Thermal Gel Degradation (*Modori*) in Sardine Surimi Gels

C. Alvarez, I. Couso and M. Tejada

ABSTRACT

Sardine (*Sardina pilchardus*) surimi gels set (50°C or 60°C), or set and cooked (50°C or 60°C + 90°C) for different times were studied in order to evaluate *modori* (thermal gel degradation). The texture data of the set gels were similar to data obtained previously in gels set at lower temperature that produced good *kamaboko* gels. However, in gels set at 50°C and 60°C *modori* occurred upon cooking. Microscopically the set gels exhibited globular aggregated structures that became more compact when *modori* occurred. Results suggested that at *modori* temperature protein-protein bonding caused massive protein coagulation preventing the formation of a fibrillar matrix upon cooking.

Key Words: modori, surimi, sardine, texture, structure, gel degradation

INTRODUCTION

MODORI IS A COMMON TERM FOR THERMAL GEL DEGRADATION occurring when the gel structure is irreversibly destroyed during heating when surimi paste (surimi ground with salt to form a sol) is incubated at temperatures close to 60°C. The result is a very brittle, non elastic gel. *Modori* has been reported at lower temperatures (Nomura et al., 1993, 1995) and at higher temperatures (Tsukamasa and Shimizu, 1991). The *modori* phenomenon is highly dependent on species, which can be classified in terms of the facility with which degradation occurs at various temperatures in washed and unwashed muscle (Nomura et al., 1993). Sardine (*Sardinops melanosticta*) is considered to be a species highly susceptible to *modori* (Shimizu et al., 1981; Tsukamasa and Simizu, 1989).

It has been hypothesized that *modori* occurred when the formation of stable and temporary cross-links among proteins exceeded that optimal for *kamaboko* (thermally irreversible surimi gels) forming ability, and also that it was caused by the hydrolysis of protein molecules due to fish muscle proteinases (Takagi, 1973). Niwa (1992) described three mechanisms responsible for *modori*: proteolytic degradation due to enzymes; thermal coagulation of myofibrillar proteins during heating and involvement of specific non-enzymic proteins. Enzymatic degradation of the gel with breakdown of myosin heavy chain (MHC) has been shown to depend on species and season. For the same species there were different types of heat activated proteolytic enzymes of myofibrillar and/or sarcoplasmic origin that produced *modori* at different temperatures (Itoh et al., 1995). Another type of *modori*-inducing enzymes was involved in the degradation of fish species infested with parasites of myxosporidia spp (Niwa, 1992). Hydrophobic interactions and disulfide bridges have been found to increase among myofibrillar proteins at *modori* temperatures (Sano, 1988). Such increase was reported in sardine surimi pastes set at 60°C (Careche et al., 1995). The activating mechanism in the myofibrillar protein network of non enzymatic proteins that induce *modori* is not understood. It was hypothesized to be due to a non-enzymatic interaction between such proteins and myofibrillar proteins (Iwata et al., 1977).

The quality of directly cooked gels is in most cases poorer than those with prior setting, but *modori* does not occur. The lower quality of such directly cooked gels is thought to be due to rapid formation of disulfide and hydrophobic protein-protein bonds in the absence of the conditions required for the proteins to orient to form a network. Protein coagulation becomes more general than in set gels (Niwa, 1985)

In sardine surimi, the *modori* effect is mainly attributed to heat denaturation of myofibrillar proteins (Toyohara and Shimizu, 1988). Tsukamasa and Shimizu (1991) described two types of proteinase-independent *modori* in sardine meat, according to their intensity when raising the temperature. However, no evidence has been reported for involvement of proteinases in *modori* breakdown of sardine gels (Suzuki, 1981) or for rupture of the myosin heavy chain (Tsukamasa and Simizu, 1989; Careche et al., 1995).

In previous studies, thermal scanning rigidity monitoring of surimi sardine paste indicated a zone of minimum rigidity values (between 46°C and 50°C) related to a heat-induced change. This could be responsible for the occurrence of *modori* at higher temperatures. When setting at 40°C was prolonged, the gel strength and texture profile analyses of the set gels decreased, indicating set and cooked gels with poorer texture characteristics (Alvarez and Tejada, 1997). In structural terms this was observed as a modification, in which areas of disordered aggregation and lateral compacting occurred, while at lower temperatures fiber-like structures were formed (Alvarez et al., 1999).

