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Thermal gelation properties of two different composition sardine (*Sardina pilchardus*) muscles with addition of non-muscle proteins and hydrocolloids

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A Thermal Scanning Rigidity Monitor was used to study heat gelation in two sardine minces (S1 and S2) differing in compositional properties (fat content (S1 = 4.3%, S2 = 9.6%) and water content (S1 = 76.5%, S2 = 72.0%)), protein functionality (soluble protein (S1 = 62.6%, S2 = 49.8%) and viscosity (S1 = 3140 cP, S2 = 2323 cP)), as well as the contribution to gelation of a number of non-muscle proteins (egg white, soy protein, sodium caseinate, wheat gluten) and hydrocolloids (iota-carrageenan and starch). The batter of the mince, characterised by higher protein solubility and viscosity and lower fat content (S1), exhibited greater structural stability at temperatures above 50°C. The addition of gelling ingredients always produced an increase in maximum rigidity values, except in the case of sodium caseinate or starch-containing batters of mince with low protein solubility and viscosity (S2), where the rigidity is considerably inferior throughout the experimental temperature range. Of the non-muscle proteins assayed, batters contanining egg white presented the highest rigidity at maximum gelation peak, mainly in the low quality mince (S2). Iota-carrageenan also increased rigidity considerably with respect to the controls. Addition of starch gave less rigidity than iota-carrageenan, although it helped stabilise the formed gel at high temperatures (80°C). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The development of gel-based analogue products does not necessarily require *surimi*, which is not always easy to produce and is relatively expensive. There are products in which neither colour nor flavour present obstacles and in these cases, if high gel-forming capacity is not required, unrefined fish mince may be used after only a cursory wash. The result is a base product offering higher yield than *surimi* and hence more economical. Gelation of sardine mince sometimes present problems owing to intrinsic characteristics of the muscle (Ishikawa, 1978; Leinot & Cheftel, 1990). Gel-forming capacity can be improved by adding certain biopolymers, such as proteins and hydrocolloids, before processing (Ikeuchi, 1964; Akahane *et al.*, 1984; Iso *et al.*, 1985; Lee & Kim, 1985; Kim & Lee, 1987; Niwa *et al.*, 1988; Chung & Lee, 1990). However, the effect of these gelling ingredients is highly conditioned by the quality of the minced muscle (Westerly *et al.*, 1980; Nakayama *et al.*, 1988; Burgarella *et al.*, 1985*a*; Chung & Lee, 1991; Lee *et al.*, 1992; Gómez-Guillén *et al.*, 1996*a*).

The thermal scanning rigidity monitor (TSRM) is considered as a nondestructive test for monitoring the gelation of food proteins, being also valuable in studying possible interactions within different food systems (Hamann, 1987). Continuous rigidity scanning (as a function of temperature) is a more sensitive method for detecting protein sol-gel transformation transitions than measuring at constant temperature.

The objectives of this work were (1) to compare the gelation in the heating process of two sardine minces with different compositional properties and protein functionality, and (2) to examine the effect of various gelling ingredients on muscle protein thermal aggregation and gelation, for better understanding of the physical properties of heat-set gel formation and the functional role of these ingredients in processed products.

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Table 1. Proximate analysis and functional properties of S1 and S2 minces

Mince	Moisture (%)	Ash (%)	Fat (%)	Crude protein (%)	Soluble protein (%)	Apparent viscosity (cP)
S1	76.5±1.6	0.61±0.01	4.3±0.3	14.7±0.0	62.6±1.1	3140±34
S2	72.0±0.1	0.68±0.01	9.6±0.1	14.0±0.0	49.8±0.1	2323±35

MATERIALS AND METHODS

The fish used were sardine of the species *Sardina pilchardus* (Walbaum), caught off Nantes (France) in two different seasons: October (S1) and June (S2), with the aim to obtain two minces from muscles with differences in fat content and protein functionality (Table 1). The S1 mince presented lower fat content and higher water content than S2. With regard to protein functionality, the protein solubility and viscosity in S1 was higher than in S2. According to these values S1 was characterised as a high quality mince and S2 as a lower quality mince. These differences are related to physiological changes of the muscle due to the different seasons of capture, but also to some non-controlled factors during fish processing. 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, and the resulting mince was held for 10 min at 0–3°C in an aqueous solution of 0.5% bicarbonate, proportion 3:1 (solution:mince), 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 centre 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, in order to minimize alteration during frozen storage.

