-disulfide covalent bonds which would intervene in the formation of the network without being ruptured in the experimental conditions of solubilization. In such conditions greater network solubility would mean a weaker structure, which is generally consistent with the textural data found. The reason for low solubility could also be that our solubilization conditions, though in the line with those found in the literature, were not harsh enough. Possibly more aggressive treatments would be required, which we did not contemplate owing to the likelihood of protein degradation.

Electrophoresis. The electrophoretic profile of sardine surimi is shown in Figure 3. The majority of bands appear to be myosin heavy chain (MHC) (200 000 Da), actin (42 000 Da), and tropomyosin (37 000 Da), as described in sardine surimi by other authors (Roussel, 1988; Roussel and Cheftel, 1990; Leinot, 1991).

The electrophoretic profiles of the suwari and kamaboko gel fractions solubilized with the different solutions are shown in Figures 4–9. The electrophoretic profiles of all the S1 fractions (Figure 4) show a band corresponding to tropomyosin, and the fractions of the suwari gels set at 35 and 40 °C (lanes 6 and 4) show an actin band; this would suggest either that part of the actin was linked to the suwari network by electrostatic bonds or that only after treatment at temperature ≥60 °C was actin bound to the gel network. The presence of actin coincided with a slightly higher level of protein solubilization with solution 1 in these gels (Figure 2A). Also, although in a lower proportion, proteins of MM under 37 000 Da were detected, corresponding to troponins and myosin light chains.

The electrophoretic profiles of the proteins solubilized with solution 2 (Figure 5) were similar to those treated

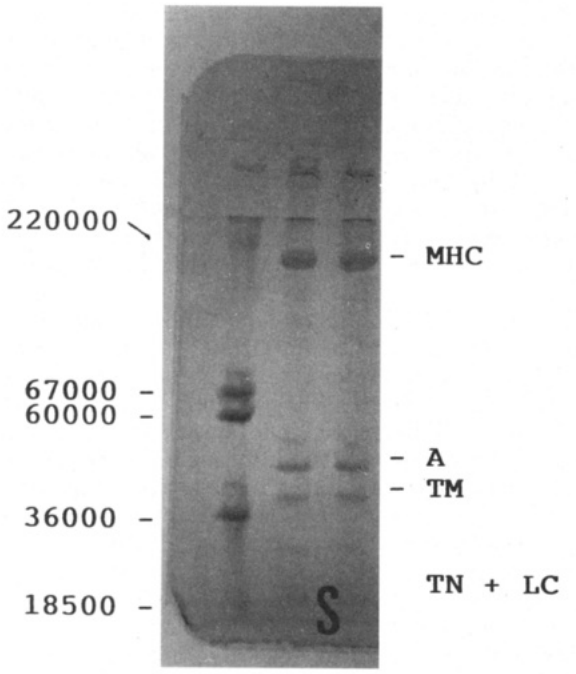

Figure 3. SDS–PAGE (12.5%) pattern of sardine surimi proteins. The sample was treated with 2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue, heated for 5 min in a boiling water bath, and centrifuged at 12 000*g* for 1 min. MHC, myosin heavy chain; A, actin; TM, tropomyosin; TN, troponin; LC, myosin light chains. Standard: high-MM protein mix (Pharmacia LKB Biotechnology). Each lane is a replicate of the sample.

with solution 1 for each gel type and setting temperature. The low-MM bands (<37 000 Da) in the suwari gels set at 35 and 40 °C were more pronounced (lanes 6 and 4), which was consistent with the greater solubility of these gels in solution 2.

The electrophoretic profile of proteins solubilized in 0.6 M NaCl plus 8 M urea (S3) (Figure 6) revealed the presence of actin, tropomyosin, and a number of low-MM (<37 000 Da) bands in all suwari and kamaboko gels at all setting temperatures, although the actin band was less apparent in kamaboko gels set at 40 °C (lane 3). Noteworthy was the appearance of bands in the interphase zone (between the stacking and resolving gels) in suwari and kamaboko gels set at 60 °C and also of protein held in the application zone that did not enter the gel, although it was extracted with the supernatant.