Our objective was to determine how setting time and temperature influenced the development of *modori* in final sardine gels and corresponding changes in texture and microstructure of the gels.

MATERIALS & METHODS

Materials

Frozen sardine (*Sardina pilchardus*) surimi (SA grade), prepared in one batch for this study by SCOMA (Lorient, France), was air-freighted on solid CO_2 to the laboratory, cut into blocks, vacuum-packed (10.66 kPa) in bags (Cryovac BB-1, Duncan, SC; oxygen permeability at 23°C: 60 $cm^3/24h/m^2/at$) and stored at –20±1°C for 1 mo. Cryoprotectants added to the surimi were 4% sucrose, 4% sorbitol and 0.3% Na tripolyphosphate. One 10 kg block was used. Crude protein was measured by Kjeldahl method (AOAC, 1984), crude fat by Knudsenet al. (1985) and moisture and ash as recommended by AOAC (1984).

Preparation of samples

Surimi was tempered for about 2 h at 20±2°C until it reached –5±1°C, then ground for 1 min (Stephan UM12 refrigerated vacuum cutter mixing machine [Stephan u Söhne GmbH & Co., Hameln, Germany], 10 kPa, coolant temperature –2°C). The surimi was chopped for 1 min at high speed (setting 1). Salt was added (NaCl, 3% of surimi, equivalent to 2.44% in the final gel). Ice flakes (as necessary to adjust moisture content to 78%) were added and the mixture stirred slowly (setting 2) for 5 min. The surimi sol was kept at <10°C at all times. The sols were heated in stainless steel cylinders (30 mm i.d., 30 mm ht) with screw-on tops and bottoms with

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Teflon sealing rings, taking special care to ensure that sols were well packed and free of air bubbles. Different heat treatments were used: sols were heat-set at 50°C or 60°C for 30 or 60 min in a water bath (Julabo F10, Labortechnik GmbH, Seelbach, Germany) (heat-set treatment). Other sols were heat-set in the stated conditions then cooked at 90°C for 30 or 60 min (heat-set and cook treatment) using a saturated steam oven (Rational Combi-Master CM6, Grossküchentechnik GmbH, Landsberg Lech, Germany). Gels were also prepared by heating the sols directly at 90°C for 15, 30 or 60 min using the saturated steam oven (direct cook treatment). After treatment, the gels were cooled with ice water and stored at 4±1°C for 24 h before analysis.

Rheological analyses

Folding test (FT) was determined on gel slices (3 mm ht, 30 mm dia, 20±1°C) by the method of Tanikawa et al. (1985). The maximum score (FT=5) indicated no cracks were observed when the slice was folded twice without breaking. The minimum score (FT=1) was assigned if the slice broke into fragments when folded in half. According to Kinoshita et al. (1990), this test indicates that *modori* has occurred when the score is 1.

A **penetration test** was performed only in those gels with FT values >1. Samples (30 mm ht, 30 mm dia, 20±1°C) were measured using a cylindrical stainless-steel spherical probe (dia 5 mm) attached to a 100 N load cell connected to an Instron Universal Testing Machine model 4501 (Instron Engineering Corporation, Canton, MA) as described by Alvarez and Tejada (1997). Gel strength (GS) (N.mm) was determined as the product of yield deformation (YD) (mm) and yield strength (YS) (N) measured at the point of gel breakage.

Texture profile analysis (TPA) was performed as described by Bourne (1978) only on those gels with FT values >1. The gel sample (30 mm ht 30 mm dia, 20±1°C) were axially compressed to 30% of original height by a cylindrical plunger (diam 36 mm) (Instron Model 4501). Force-time deformation curves were derived with a 5 kN load cell applied at a crosshead speed of 50 $mm.min^{-1}$. Hardness (N), springiness (mm), cohesiveness(dimensionless), gumminess (N) and adhesiveness (g.cm) were calculated as in Alvarez and Tejada (1997).

