# Rheological Properties of Gels Made from High- and Low-Quality Sardine (*Sardina pilchardus*) Mince with Added Nonmuscle Proteins

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The effect of added nonmuscle proteins on minced sardine gels is highly dependent on mince quality. In high functional quality mince, any protein addition interfered with gelation of muscle proteins. In low-quality mince, however, egg white or soy protein considerably improved gel strength. Casein reduced all of the rheological parameters studied. Regarding salt concentration, high-quality mince did not gel with low salt (1.5%), whereas low-quality mince gelled with both 1.5% and 2.5% salt concentrations. Gels made with 1.5% NaCl exhibited lower gel strength, elasticity, and cohesiveness than gels with the higher salt concentration.

Keywords: Sardine mince; gelation; rheological properties; expressible moisture

## INTRODUCTION

A large variety of seafood analogues have been developed by modifying the functional and textural properties of surimi proteins through the addition of biopolymers with gel-forming or water-holding capacities (Okada, 1963; Ikeuchi, 1964; Akahane et al., 1984; Iso et al., 1985; Lee and Kim, 1985; Kim and Lee, 1987; Niwa et al., 1988; Chung and Lee, 1988, 1990). For some products in which neither color nor flavor is an impediment and no great gel-forming capacity is required, unrefined muscle mince can be used with only cursory washing. The base product thus obtained gives greater yield than surimi and is therefore more economical.

The effect of adding nonmuscle proteins to fish gels depends on the quality of the fish protein or the surimi. As a rule, if a surimi or fish mince is of high gel strength, the gel tends to lose cohesiveness, strength, and rubberiness when nonmuscle proteins are added (Westerly et al., 1980; Burgarella et al., 1985; Lee and Kim, 1986; Chung and Lee, 1990, 1991; Montero et al., 1992a; Alvarez, 1993). However, such an effect may actually improve sensory acceptability of a product which might otherwise be too rubbery and tough. In low gel strength surimi or fish mince, on the other hand, addition of some nonmuscle proteins considerably increases gel strength and other texture parameters (Montero et al., 1992a,b; Lee et al., 1992; Gnanasambandam and Zayas, 1992; Westerly et al., 1980; Yasumatsu et al., 1972).

Considering that, unlike surimi, minced sardine muscle does not always gel properly, this research was intended to improve gelation by addition of nonmuscle proteins, with a view to the fabrication of fish analogue products with a variety of textural characteristics. The present work examined the importance of functional quality of sardine mince for gel texture and the effect of adding a number of nonmuscle proteins (egg white, soy protein, casein, and wheat gluten), with two levels of salt (2.5% and 1.5%) in each case.

## MATERIALS AND METHODS

The fish used in these experiments were sardines of the species *Sardina pilchardus* (Walbaum), caught off Nantes (France) in two different seasons: October (S1) and June (S2).

Fish mince was prepared at the fishery, using the following procedure: 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), with constant stirring. 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 center reached -20 °C. The various lots were sent frozen to our laboratory, where they were stored at -80 °C in a Revco vertical freezer cabinet to minimize alteration during frozen storage for the duration of the experiment.

NaCl was supplied by Panreac, Montplet & Esteban S.A. (Riera de San Cugat, Barcelona, Spain). Atomized-dried egg white was supplied by Sanofi, S.A. (Barcelona, Spain). For soy protein, a soy isolate was used, supplied by Protein Technologies International (Gerona, Spain) under the product name PP 500 E. Atomized-dried sodium caseinate was supplied by La Pilarica, S.A. (Valencia, Spain.). Wheat gluten was supplied by Levantina Agrícola Industrial, S.A. (Barcelona, Spain), under the product name Vital L wheat gluten.

**Proximate Analysis and Protein Functionality.** To characterize the two washed mince types (S1 and S2), moisture, ashes, crude fat, and crude protein were determined by according to AOAC (1975) methods. Analyses do not show 4% added sorbitol as cryoprotectant. Results were averages of three determinations and expressed as percent of muscle mince. As protein functionality index, apparent viscosity (Borderías et al., 1985) and protein solubility (Ironside and Love, 1958) were determined.