NaCl was supplied by PANREAC, Montplet and Esteban S.A. Barcelona (Spain). Roquette Freres CLEARAM CH 20 starch was supplied by Levantina Agrícola Industrial, S.A. (LAISA) (Barcelona, Spain). This is a modified waxy corn starch (acetylated starch adipate), which is especially indicated in processed foods, because it avoids retrogradation during chilled and frozen storage; i-carrageenan was supplied by LITEX A/S (Denmark), product reference GELCARIN XP 8009. Atomized-dried egg-white was supplied by SANOFI, S.A. For soy protein, a soy isolate was used, supplied by Protein Technologies International, product reference PP 500 E. Atomized-dried sodium caseinate was supplied by 'La Pilarica, S.A.' Wheat gluten was supplied by Levantina Agrícola Industrial, S.A. (LAISA), product name VITAL 'L' Wheat Gluten.

Compositional properties and protein functionality

To characterize the quality of the two washed mince types (S1 and S2), moisture, ash, crude fat and crude protein were determined by A.O.A.C. (1975). Results were averages of three determinations and expressed as a % of muscle mince. Protein solubility was analyzed in S1 and S2 by the method of Ironside and Love (1958). Results were averages of three determinations and expressed in % of 5% NaCl soluble fraction in relation to the total protein. Apparent viscosity was determined in S1 and S2, by the method of Borderías *et al.* (1985). Results were average of four determinations and expressed in centipoises (cps).

Homogenization of muscle with ingredients

Washed sardine mince was semi-thawed and placed in a refrigerated vacuum homogenizer (Stephan mod. UM5, Stephan u. Söhne GmbH and Co., Germany). Muscle was ground for 1 min (rotor angular velocity 3000 rpm). Sodium chloride (2.5%) was added with sufficient crushed ice to give the required final gel moisture and the mixture was homogenised for 5 min at 1500 rpm in vacuum conditions. The ingredient was then incorporated and the dough homogenised again for 5–7 min. The different gelling ingredients were added in the following proportions with respect to total weight: non-muscle proteins 2%; starch 8%; i-carrageenan 2%. In order to standardize the different samples, final moisture was adjusted to 74% in batters with gelling ingredients. The ingredient concentrations and moisture levels were selected according to previous work carried out in our laboratory. In samples without gelling ingredients (controls: S1 and S2) moisture was not adjusted, to prevent any modification in compositional properties.

Thermal scanning rigidity monitor (TSRM)

Modulus of rigidity was determined according to the model of Wu *et al.* (1985*a*) as modified by Carballo *et al.* (1992). Batter composed of muscle homogenized with salt and one of the ingredients was placed in a stainless steel cylinder (IR 2.2 cm). This was fitted to a cylindrical chamber with double walls for recycling water (diameter 2 cm, height 7.5 cm), part of the small-sample accessory for the Brookfield rotary viscosimeter,

model LVTD, MAB Industrial (UK). This chamber was mounted on an Instron Universal Texturometer model 4501 (Instron Engineering Corp., Canton, MA.). A grooved shaft with 9 mm diameter flat head was attached to a 100-N load cell connected to the texturometer. Water was recycled through the double chamber wall using a JULABO model F10 waterbath from Julabo Labortechnik GmbH (Germany), fitted with a JULABO model PRG1 temperature programmer. A few drops of oil were spread over the sample surface to prevent dehydration and skin formation (Montejano *et al.*, 1984). The sample was heated from 10 to 90°C at a rate of 1°C/min. A thermocouple was fitted to measure actual sample temperature. A Hewlett–Packard model Vectra ES/12 computer was used to move the head 0.2 mm every 2 min, at a rate of 0.5 mm/min, returning after each measurement to the original position.