Water-holding capacity (WHC) was determined in those gels with FT values >1, following the method described by Roussel and Cheftel (1990) as modified by Alvarez et al. (1992). Sample (2 g cut into cubes of about 3 mm) was centrifuged (Sorvall RT6000B Du Pont Co., Wilmington, DE) for 15 min at 1000 × *g* and the exudate collected on Whatman filter paper. WHC was expressed as the percentage of water retained with respect to the water present in the gel prior to centrifuging. Rheological and WHC determinations were performed at least five times.

Scanning electron microscopy (SEM). Small gel blocks (2 mm cubes) were cut from the center of the cylindrical gel specimen, fixed with 2% glutaraldehyde in a phosphate buffer (pH 7.2) then dehydrated in solutions of acetone of increasing strength, and critical-point dried (Balzers mod. CPD 030, Balzers Process Systems, Liechtenstein) using CO_2 as transition fluid. The prepared samples were mounted on copper specimen holders, sputter-coated with gold (Balzers mod. SCD 004) and examined on a JSM 6400 (JEOL, Ltd., Akishima, Japan) scanning electron microscope operated at up to 15 kV. Replications were performed at different magnifications (×500, ×6000, ×10000 and ×20000).

Statistical analysis

Two-way analyses of variance were performed using the program Statgraphics STSC Inc (Rockville, MD). The differences of means between pairs were resolved by LSD test to obtain the confidence intervals. Significance was defined at $P<0.05$.

RESULTS

THE PROXIMATE COMPOSITION OF THE SURIMI WAS: PROTEIN 13.13%; crude fat 3.05%; moisture 75.82%; ash 0.63%. The pH was 6.6.

Table 1— Folding test values of sardine surimi gels made under different conditions[a]

	Heat-set (S)		Heat-set and /or cooked at 90°C (K)		
Temperature	30 min	60 min	15 min	30 min	60 min
50 °C	3	4	–	1	1
60 °C	2	2	–	1	1
90 °C			4	5	5

[a]Score (1 indicate modori effect, unacceptable gels; Score 2-5 indicates increasing acceptability of gels.—No attempt was made to produce gels in these conditions

Folding test

The FT scores were compared (Table 1) for heat-set (50°C or 60°C), heat-set and cooked (50°C or 60°C + 90°C) and directly cooked gels (90°C) obtained at different setting times (30 or 60 min) and/or cooking times (15, 30 or 60 min). In the heat set gels, when raised from 50°C to 60°C the folding test score was lower. When setting was prolonged from 30 to 60 min, set gel scores increased at 50°C but did not change at 60°C. When heat-set gels were cooked at 90°C, they scored 1 in the FT irrespective of setting time and temperature or cooking time which indicates that *modori* had occurred (Kinoshita et al., 1990). Because the gel was destroyed, no other rheological measurements could be made on those samples. Directly cooked gels attained increasing FT scores when cooking at 90°C was prolonged and consistently scored the maximum (5) after 30 min cooking.

Rheological analyses

Penetration test. GS (Fig. 1), YD (Fig. 2) and YS (Fig. 3) for gels scoring FT>1, that is heat-set gels (not subsequently cooked at 90°C) (S) and directly cooked gels (K)were compared and data analyzed (Table 2). In heat-set gels, GS decreased when setting temperature increased. Prolonging of setting negatively affected the GS of gels set at 60°C, while there was an increase in gels set at 50°C. The same tendency was found for YD and YS when the temperature was raised or setting prolonged, although greatest changes were due to a