**Homogenization of Muscle with Ingredients.** Washed sardine mince was semithawed and placed in a refrigerated vacuum homogenizer (Stephan Model UM5, Stephan u. Söhne GmbH & Co., Germany). Muscle was ground for 1 min (rotor angular velocity 3000 rpm). Sodium chloride was then added with sufficient crushed ice to give the required final gel moisture, and the mixture was homogenized for 5 min at 1500 rpm in vacuum conditions. One of the nonmuscle proteins was then added (2%) and the sample homogenized again for 5–7 min. Final moisture content was adjusted to 75% in samples with nonmuscle proteins.

**Heat Treatment.** The resulting batters were stuffed into stainless steel cylinders (i.d. 3 cm, height 3 cm) with hermetic screw-on cap and rubber gasket. Cylinders were first placed

in a water bath at 37 °C for 30 min and then moved to another bath at 90 °C for 50 min. Finished gels were kept overnight at 4 °C.

**Puncture Test.** This was performed on pieces of sample molded in the cylinders. Gels were removed and tempered to about 20 °C. The gel was axially penetrated to breaking point using an Instron Model 4501 texturometer (Instron Engineering Corp., Canton, MA): 5 mm diameter metal probe with rounded head; speed setting,10 mm/min; load cell, 100 N. *Gel strength* was determined by multiplying *breaking force* (N) by *breaking deformation* (mm). All determinations were performed at least in quadruplicate.

**Compression Tests.** Both texture profile and compression–relaxation tests are described by Bourne (1976).

(a) Texture Profile Analysis. Samples were placed on the flat plate of the Instron texturometer. Axial compression was applied by a cylindrical plunger (diameter 36 mm) adapted to a 5 kN load cell at a deformation rate of 50 mm/min. On the basis of previous trials to establish a compression limit that would ensure no cracking and recoverability of most samples, it was decided to compress samples to 50% of height. In the test, each sample was compressed twice running. The following parameters were determined: hardness (N), maximum height of first peak on first compression; cohesiveness ( $A_2/A_1$ ), ratio of second-compression to first-compression positive areas.

(b) Compression–Relaxation Test. Compression procedure was as in point a, except that sample was compressed once only for 1 min and the force exerted on the sample recorded. Percent relaxation was calculated as  $Y_T = 100 \times (F_0 - F_1)/F_0$ , where  $F_0$  is force registered at the onset of relaxation immediately after sample compression and  $F_1$  is force registered after 1 min of relaxation. Thus,  $(100 - Y_T)$  is taken as an index of elasticity and is expressed as percent elasticity of the gel.

All determinations were carried out at least in quadruplicate.

**Expressible Moisture.** The method used was a modification of the method of Roussel and Cheftel (1990). Chopped sample (1.5 g) was placed in a centrifuge tube along with a Gilson Pipetman pipet filter. A Sorvall RT60008 centrifuge (DuPont Co., Wilmington, DE) was used: 4000g, 10 min, ambient temperature. Expressible moisture (EM) was expressed as percent water retained per 100 g of water present in the gel prior to centrifuging. All determinations were carried out in quadruplicate.

**Statistical Analysis of Data.** One-way analysis of variance was carried out for the different samples, using the computer program Statgraphics (STSC Inc., Rockville, MD). The difference of means between pairs was resolved by means of confidence intervals using a least significant difference range test. Level of significance was set for  $p \le 0.05$ .

#### RESULTS AND DISCUSSION

**Proximate Analysis and Functional Properties** of the Minces. The two types of mince (S1 and S2) differed essentially in moisture and fat content. S2 contained significantly more fat (9.85  $\pm$  0.13%) and less moisture (72.00  $\pm$  0.06%) than S1 (4.32  $\pm$  0.34% fat,  $76.47 \pm 1.59\%$  moisture). Fat is an undesirable element in gel-making, as it interferes with formation of a protein matrix and hence hinders gelation. Crude protein contents of minces were  $14.74 \pm 0.02\%$  and  $13.95 \pm 0.01\%$  for S1 and S2, respectively. Ash was 0.61  $\pm$  0.01% for S1 and 0.68  $\pm$  0.01% for S2. S1 exhibited considerably higher protein solubility ( $62.61 \pm 1.15\%$ ) and apparent viscosity (3140  $\pm$  34.64 cP) than did S2  $(49.75 \pm 0.01\%$  and  $2323 \pm 35.11$  cP). Mince S1 may therefore be considered of high functional quality and S2 of low functional quality.