Modulus of rigidity (G), expressed as kPa, was the average of two determinations carried out in different batters. G was calculated by means of the equation $G=[F \ln(R_1/R_2)]/2\,DL$; where F is maximum force (N); D is shaft displacement (0.0002 m); L is shaft length in contact with sample (0.05 m); R_1 is shaft radius (0.009 m) and R_2 is cylinder inner radius (0.022 m).

RESULTS AND DISCUSSION

Moduli of rigidity (G) of muscles S1 and S2 in relation to internal sample temperature are shown in Fig. 1. Development of gel rigidity as a function of temperature was broadly similar in both lots to that reported in Alaska Pollack *surimi* by Montejano *et al.* (1983).

The peak associated with the setting phenomenon appeared between 33 and 37°C in the two minces, which corresponds to the range where it normally takes place in *surimi* from other species (Lanier *et al.*, 1982; Montejano *et al.*, 1983). As reported by Wu *et al.* (1985*a*), such behaviour probably marks a degree of unfolding of protein molecules giving rise to protein–protein association, binding together via hydrophobic interactions. The decline of rigidity immediately after setting took place in the same manner for both muscles, reaching a minimum value at 40°C. This texture degradation could be a consequence of a protein conformational change caused by an increase in heat energy (Montejano *et al.*, 1983), but it also has been ascribed to an early stage of modori (Sano *et al.*, 1988). From 42°C upwards, both muscles showed an increase in rigidity (even and continuous), indicating gradual formation of a stable network by cross-linked protein aggregation (Montejano *et al.*, 1984).

Comparing the two minces, S2 exhibited higher values of G throughout the temperature range 10–60°C, but S1 displayed better capacity to form a stable three-dimensional network at temperatures above 60°C, since rigidity proved stable between 60 and 82°C, with a

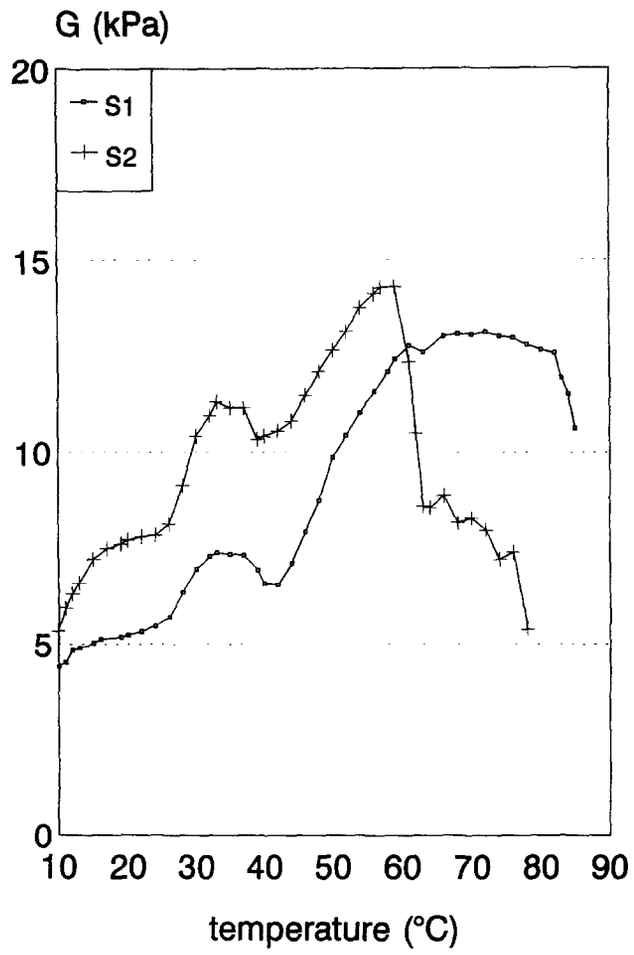

Fig. 1. Modulus of rigidity of S1 and S2 minces.

minor peak at 76°C. Maximum rigidity in S2 occurred at a lower temperature (58°C) and was followed by a sharp drop; this could be due to irreversible deterioration of texture as the structure has a strong tendency to be destabilised by heat energy and proteolysis. Barbut and Mittal (1990) ascribed this event to structural breakdown of unstable gels caused by the application of some external force during TSRM.