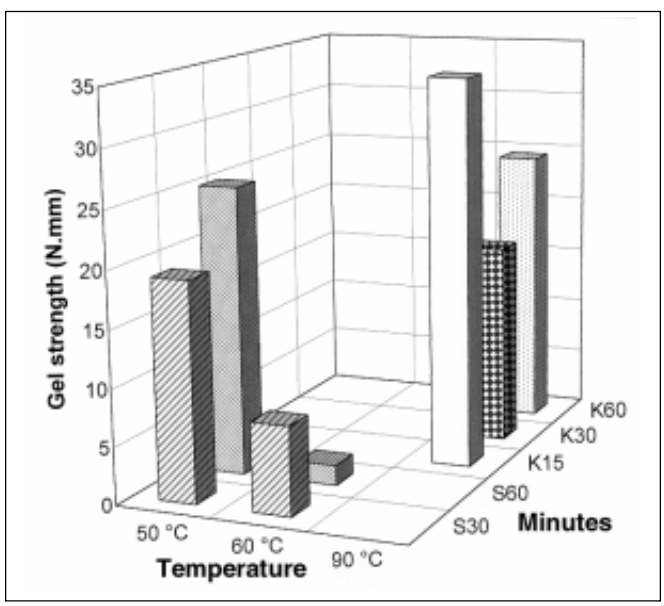

Fig 1—Gel strength (N mm) of sardine surimi gels set (S) at different temperatures (50°C and 60°C) for different times (30 or 60 min) or directly cooked (K) at 90°C for different times (15, 30 or 60 min).

Table 2—Analyses of variance for the data*

	Temp °C	Heat-set (S) 30 min	Heat-set (S) 60 min	Heat-set and/or cooked at 90°C (K) 15 min	Heat-set and/or cooked at 90°C (K) 30 min	Heat-set and/or cooked at 90°C (K) 60 min
Gel strength	50	1a	2a	–	—	—
	60	1b	2b	–	—	—
	90			1	2	3
Yield deformation	50	1a	2a	–	—	—
	60	1b	2b	–	—	—
	90			1	2	3
Yield strength	50	1a	2a	–	—	—
	60	1b	2b	–	—	—
	90			1	2	3
Water-holding capacity	50	1a	2a	–	—	—
	60	1b	2b	–	—	—
	90			1	2	1

*Different numbers in the same line, indicate significant differences at different setting or cooking times. Different letters in the same column, within each parameter, indicate significant differences at different temperatures (P<0.05). – No attempt was made to produce gels in these conditions. — No measurement was performed as folding test was 1.

Table 3—Texture profile analyses (TPA) of sardine gels heat set or directly cooked at different temperature and time conditions*

TPA	Temp °C	Heat-set 30 min	Heat-set 60 min	Heat-set and/or cooked at 90°C 15 min	Heat-set and/or cooked at 90°C 30 min	Heat-set and/or cooked at 90°C 60 min
Hardness (N)	50	7.38^{1a}	6.82^{2a}	–	—	—
	60	6.51^{1b}	6.14^{1b}	–	—	—
	90			4.5^{1}	7.61^{2}	5.48^{3}
Springiness (mm)	50	7.22^{1a}	6.79^{2a}	–	—	—
	60	7.27^{1a}	7.65^{1b}	–	—	—
	90			7.34^{1}	7.09^{2}	6.38^{3}
Cohesiveness	50	0.70^{1a}	0.68^{1a}	–	—	—
	60	0.66^{1a}	0.56^{2b}	–	—	—
	90			0.75^{1}	0.69^{2}	0.72^{3}
Gumminess (N)	50	5.21^{1a}	4.66^{2a}	–	—	—
	60	4.22^{1b}	3.60^{2b}	–	—	—
	90			3.42^{1}	5.29^{2}	3.96^{1}
Adhesiveness (g.cm)	50	122.0^{1a}	112.0^{1a}	–	—	—
	60	121.1^{1a}	143.6^{2b}	–	—	—
	90			51.56^{1}	111.4^{2}	65.7^{3}

*Different numbers in the same line, within each type of gel (set or directly cooked) indicate significant differences at different setting times. Different letters in the same column, within each TPA parameter, indicate significant differences at different temperatures. (P<0.05). Compression: 30%. – No attempt was made to produce gels in these conditions. — No measurement was performed as folding test was 1.

higher relative decrease of YS. Prolonging direct cooking produced minimum GS in gels cooked for 30 min. Maximum YS and YD values occurred at 15 min, although that gel did not attain the maximum FT score.

Hardness. In heat-set gels, there was a decrease in hardness (Table 3) when setting at 50°C was prolonged or the setting temperature was raised to 60°C. Prolonged setting at 60°C produced no differences in hardness. In directly cooked gels hardness reached maximum in 30 min. Gels cooked 15 min gave the lowest values, which were even lower than those for heat-set gels.