In the suwari and kamaboko gels solubilized with 0.6 M NaCl and 8 M urea plus 5% ME (S4) (Figure 7), actin bands were clearly visible as were, to a much lesser extent, tropomyosin bands. The scantness of the latter may be due to the prior extraction of the tropomyosin. In gels set at 60 °C (lanes 1 and 2), the actin band was more intense and more high-MM bands are observed. In these gels, small amounts of myosin and a larger amount of retained high-MM protein were found both in the application zone and the interphase zone.

Four 15% gradient SDS–PAGE of the proteins solubilized with solution 4 (Figure 8) showed MHC bands in suwari and kamaboko gels set at 60 °C (lanes 2 and 1) and, to a lesser extent, in kamaboko gels set at 35 °C (lane 5). They were virtually undetectable in suwari gels set at 35 °C (lane 6) and in both types set at 40 °C (lanes 3 and 4). Myosin dimers and trimers were not found, although proteins were retained in the application zone and in smaller amounts in the interphase; these did not enter the gel but were solubilized with solution 4. In all of the kamaboko gels and in the suwari gels set at 60 °C, a band appeared above the actin (MM ca. 50 000 Da), which has already been reported in actomyosin gels by other authors (Jiménez-Colmenero *et al.*, 1994). The presence of MHC in gels set at 60 °C may be related to the fact that myosin molecules, in some species, dissociate in the gel network at temperatures above 40 °C, forming a "myosin-poor" actomyosin network (Sano, 1988). In this case myosin would be more easily released.

Four 15% gradient SDS–PAGE of the proteins remaining undissolved in solution 4 but soluble under the

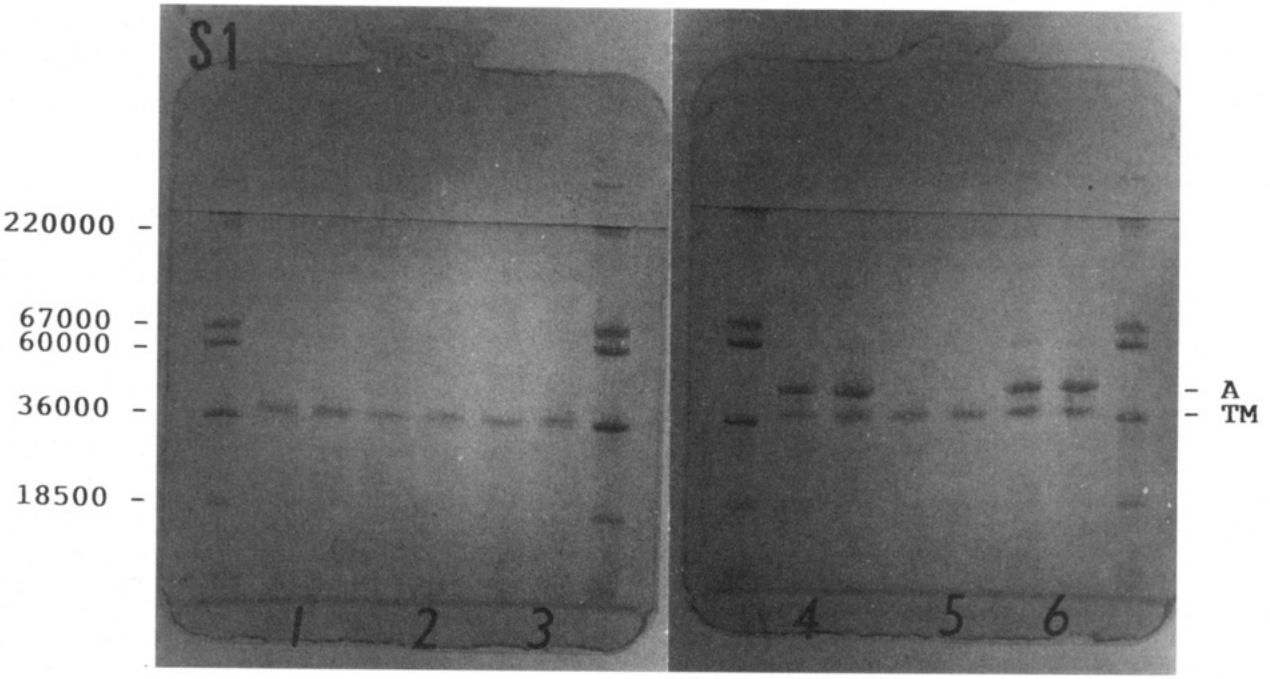

Figure 4. SDS–PAGE (12.5%) of proteins from suwari and kamaboko gels solubilized with solution 1 (0.6 M NaCl). Gels were obtained by heating at (1) 60 + 90 °C, (2) 60 °C, (3) 40 + 90 °C, (4) 40 °C, (5) 35 + 90 °C, and (6) 35 °C. Setting and cooking times were each 30 min in all cases. Electrophoretic conditions and abbreviations used are the same as in Figure 3. The two lanes above each number are replicates of the same gel.