**Rheological Properties and Expressible Moisture of Gels.** The concentration of salt used to solubilize myofibrillar proteins during muscle homogenization had a pronounced effect on the rheological characteristics of the various gels. With both mince types (S1 and S2), samples containing 2.5% salt scored full marks (5) in the folding test. With 1.5% salt, however, S1 sample did not gel, scoring "1" in the folding test. (This is the reason no penetration or compression tests were performed on S1 gels with low salt.) We do not find a clear explanation for this fact. Possibly 1.5% NaCl is not enough to solubilize the actomyosin and so ensure proper gelling. In the case of S2, because it has much more fat, it may be that a gel emulsion is formed rather than a real gel.

Figure 1 shows the puncture test (gel strength, breaking force, and breaking deformation) and compression test (hardness, elasticity, and cohesiveness) of gels made from the two minces with both salt concentrations. With 2.5% salt, S1 exhibited significantly higher gel strength than S2. This was due primarily to much greater breaking force, but breaking deformation was also significantly higher. In S2 samples with 1.5% NaCl, although gels scored full marks in the folding test, gel strength was lower, largely because breaking force was significantly lower. Breaking deformation did not differ significantly from that of gels with 2.5% salt (eliminar). Values of expressible moisture were significantly lower in gels made from either mince with 1.5% salt than with 2.5% salt (Tables 1 and 2). There were no differences in this respect between minces S1 and S2.

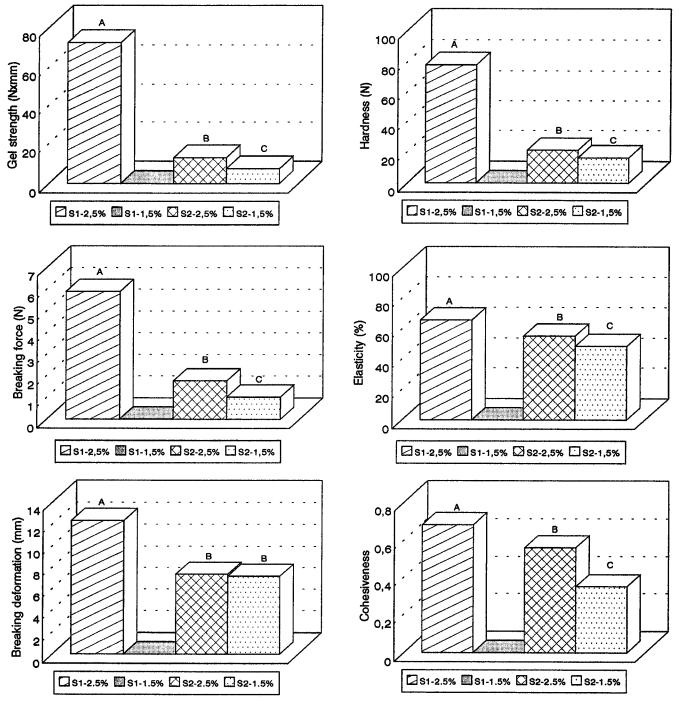
With 2.5% salt, S1 gels scored higher than S2 gels in compression tests, particularly on hardness but also on elasticity and cohesiveness. Hamann and MacDonald (1992) noted differences in gel cohesiveness and hardness depending on freshness, and hence quality, of the fish mince used. The gel made from mince S2 with the lower salt concentration exhibited significantly lower hardness, elasticity, and cohesiveness than the equivalent gel with higher salt. Homogenization with a higher salt concentration raises gel strength by increasing the amount of soluble actomyosin, causing myofibrillar protein to swell and thus increasing the intrinsic friction in the system (Rizvi, 1981; Hamm, 1986).