Springiness. As with hardness, springiness decreased (Table 3) when setting at 50°C was prolonged. However, springiness was unaffected by prolonged setting at 60°C. In directly cooked gels the highest values coincided with shortest cooking times and values decreased beyond that time. Springiness values were within the same range for heat-set and directly cooked gels.

Cohesiveness and Gumminess. In heat-set gels, cohesiveness only decreased in samples set at 60°C for 60 min. In directly cooked gels maximum values were recorded in samples cooked 15 min; values decreased and reached minimum when cooking was prolonged to 30 min. In heat-set gels gumminess decreased when setting time or temperature increased, so that maximum values were recorded at 50°C and 30 min. In directly cooked gels gumminess reached maximum in samples cooked 30 min; gumminess values were lower in samples cooked for 15 or 60 min and similar to each other.

Adhesiveness. Adhesiveness was only affected by time/temper-

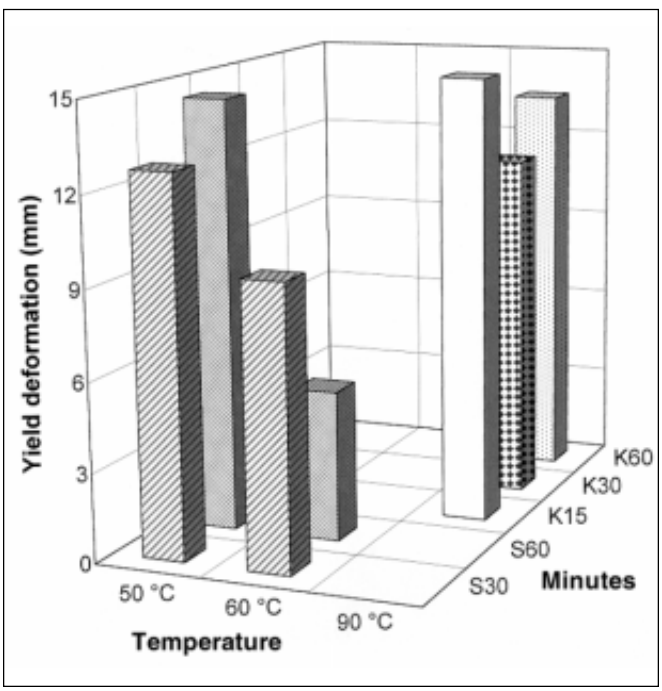

Fig 2—Yield deformation (mm) of sardine surimi gels set (S) at different temperatures (50°C and 60°C) for different times (30 or 60 min) or directly cooked (K) at 90°C for different times (15, 30 or 60 min).

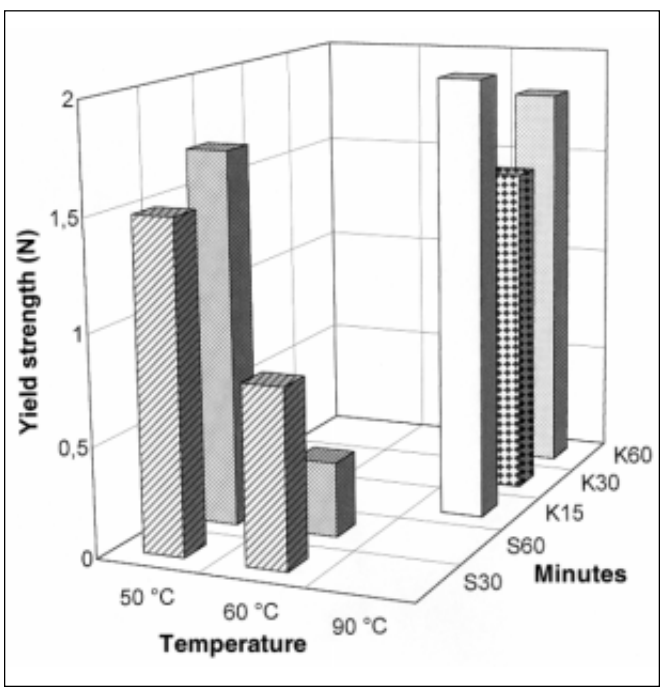

Fig 3—Yield strength (N) of sardine surimi gels set (S) at different temperatures (50°C and 60°C) for different times (30 or 60 min) or directly cooked (K) at 90°C for different times (15, 30 or 60 min).

ature in gels set for 60 min at 60°C. In directly cooked gels the highest values were measured in samples cooked for 30 min. These values were in the range of heat-set gels, while they were 50% lower in samples cooked for 15 min.