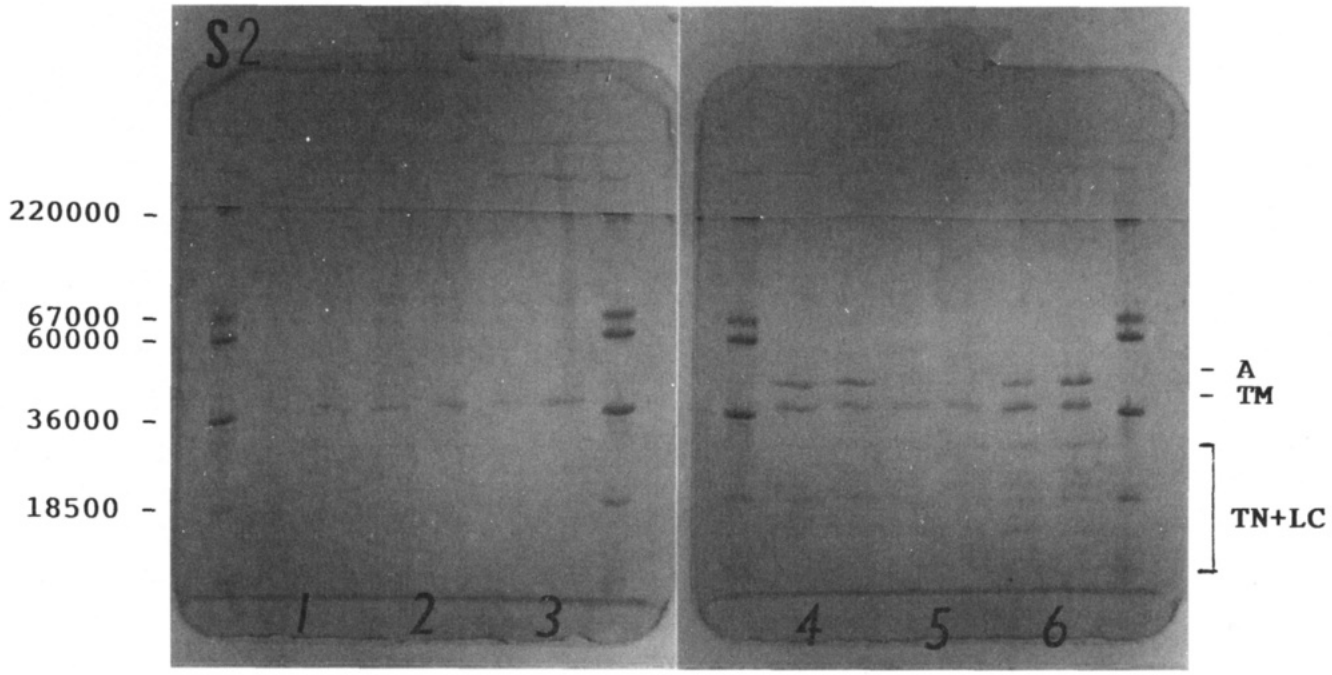

Figure 5. SDS–PAGE (12.5%) of proteins from suwari and kamaboko gels solubilized with solution 2 (0.6 M NaCl; 1.5 M urea). Conditions and abbreviations used are the same as in Figures 3 and 4.