Effect of Nonmuscle Proteins on Rheological Properties and Expressible Moisture of Gels. The effect of adding a number of nonmuscle proteins (egg white, soy protein, casein, and gluten) to both mince types while maintaining standard moisture and heat treatment was analyzed with the two experimental salt concentrations.

(a) High Functional Quality Mince (S1). Puncture and compresion tests of gels made from mince S1 with 1.5% and 2.5% NaCl and each of the nonmuscle proteins are shown in Table 1. With 1.5% NaCl, addition of egg white or soy protein did not significantly improve the texture of gels made from mince S1: the folding test score (2) was only one point higher than the control score. Gels containing casein or gluten, on the other hand, scored top marks (5) in the folding test. These further exhibited higher gel strength and breaking force than gels with egg white or soy. Gels with casein or gluten did not differ ( $P \le 0.05$ ) between high- and lowsalt gels in terms of gel strength and breaking force. With casein, reduced salt content brought a slight but significant decrease in breaking deformation.

Low-salt gels with egg white or soy protein were too fragile to run a compression test. Gels with casein and gluten did not differ significantly from one another with regard to hardness or cohesiveness, although the casein-containing gel was slightly less elastic than the gluten-containing gel. Low-salt gels were a little harder but less elastic and cohesive than high-salt gels ( $P \le 0.05$ ).

With 2.5% NaCl, all gels scored "5" in the folding test but registered lower gel strength than the control (S1



**Figure 1.** Gel strength, breaking force, breaking deformation, hardness, elasticity, and cohesiveness of sardine gels made from minces S1 and S2, with 2.5% and 1.5% NaCl, 75% moisture, and heat treatment 37 °C 30 min/90 °C 50 min. Different letters in the same graph indicate significant differences ( $P \le 0.05$ ) among gels.

without 2.5% NaCl), as a result of diminished breaking force and breaking deformation. Gel strengths of samples with egg white and soy protein were similar and lower ( $P \le 0.05$ ) than in samples with casein or gluten.

In compression tests, addition of nonmuscle protein did not appear to alter hardness or elasticity of gels except for the one with soy protein, for which values were lower than control ( $P \leq 0.05$ ). Cohesiveness, however, was significantly lower than that of the control for all four proteins. According to Hamann and Mac-Donald (1992), cohesiveness and hardness can vary independently. Lanier (1986) suggested that cohesiveness was the most sensitive indicator of quality or functionality of surimi proteins. In the present case, the puncture test appeared to be the most sensitive rheological test. Many authors dealing with surimi gels have reported that adding a nonmuscle protein to a high-quality surimi weakens texture (Westerly et al., 1980; Chung and Lee, 1990, 1991), tending to reduce cohesiveness and gel strength (Lee and Kim, 1986). This is probably because these nonmuscle proteins interfere with gel formation by preventing actomyosin crosslinking, in which retained water is more loosely bound (Okada, 1964; Shimizu and Nishioka, 1974; Chung and Lee, 1991). Burgarella et al. (1985) suggested that loss of strength of gels with ingredients could be due to "dilution" of myofibrillar protein, the most active agent in gel formation.

Expressible moisture (EM) of gels with nonmuscle proteins and both salt levels is shown in Table 1. EM of low-salt gels was generally significantly lower than in corresponding high-salt gels; the sole exception were

Table 1. Rheological Parameters and Expressible Moisture of Sardine Gels Containing Nonmuscle Protein, Made fromS1 Muscle, with 1.5% NaCl or 2.5% NaCl, 75% Moisture, and Heat Treatment 37 °C 30 min/90 °C 50 min <sup>a</sup>