Water-holding capacity. In heat-set gels, the maximum WHC was recorded for samples set at 50°C with setting prolonged to 60 min Fig. 4, Table 2). At 60°C, however, prolonged setting caused a considerable decrease in WHC. In direct cooked gels there were smaller time-dependent differences upon cooking, although the WHC of gels cooked for 30 min at 90°C was lower.

Microstructure

At low magnification (×500), irrespective of thermal treatment, the structure of heat-set gels was rather less continuous and grainy (results not shown) than had been reported for sardine surimi gels made at ≤40 °C, which was more compact (Alvarez et al., 1999). At higher magnification, gels made by setting for 30 min at 60°C (Fig. 5) exhibited an aggregated structure consisting of clusters of globules joined without definite orientation forming large masses. These were separated by interconnecting grooves that formed cavities and cracks and seemed to constitute a network of inter-protein spaces. The gel made by setting for 60 min at 60°C (Fig. 6) also exhibited globules and grooves but had some fibrillar structures with a globular surface.

In heat-set (60°C, 30 min) and cooked (90°C 30 min) gels (Fig. 7), where *modori* was clearly indicated by the folding test, there was a globular aggregated structure resembling that observed in the heat-set gel, although the globules were less clearly outlined. When gels set at 60°C for 60 min were cooked (90°C 30 min) (Fig. 8), the fiber-like structure observed in the set gels was lost, and a globular aggregated structure was formed. Directly cooked gels (90°C, 30 min) exhibited a morphology consisting largely of globular aggregates accompanied by some structures of fibrillar appearance which formed links (Fig. 9).

DISCUSSION

THE RHEOLOGICAL PARAMETERS AND WHC OF SET GELS MADE at 50°C (30 or 60 min) were similar to those reported for the same batch of sardine surimi set at 40°C for 60 min (Alvarez and Tejada, 1997). However, while that sample produced set and cooked (*kamaboko*) gels with acceptable rheological characteristics, gels set at 50°C fell apart when cooked at 90°C although when setting was prolonged, the rheological characteristics of the gels set at 50°C were better than when setting occurred at 40°C. Prolonged setting at 60°C caused degradation of the gel formed in 30 min, and in both cases the gel fell apart when cooked at 90°C.

Under SEM, the gels set for 30 min exhibited aggregation with a globular surface, lacking the fibrillar structures typical of gels with good texture characteristics described for sardine surimi gels (Alvarez et al., 1999). Although some fibrillar structures were formed when setting was prolonged, they disappeared when gels were cooked at 90°C. Macroscopically, they appeared as loose granulated masses lack-

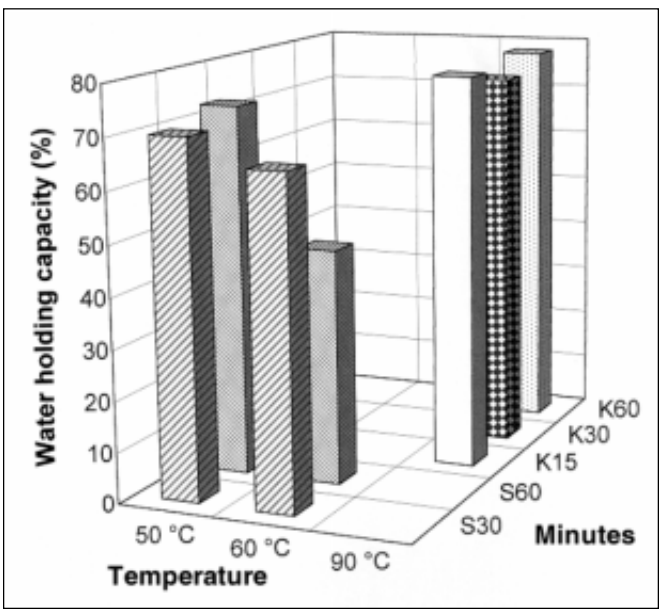

Fig 4—Water-holding capacity (% retained/total water) of sardine surimi gels set (S) at different temperatures (50°C and 60°C) for different times (30 or 60 min) or directly cooked (K) at 90°C for different times (15, 30 or 60 min).