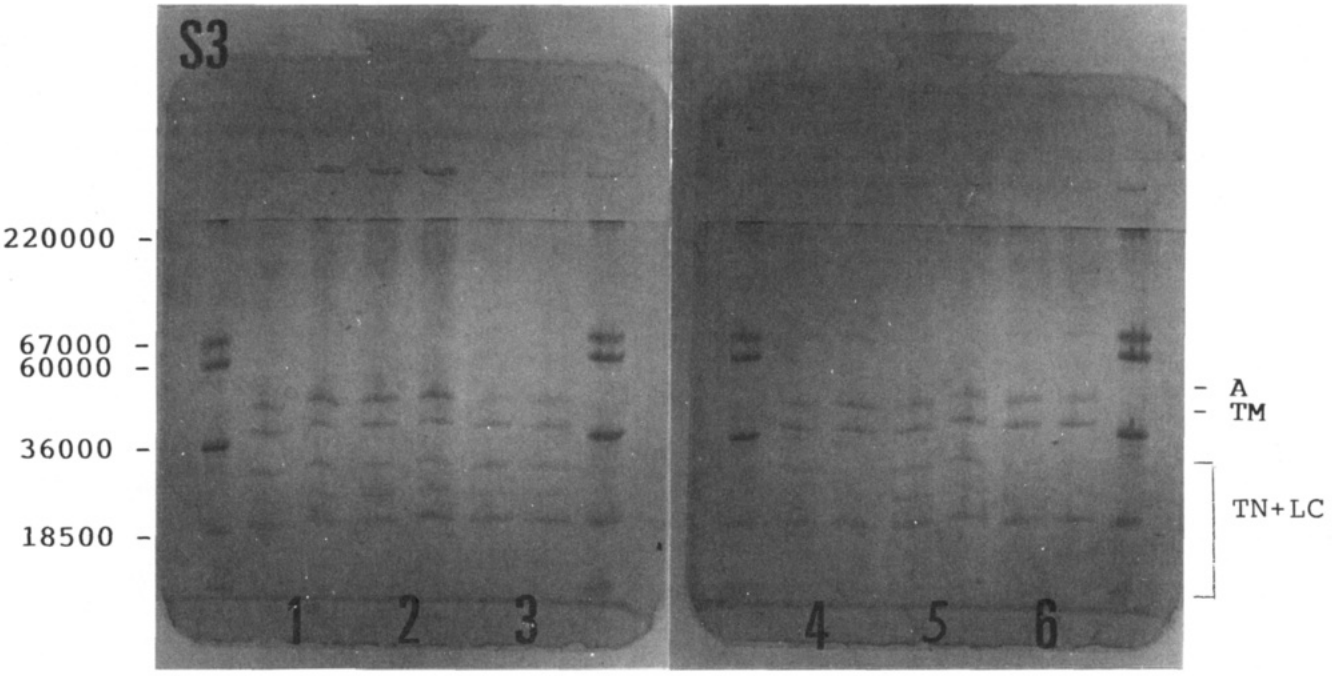

Figure 6. SDS–PAGE (12.5%) of proteins from suwari and kamaboko gels solubilized with solution 3 (0.6 M NaCl; 8 M urea). Conditions and abbreviations used are the same as in Figures 3 and 4.

electrophoretic sample preparation conditions (PP) (Figure 9) revealed the presence of myosin in all of the kamaboko gels and in the suwari gels set at 60 °C and of protein in the application zone that did not enter the gel. Actin was detected in all gels. It is important to note that hardly any myosin was detected in suwari gels set at 35 and 40 °C; this means that the network formed with sardine surimi in these conditions does not rupture even in the harsh conditions of sample treatment for electrophoresis.

Our results showed that tropomyosin appeared in all fractions of suwari and kamaboko gels irrespective of setting temperature. TM was less evident in fraction 4, probably because it had been solubilized in previous solutions. Roussel and Cheftel (1990) found a marked decrease in tropomyosin in solutions that acted in the same way as solution 4. A number of authors have reported that tropomyosin is not involved in network formation (Cheng *et al.*, 1979; Samejima *et al.*, 1982; Shiga *et al.*, 1988; Jiménez-Colmenero *et al.*, 1994). However, in gels made from Alaska pollock surimi, surimi TM content has been found to correlate inversely with the stiffness of the gel (French, 1986). The fact that it was successfully solubilized with the various agents used suggests that when the network was ruptured, TM was released from the zones where it had been trapped upon formation of the gel.