The difference in behaviour of the two mince types during gelation are related to the differences noted in proximate composition and protein functionality (protein solubility and viscosity) (Table 1). The lower absolute values of G found in S1 over the range 10–60°C may be due to higher moisture content in S1 (Table 1), given that moisture is one of the factors determining rigidity (G) (Alvarez, 1993). The occurrence of a maximum rigidity peak at lower temperatures in S2 muscle and the subsequent sharp decline, may be connected to its lower protein solubility and viscosity (Table 1), but also to the lower protein concentration in the salted homogenized batter (Wu *et al.*, 1985*a*; Burgarella *et al.*, 1985*a*). Final concentrations of muscle crude protein in each batter were 14.4 g per 100 g sample in S1 and 13.6 g per 100 g sample in S2. On the other hand, the higher fat content in S2 (Table 1) may also be decisive, since fat is an undesirable element in gel making, as it

interferes with formation of a protein network. After removing a major part of triglycerides with the washing process, the remaining unstable phospholipid fraction in the muscle could participate in protein denaturation to some extent (Sikorski *et al.*, 1976). This might also explain why the network formed at high temperature was less stable in S2 than S1 batters.

Effect of adding non-muscle proteins

Figure 2 shows the modulus of rigidity (*G*) of batter S1, containing the different non-muscle proteins, with final moisture adjusted to 74%. Addition of non-muscle proteins to mince S1 raised rigidity with respect to control throughout the experimental temperature range. This may be due to the role of non-muscle proteins in the gel matrix, acting as simple fillers or forming interpenetrating networks (Ziegler & Foegeding, 1991), but also to lower final moisture in samples made with non-muscle protein (74 vs 76% in control), even although muscle protein concentration in this case (12.8 g per 100 g sample) was slightly lower than control (14.4 g per 100 g sample).

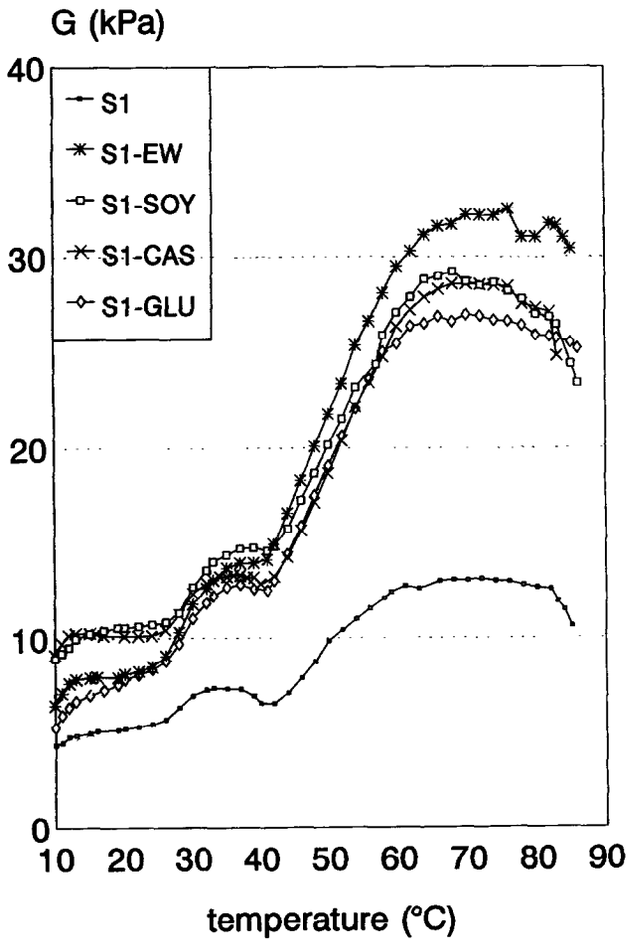

Fig. 2. Modulus of rigidity of S1 mince, with addition of non-muscle proteins, at 74% moisture. (EW = egg white; SOY = soy protein; CAS = sodium caseinate; GLU = gluten).