treatment	gel strength (N × mm)	breaking force (N)	breaking deformation (mm)	hardness (N)	elasticity (%)	cohesiveness	expressible moisture (%)
1.5% NaCl							
control							$75.85_{a}$
EW	16.73 <sub>a</sub>	$2.62_{a}$	6.41 <sub>a</sub>				$67.88_{b}$
soy	21.35 <sub>ab</sub>	$2.62_{a}$	$8.25_{b}$				$71.02_{b}$
Cas	$40.15_{cd}$	$4.34_{b}$	8.72 <sub>bc</sub>	91.33 <sub>a</sub>	58.60 <sub>a</sub>	$0.57_{a}$	87.18 <sub>c</sub>
Glu	$43.95_{c}$	$4.76_{b}$	$9.22_{bcd}$	89.72 <sub>a</sub>	61.89 <sub>b</sub>	$0.57_{a}$	$80.30_{\rm d}$
2.5% NaCl							
control	$72.36_{e}$	5.88 <sub>c</sub>	$12.88_{e}$	$77.88_{bc}$	$65.57_{c}$	$0.68_{b}$	87.77 <sub>c</sub>
EW	$30.92_{df}$	$3.79_{de}$	$8.53_{b}$	77.47 <sub>b</sub>	$65.22_{c}$	0.66 <sub>c</sub>	$81.29_{d}$
soy	$30.17_{bf}$	$3.55_{d}$	$8.48_{b}$	$67.75_{d}$	55.81 <sub>d</sub>	$0.65_{c}$	79.52 <sub>ad</sub>
Cas	43.03 <sub>d</sub>	$4.16_{be}$	$10.45_{d}$	75.71b	62.06b	$0.62_{\rm d}$	86.25 <sub>c</sub>
Glu	$45.75_{d}$	$4.53_b$	10.05 <sub>cd</sub>	81.89 <sub>c</sub>	$65.18_{c}$	$0.62_{d}$	86.95 <sub>c</sub>

<sup>*a*</sup> EW, egg white; soy, soy protein; Cas, casein; Glu, gluten. Different letters in the same column indicate significant differences ( $P \le 0.05$ ).

Table 2. Rheological Parameters and Expressible Moisture of Sardine Gels Containing Nonmuscle Protein, Made fromS2 Muscle, with 1.5% NaCl or 2.5% NaCl, 75% Moisture, and Heat Treatment 37 °C 30 min/90 °C 50 min <sup>a</sup>

treatment	gel strength (N × mm)	breaking force (N)	breaking deformation (mm)	hardness (N)	elasticity (%)	cohesiveness	expressible moisture (%)
1.5% NaCl							
control	7.35 <sub>ab</sub>	1.02 <sub>ab</sub>	7.16 <sub>ab</sub>	16.18 <sub>a</sub>	$48.45_{a}$	$0.35_{a}$	$77.48_{a}$
EW	19.86 <sub>c</sub>	$2.34_{c}$	8.42 <sub>c</sub>	38.09 <sub>b</sub>	$59.46_{b}$	0.63 <sub>b</sub>	79.58 <sub>ab</sub>
soy	$30.40_{d}$	$3.87_{de}$	7.85 <sub>bc</sub>	$32.80_{c}$	$53.35_{cd}$	0.61 <sub>c</sub>	78.22 <sub>ab</sub>
Cas	$4.29_{b}$	0.76 <sub>a</sub>	$5.63_{d}$	$13.53_{a}$	$44.32_{e}$	$0.43_{d}$	72.70 <sub>c</sub>
Glu	8.34 <sub>ab</sub>	$1.29_{b}$	$6.44_{ad}$	$26.34_{d}$	55.08 <sub>c</sub>	$0.61_{bc}$	75.98 <sub>ac</sub>
2.5% NaCl							
control	$12.92_{a}$	$1.76_{\rm f}$	7.34 <sub>ab</sub>	$21.00_{e}$	55.14 <sub>c</sub>	$0.56_{e}$	$88.45_{d}$
EW	$43.44_{e}$	$3.61_{d}$	$12.00_{e}$	$40.53_{b}$	$66.60_{\mathrm{f}}$	$0.72_{\mathrm{f}}$	$88.80_{d}$
soy	$43.67_{e}$	$4.12_{e}$	$10.37_{f}$	$30.35_{c}$	$62.44_{ m f}$	0.65g	86.88 <sub>de</sub>
Cas	$4.66_{b}$	$0.67_{a}$	6.90 <sub>a</sub>	$15.24_{a}$	51.82 <sub>d</sub>	0.51 <sup>o</sup> <sub>h</sub>	$82.34_{be}$
Glu	$11.78_{a}$	$1.29_{b}$	8.62 <sub>c</sub>	$23.90_{\mathrm{de}}$	59.95 <sub>b</sub>	0.66g	82.76 <sub>be</sub>