A certain percentage of actin would appear to form part of the network only beyond a certain temperature, as part of the actin was released from suwari gels set

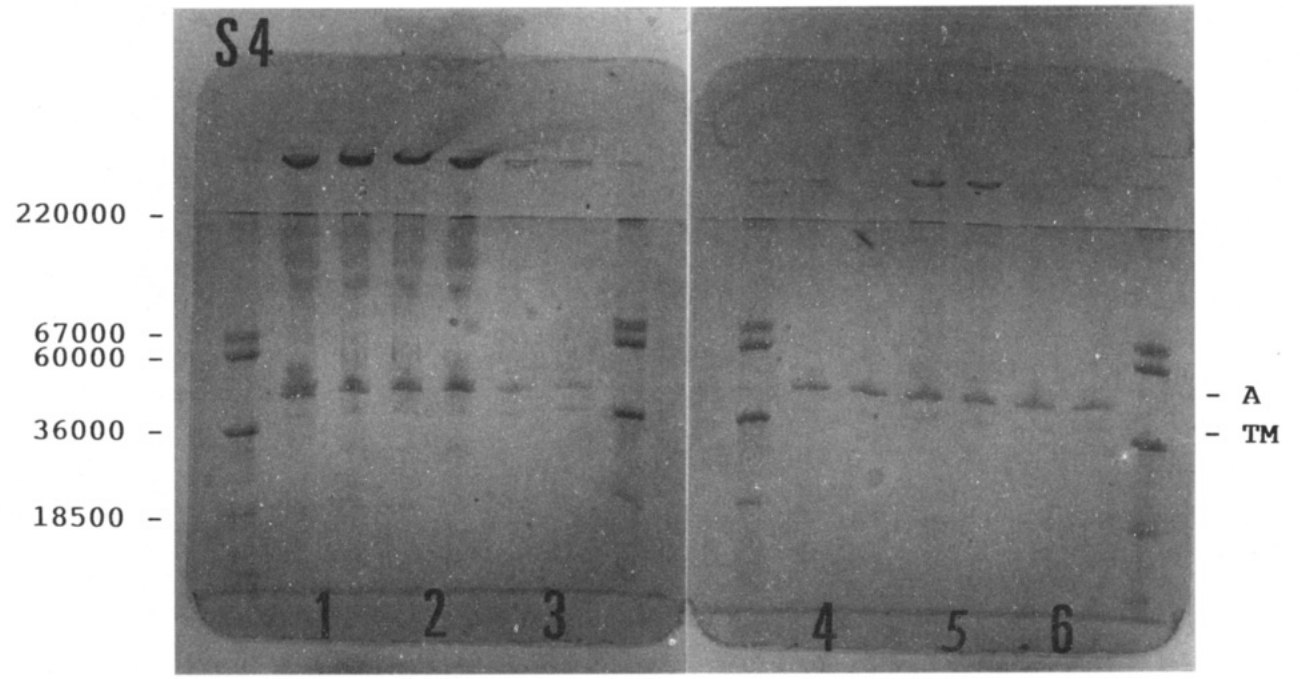

Figure 7. SDS–PAGE (12.5%) of proteins from suwari and kamaboko gels solubilized with solution 4 (0.6 M NaCl; 8 M urea; 5% ME). Conditions and abbreviations used are the same as in Figures 3 and 4.