Setting occurred within a similar temperature range to control (33–37°C) in all four proteins. Decreasing rigidity following setting, down to a minimum at around 41°C, was slightly less pronounced where non-muscle proteins were added. This could be because subsequent breakdown by increase of heat energy was prevented by the stabilising effect of a primary network formed during setting. Where the added ingredient was egg white, there was no such decrease at all and it could be the result of egg white inhibiting the onset of proteolysis at these temperatures (Lee, 1984; Gómez-Guillén, 1994).

In all cases *G* peaked at 68–72°C and declined thereafter, with the exception of batters containing egg white, where *G* remained constant between 70 and 78°C, the range at which gelation of muscle protein occurred. A final peak in this batter could be seen at 82–83°C. According to Baldwin (1977) and Goldsmith and Toledo (1985), egg white begins to coagulate at temperatures above 62°C. The last peak coincides with the temperature range within which egg white gels completely (Beveridge *et al.*, 1984; Burgarella *et al.*, 1985*b*), suggesting a posible mechanism of independent gelation of this non-muscle protein forming a net (Gomez-Guillen *et al.*, 1996*b*). However, no evident peaks were detected in the gelation profiles of S1 batters which might correspond to gelation of soy protein, sodium caseinate or gluten. Similarly, addition of soy protein has not been found to make any significant difference to gelation of meat (chicken and veal) batters (Patana-Anake & Foegeding, 1985). The role of these non-muscle proteins (soy, casein and gluten) in mince gelation seems to be less outstanding than that observed for egg white.

With the S2 mince (lower protein functionality and higher fat content) (Fig. 3), addition of egg white, soy protein or gluten resulted also in higher rigidity than control, being much more pronounced in the case of the egg white. With casein, *G* was lower than control throughout the experimental temperature range. In S2 mince, enhanced gelation by egg white, soy protein or gluten, was clearly a result of ingredient addition, since increased rigidity could not be attributed in this case to lower moisture (74% in ingredient-containing batters vs 72% in control) and, moreover, muscle protein concentration (10.2 g per 100 g sample) was considerably lower than in control (13.6 g per 100 g sample).

As in S1 batters, the setting phenomenon (between 33 and 37°C) with all four proteins seems not to be influenced by the characteristics of the mince nor the type of non-muscle protein incorporated.

Where the added protein was egg white, rigidity continued to increase up to 82–83°C, the peak associated with complete gelation of egg white. With soy protein and gluten rigidity remained stable up to 68–72°C, declining thereafter. With casein, there was no improvement in stability of S2 to high temperatures, rigidity commencing to decline from 61°C upwards.