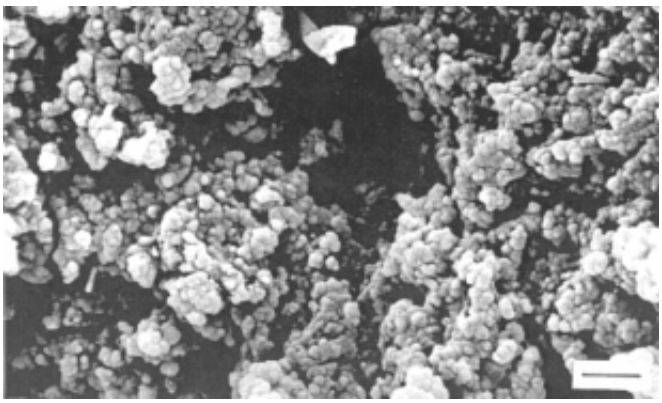

Fig 5—Ultrastructure of gels set at 60°C for 30 min. *Bar* = 1 µm.

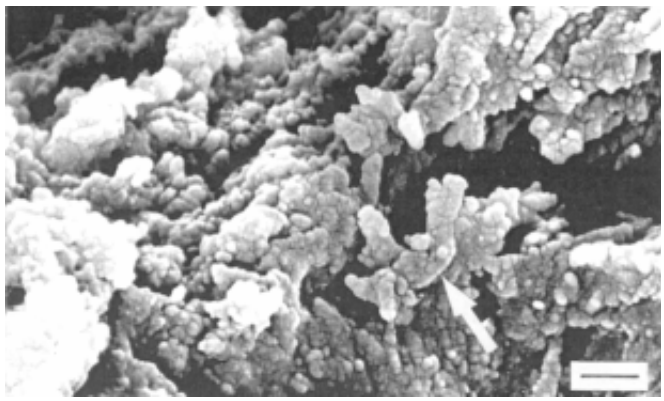

Fig 6—Ultrastructure of gels set at 60°C for 60 min. Arrow: fiber-like structure. *Bar* = 1 µm.

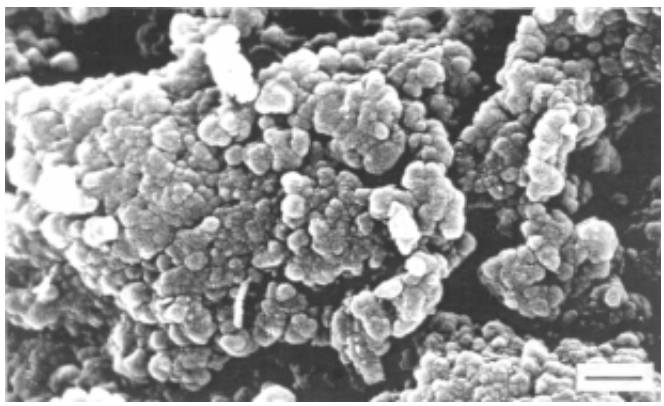

Fig 7—Ultrastructure of gels set at 60°C for 30 min and cooked at 90°C for 30min. *Bar* = 1 µm.

ing the gel appearance that are described to be typical of *modori*.

It has been reported (Careche et al., 1995) that in set gels at 60°C and set and cooked gels, both types of networks were more soluble in solutions that preferentially ruptured hydrophobic interactions and disulfide bridges than when setting took place at ≤40°C. A study of the major proteins extracted from the networks with different agents showed that actin became fixed in a different way at temperatures above or below 40°C. At >40°C the actin was only extracted from set gels when agents capable of cleaving disulfide or hydrophobic bonds were used. At ≤40°C actin was extracted when electrostatic or hydrogen bonds were ruptured. Moreover, myosin was released by solutions with mercaptoethanol in gels where setting had taken place at 60°C, whereas no myosin was extracted when setting took place at lower temperatures.