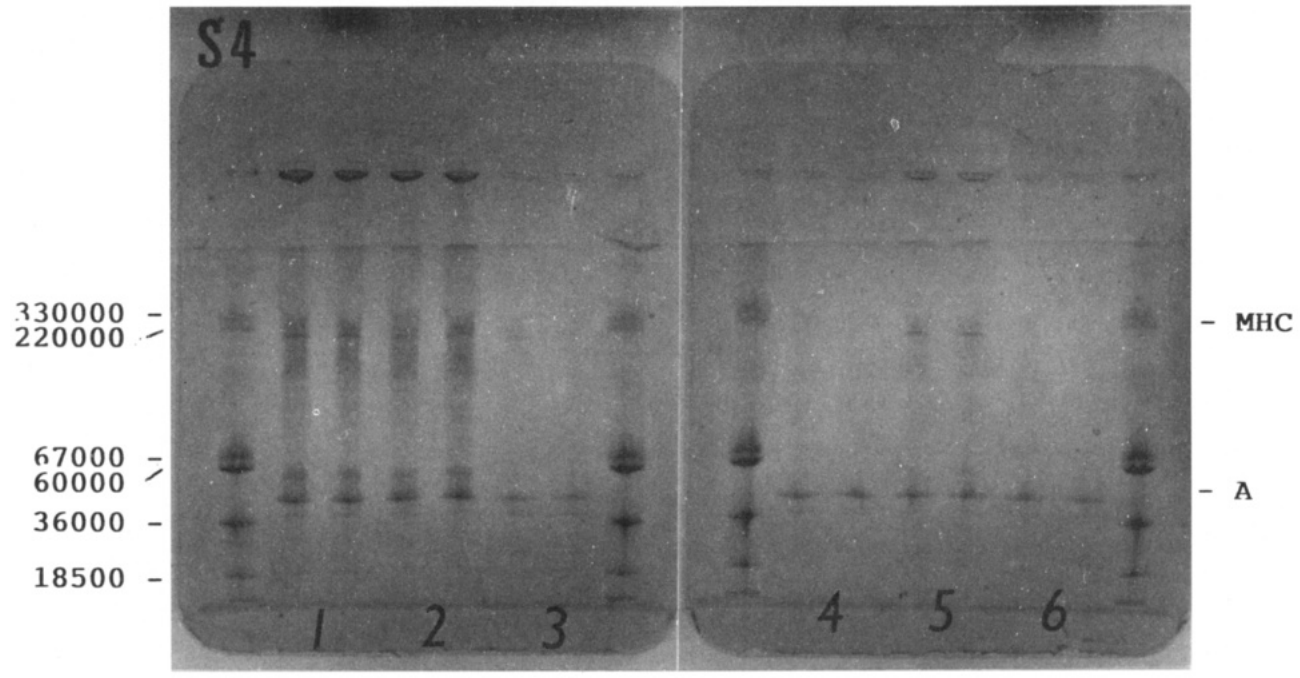

Figure 8. Four 15% gradient SDS–PAGE of proteins from suwari and kamaboko gels solubilized with solution 4 (0.6 M NaCl; 8 M urea; 5% ME). Conditions and abbreviations used are the same as in Figures 3 and 4.

at 35 and 40 °C when treated with 0.6 M NaCl (solution 1) and 0.6 M NaCl plus 1.5 M urea (solution 2), whereas to release actin from kamaboko gels or gels set at 60 °C, treatments breaking hydrophobic (solution 3) or disulfide bridges (solution 4) are required.

MHC proved to be a very stable part of the suwari and kamaboko networks formed at 35 and 40 °C settings. A MHC band appeared in the kamaboko gel set at 35 °C only where disulfide bridges were ruptured (solution 4), whereas no MHC was released in either suwari or kamaboko gels set at 40 °C. In suwari and kamaboko gels set at 60 °C, on the other hand, MHC was clearly apparent upon solubilization in solution 4, which means that in modori conditions MHC must be bound to the network by disulfide bridges. According to Niwa (1992), disulfide bridges are formed massively when the temperature is increased. The fact that no MHC bands appeared in the insoluble precipitates from networks formed at ≤40 °C (suwari) suggests that at these temperatures the MHC forms bonds—presumably non-disulfide covalent bonds—which prevent solubilization even under electrophoretic sample preparation conditions. Also, in the precipitate from these suwari gels there was practically no apparent release of aggregates that did not enter in the SDS–PAGE gel (4–15%).

The possibility of some proteolysis in networks formed at modori temperatures is not discarded, given that