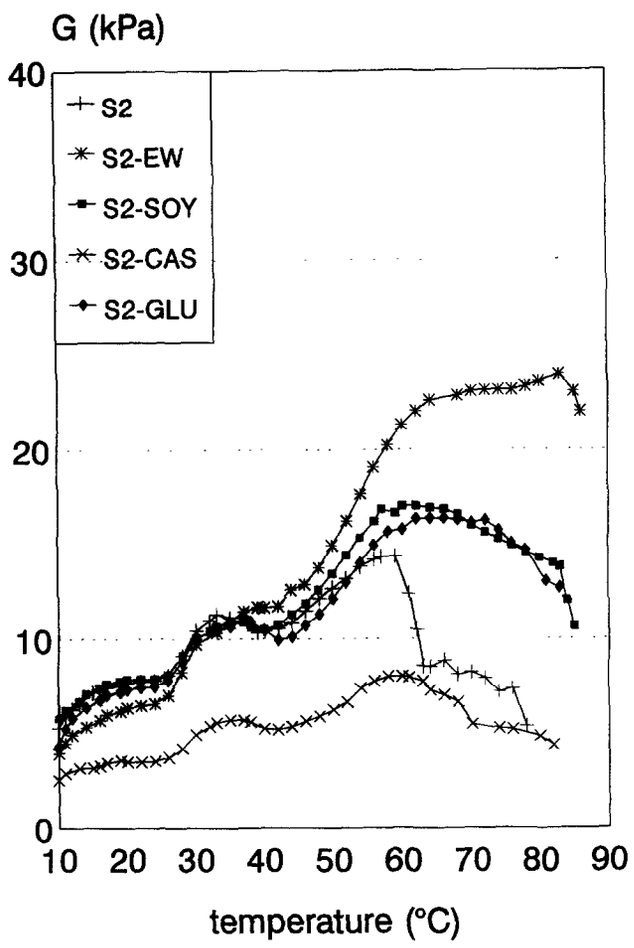

Fig. 3. Modulus of rigidity of S2 mince, with addition of non-muscle proteins, at 74% moisture. (EW = egg white; SOY = soy protein; CAS = sodium caseinate; GLU = gluten).

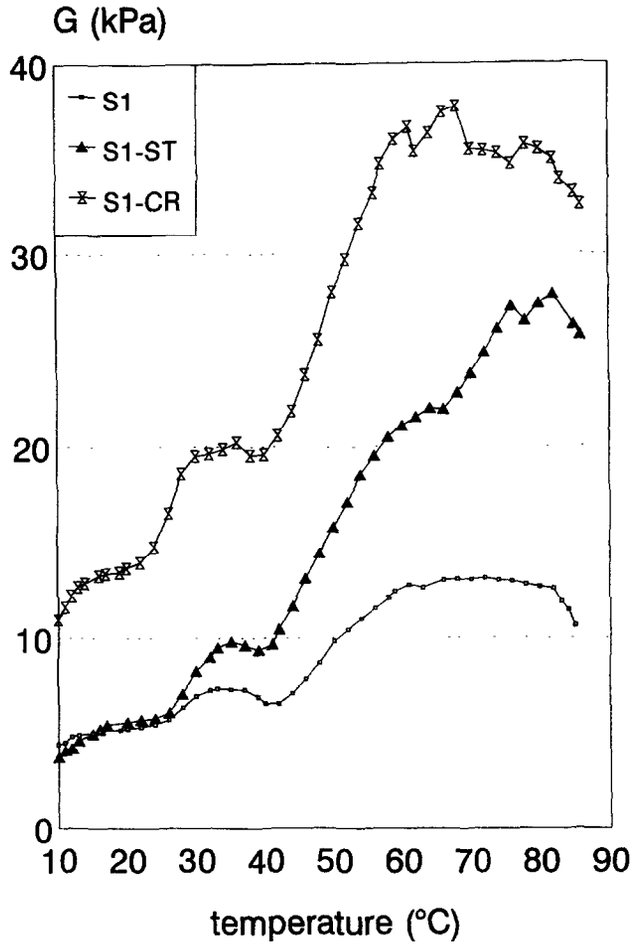

Fig. 4. Modulus of rigidity of S1 mince, with addition of hydrocolloids, at 74% moisture. (CR = i-carrageenan; ST = starch).

Effect of adding hydrocolloids

Addition of either i-carrageenan or starch to S1 mince improved *G* values throughout the experimental temperature range in the case of carrageenan and from 26°C upwards in the case of starch (Fig. 4). As in batters with non-muscle proteins, this may be due in large part not only to the added ingredient, but also to lower moisture content. Muscle protein concentration in these batters was also lower than in the S1 control (batter with starch = 10.9 g per 100 g sample; batter with i-carrageenan = 11.9 g per 100 g sample; control = 14.4 g per 100 g sample). Thus the use of those ingredients that enhace rigidity of batters with a lower proportion of myofibrillar protein, is interesting. With the two hydrocolloids, setting occurred within the same temperature range as the control (33–37°C) and as in the corresponding batters with non-muscle proteins.

In the sample with starch there was minor loss of stability at 64°C, as in the S1 control, and a final peak at 81°C. This last peak is ascribed to complete gelatinization of starch, which according to Wu *et al.* (1985*b*) occurs after gelation of fish protein. With carrageenan, maximum occurred at 70°C, the temperature at which this hydrocolloid gels (Foegeding & Ramsey, 1987).