Protein-protein bonds of varying heat stability are formed at different rates among the major proteins in set gels made in different time/temperature conditions. This could explain the possibility of formation of different types of networks during cooking at higher temperatures, as suggested (Alvarez et al., 1999). In sardine surimi *modori* may be due to the effect of high temperature during setting causing large-scale protein-protein interactions consisting largely of hydrophobic interactions and disulfide bonds (Careche et al., 1995). This would prevent further reorganization of proteins to form the final network in the gel. Thus, when gels set at *modori* temperature were cooked at 90°C, the framework of the network required for acceptable texture characteristics would not have been formed. The proteins would coagulate producing globular formations aggregated in an amorphous structure, instead of forming superior fibrillar structures as in sardine surimi gels with good texture characteristics (Alvarez et al., 1999). However, where sols were directly cooked without prior setting, although the ordered fibrillar structure was lacking, some fibrillar structures connecting globules were observed. This suggests that in directly cooked gels, although the orientation of the proteins in the network was restricted, other heat-stable protein-protein bonds were formed to prevent breakdown of the gel. Differences in texture values for directly cooked gels, suggested that the rate of intermolecular disulfide bonds and hydrophobic interactions involved in gel formation at cooking temperature had been modified after prolonged cooking.

The *modori* occurring in sardine surimi may be due basically to the effects of temperature on the proteins, as has been suggested (Toyohara and Shimizu, 1988). Such temperature effect would cause the major proteins in the gel to have a different rate of formation of bonds among major proteins at *modori* temperatures than they would at lower temperatures. There would be more involvement of hydrophobic interactions and disulfide bonds in the gel set at *modori* temperatures. This would prevent subsequent reorganization at higher temperatures, giving rise to an amorphous morphology in cooked gels and loss of texture characteristics.

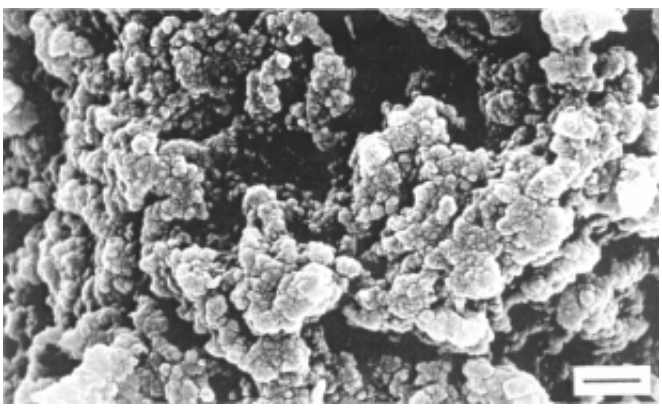

Fig 8—Ultrastructure of gels set at 60°C for 30 min and cooked at 90°C for 60 min. *Bar* = 1 µm.

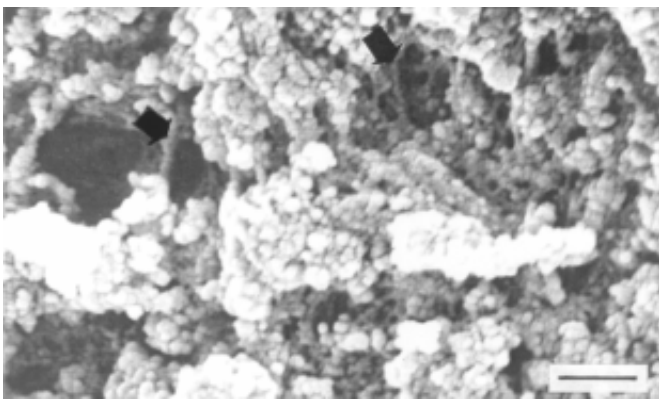

Fig 9—Ultrastructure of gels directly cooked at 90°C for 30 min. Arrows: fibrillar structure. *Bar* = 1 µm.

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