<sup>*a*</sup> EW, egg white; soy, soy protein; Cas, casein; Glu, gluten. Different letters in the same column indicate significant differences ( $P \le 0.05$ ).

gels containing casein, in which salt concentration made no significant difference. With 2.5% NaCl, EM was similar between gels with egg white and soy and significantly lower than the control (S1 mince with 2.5% NaCl). On the other hand, EM of high-salt gels with casein and gluten was not significantly different from the control. In high-quality minces, egg white and soy protein compete for water immediately upon being added (Chung and Lee, 1991; Lee et al., 1992), thus interfering in the formation of an actomyosin network. This does not occur when the added proteins possess poorer gel-forming capacity (casein and wheat gluten). These findings are consistent with the present texture assays.

(b) Low Functional Quality Mince (S2). Low-salt gels made from low functional mince all scored lower than the control in the folding test. Egg white and soy dropped from "5" to "4" and casein and gluten to "2". Whiting (1984a,b) also reported little success in making low-salt gels with added gluten. Gel with soy protein was the one that showed significantly improved gel strength, largely because its breaking force was greater than the latter's ( $P \le 0.05$ ) (Table 2). When soy and egg white were added, gel strength was significantly lower than in equivalent high-salt gels. With casein or gluten, gel strength was similar to that of the control. In low-salt gels with casein or gluten there were no significant differences from high-salt gels with regard to gel strength or breaking force, but breaking deformation was significantly lower in low-salt gels. This may explain the pronounced drop in folding test scores in gel with gluten.

Gel with egg white exhibited the most significant increase in hardness and elasticity, followed by gels with soy protein and gluten, all of them higher in these rheological properties than control (Table 2). With casein, however, hardness was equal to control and elasticity lower. Gels with egg white, soy, and gluten exhibited similar cohesiveness and significantly more than gel with casein. The latter in turn showed significantly greater cohesiveness than control.

Gels made by adding egg white or soy protein to mince S2 homogenized with 2.5% NaCl scored full marks (5) in the folding text, as did the control (S2 without 2.5% NaCl). Adding casein or gluten, on the other hand, reduced scores to "2" and "4", respectively. Gel strength was similar in gels with egg white and soy protein ( $P \leq$ 0.05) and much higher than control (Table 2). However, gel with soy protein exhibited significantly higher breaking force and lower breaking deformation than gel with egg white. Many authors reported that with lowquality surimi or fish mince, gel strength can be considerably improved by adding a nonmuscle protein such as egg white (Westerly et al., 1980; Montero et al., 1992a; Lee et al., 1992; Gnanasambandam and Zayas, 1992). Gel strength was less than control with added casein and equal gluten. With casein, there was a significant drop in breaking force and breaking deformation. With gluten, breaking force was similar to that of the control but breaking deformation was significantly higher.

In compression tests gels with egg white, followed by soy protein, exhibited greater hardness than the control. Gel with gluten exhibited equal hardness to the control and gel with casein lower hardness. Elasticity of gels with egg white, soy protein, and gluten, in that order, was greater than that of the control, while in gels with casein it was lower ( $P \le 0.05$ ). Finally, gel with egg white exhibited significantly greater cohesiveness than the rest. Gels with soy protein or gluten also exhibited greater cohesiveness than the control (eliminar) and gel with casein lower cohesiveness than control. Gnanasambandam and Zayas (1992) also reported increased firmness and cohesiveness in gels with added soy protein or gluten. There were no significant differences among the four nonmuscle proteins with regard to hardness at either salt level. However, high-salt gels tend to exhibit higher elasticity and cohesiveness.

Egg white and soy protein produced no significant difference in EM with respect to control (with 2.5% NaCl) (Table 2). However, EM values were significantly lower in gels with casein or gluten. Reducing salt in gels produced significantly lower EM with all four nonmuscle proteins. In general, as was reported before, addition of the higher salt concentration noticeably improves retention of water by myofibrillar protein (Hamm, 1986).

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