In batters from S2 mince with starch (Fig. 5), beyond 62°C there followed an increase in rigidity, peaking at 81°C, the temperature at which starch gelatinization is complete. In batters with i-carrageenan, although *G* was higher than the control (S2) throughout the experimental temperature range, from 60°C upwards addition of i-carrageenan did not improve gel stability. This may be due to the fact that the i-carrageenan network needs to be supported by the one established by the myofibrillar protein which is very weak in this mince.

Throughout the experimental temperature range, in S2 batter with starch, *G* was lower than the control, whereas with i-carrageenan values were higher at all times. Again in S2 batter with starch, muscle protein concentration (8.7 g per 100 g sample) was much lower than the control (13.6 g per 100 g sample). The modified starch by itself seems not to be adequate for achieving a proper gelation in a mince without optimum properties (S2 mince). The functional state of the myofibrilar protein may be decisive in stablishing a partial network which acts as a support of starch granules before complete gelatinization. There was, however, a

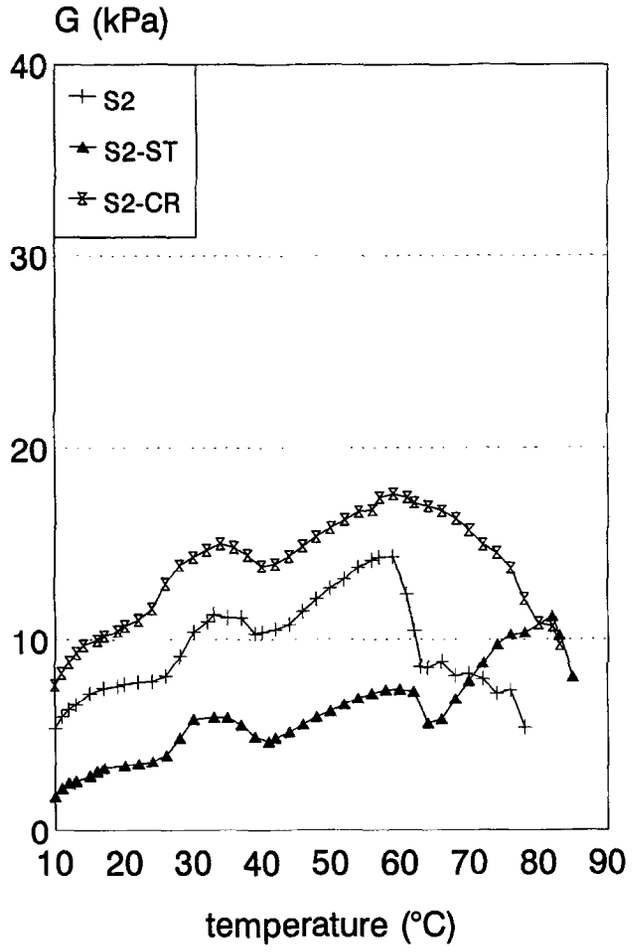

Fig. 5. Modulus of rigidity of S2 mince, with addition of hydrocolloids, at 74% moisture. (CR = i-carrageenan; ST = starch.)

final peak at 81°C, at which rigidity was comparable to batters with i-carrageenan at the same temperature. Rigidity values were higher than the control in the sample with i-carrageenan despite the fact that muscle protein content (9.46 g per 100 g sample) was lower than the control, which suggests that increased rigidity was directly due to the action of the hydrocolloid. It has been observed that i-carrageenan incorporated in meat batters presented a clear tendency to form thin networks, which act by connecting muscle structures, even at relatively low temperature (Gómez-Guillén *et al.*, 1996*b*). This also could explain the high rigidity values observed at temperatures below 42°C.

CONCLUSIONS

The compositional properties and protein functionality of the minced muscle affects its capacity to form a stable network at temperatures above 60°C. The egg white produced a major improvement in gelation of sardine muscle whereas, with the other proteins, the difference from muscle protein on its own was much less apparent. Addition of i-carrageenan always increased the rigidity but did not improve gel stability in the low quality mince. The effect of starch in muscle gelation was found to be very conditioned by the mince characteristics.

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