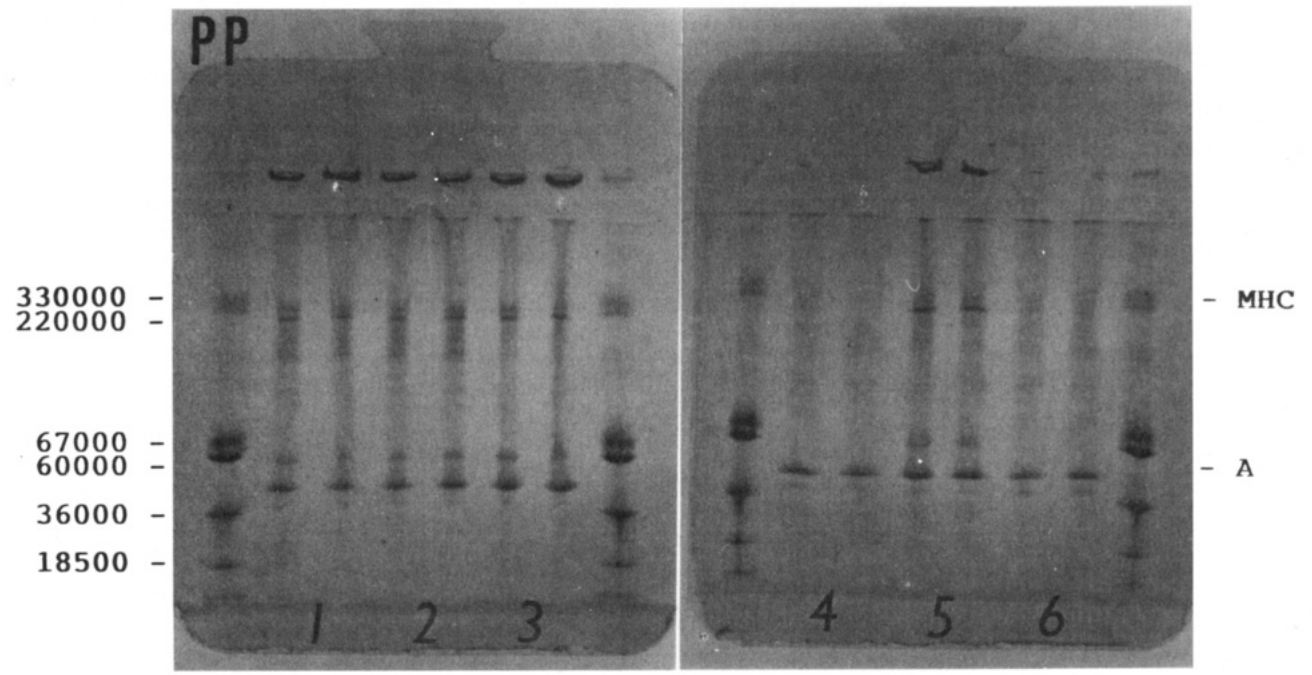

Figure 9. Four 15% gradient SDS–PAGE of proteins from suwari and kamaboko gels remaining undissolved in solution 4 but soluble under the electrophoretic sample preparation conditions. Conditions and abbreviations used are the same as in Figures 3 and 4.

diffuse zones of lower MM than MHC were apparent (Figure 8). Nevertheless, in sardine the phenomenon would appear to arise more from coagulation of myofibrillar proteins, as reported in other species (Toyohara and Shimizu, 1988), with maximum exposure of hydrophobic groups at 60 °C as shown in Figure 2C. Under scanning electron microscopy both suwari and kamaboko gels made with sardine surimi set at 60 °C present a different appearance. They lack a fibrous gel structure and show the presence of globular formations of aggregated proteins (Couso, 1994).

Conclusions. The differences in gel strength found between suwari and kamaboko sardine surimi gels set at 60 °C and those set at 35 and 40 °C correlate with solubility of the networks forming at both setting and cooking temperatures. These differences are evident in that the suwari and kamaboko networks of gels set at 60 °C are more soluble in solutions that preferentially rupture hydrophobic interactions and disulfide bridges and correspond to very low GS values. As few low-MM bands are apparent in these gels, we believe that the modori phenomenon in this species arises rather from rapid coagulation of the myofibrillar proteins at 60 °C, very much as is found in sardine surimi gels cooked directly at higher temperatures. Regarding the major proteins involved in network formation, we believe that actin becomes fixed in the network in different ways depending on whether gels are made at ≤40 °C or at higher temperatures. In the first case actin is solubilized by rupture of electrostatic or hydrogen bonds, whereas in the second case actin is solubilized only by rupture of hydrophobic or disulfide bonds. No MHC bands are observed in any of the fractions from suwari gels set at 35 or 40 °C. Myosin, on the other hand, is only released from the insoluble precipitates when temperatures over 40 °C are used in the gel preparation. This means that the structure of the set gels was altered at cooking temperatures when the kamaboko network was formed. Tropomyosin, on the other hand, seems to be released as it becomes accessible to the solubilizing agents.

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