

# Salt, Nonmuscle Proteins, and Hydrocolloids Affecting Rigidity Changes during Gelation of Giant Squid (*Dosidicus gigas*)

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A thermal scanning rigidity monitor was used to determine how certain nonmuscle proteins and hydrocolloids contribute, separately or in combination, to gelation of giant squid mantle homogenized with 1.5% or 2.5% NaCl. In batters with 1.5% salt, rigidity was higher and peak gelation also took place at a higher temperature. Of all the ingredients tested, only *ι*-carrageenan contributed significantly to rigidity. Maximum values for batter rigidity and for folding test and work of penetration in cooked gels were attained when *ι*-carrageenan was added along with starch and a nonmuscle protein, which indicates synergy among the three ingredients.

**Keywords:** *Squid muscle; gelation; proteins; hydrocolloids; rheology*

## INTRODUCTION

Given the poor gel-forming capacity of cephalopods in general (Nagashima et al., 1992) and giant squid (*Dosidicus gigas*) in particular (Gómez-Guillén et al., 1996a), addition of gelling ingredients is considered an effective means of obtaining acceptable characteristics for gels on which a number of analogues are based (Okada, 1963; Akahane et al., 1984; Niwa et al., 1988; Chung and Lee, 1990). This can enhance the economic return on what is in fact an undervalued species.

The gelling ingredients most commonly used in the fabrication of fish gels are nonmuscle proteins and hydrocolloids. Differences in the ingredient distribution pattern in the matrix of fish gels have been reported (Beveridge et al., 1984; Chung and Lee, 1991; Gómez-Guillén et al., 1996b). According to Ziegler and Foegeing (1990), the ingredient may act as a simple "filler" in the gel matrix, remaining soluble in the interstitial fluid or distributed as dispersed particles, but it may also form interpenetrating networks or could even interact with the myofibrillar fraction, as has been observed by Gómez-Guillén et al. (1996b) in this species.

Thermal scanning rigidity monitor (TSRM) is a nondestructive test which can be used to monitor the gelation of food proteins, and it is also valuable in studying possible interactions within different food systems (Hamann, 1987). Continuous rigidity scanning (as a function of temperature) is more sensitive for detecting protein sol–gel transformation transitions than measuring at constant temperature.

The objective of this research was to identify the heat gelation profile of giant squid (*D. gigas*) mantle homogenized with two levels of NaCl (1.5% and 2.5%) using a thermal scanning rigidity monitor (TSRM) and to determine how such gelation was influenced by addition of a number of ingredients, both separately and in combination. Observations were also made to determine the influence that those ingredients have on puncture-test measurements in cooked gels.

## MATERIALS AND METHODS

The species *D. gigas* (Orbigny, 1835) is a cephalopod of the *Ommastrephes* genus found off the Pacific coast of Mexico in a great abundance. Mantles were typically around 60 cm wide,

1 m long, and 3 cm thick. Gutting and removal of tentacles were performed on the spot immediately after capture, and sometimes also the outermost fascia were removed mechanically to leave clean mantles. These were then wrapped in polyethylene sheaths and frozen using an air-blast freezer set at  $-40\text{ }^{\circ}\text{C}$ , until the thermal centers reached  $-20\text{ }^{\circ}\text{C}$ , which was the storage temperature. The time elapsing from time of capture to arrival at the laboratory was estimated at between 1 and 2 months. Frozen mantles were cut into small portions of about  $3 \times 3\text{ cm}^2$  using a Braher model F2 mechanical cutter (Nordischer Mas, Germany). The portions, thus ready for homogenization, were placed in polyethylene bags in lots of 300 g and stored at  $-80\text{ }^{\circ}\text{C}$  to maintain stability over the experimental period.

NaCl was supplied by PANREAC, Montplet & Esteban S.A. Barcelona (Spain); CLEARAM CH 20 starch from Roquette Freres was supplied by Levantina Agrícola Industrial, S.A. (LAISA), Barcelona (Spain). This is a modified waxy corn starch (acetylated starch adipate) which remains largely unaltered at freezing and pasteurizing temperatures. *ι*-Carrageenan was supplied by LITEX A/S (Denmark) under the product name GELCARIN XP 8009. Atomized-dried egg white was supplied by SANOFI, S.A. For soy protein, a soy isolate from Protein Technologies International (Barcelona), with the product name PP 500 E, was used. Atomized-dried sodium caseinate was supplied by La Pilarica, S.A. (Madrid). Wheat gluten was supplied by Levantina Agrícola Industrial, S.A. (LAISA), under the product name VITAL "L" Wheat Gluten.

**Proximate Analysis.** Proximate composition of giant squid muscle was carried out by determinations of moisture, ash, crude fat, and crude protein according to AOAC (1975). Results were averages of three determinations, as percent of minced muscle.

**Homogenization of Muscle with Ingredients.** Chopped squid mantle was tempered up to  $-3$  or  $-4\text{ }^{\circ}\text{C}$  and placed in a refrigerated vacuum homogenizer (Stephan mod. UM5, Stephan u. Söhne GmbH & Co., Germany). The muscle was ground for 1 min at high speed (rotor angular velocity 3000 rpm). Sodium chloride was then added with sufficient crushed ice to give the required final gel moisture (78%), and the mixture was homogenized for 5 min at 1500 rpm under vacuum. The remaining ingredients were then added as defined for each sample, and the mixture homogenized again for 5–7 min. Ingredients were added in the following proportions with respect to total weight: nonmuscle proteins, 2%; starch, 5%; *ι*-carrageenan, 2%. Final moisture was adjusted to 78% in all cases, as dictated by previous assays. The resulting paste was stuffed into a stainless steel cylinder (inner diameter 2 cm, height 7.5 cm). At no point in this part of the process did sample temperature exceed  $10\text{ }^{\circ}\text{C}$ .

**Thermal Scanning Rigidity Monitor (TSRM).** Modulus of rigidity of muscle homogenized with sodium chloride and

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the different ingredients was measured in the batter by thermal scanning based on the model of Wu et al. (1985) as modified by Carballo et al. (1992). Sample was placed in a cylinder (inner diameter 2 cm, height 7.5 cm) which was fitted to a cylindrical chamber with double walls for recycling water, part of the small-sample accessory for the Brookfield rotary viscosimeter (model LVTD, MAB Industrial, U.K.). This chamber was mounted on an Instron Universal Testing Machine (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 testing machine. Water was recycled through the double chamber wall using a JULABO model F10 waterbath (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). Sample was heated from 10 to 90 °C at 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 at 0.5 mm/min every 2 min.

The modulus of rigidity ( $G$ ) was expressed as kPa.  $G$  was calculated by means of the equation  $G = [F \ln(R_1/R_2)]/2DL$ , where  $F$  is the maximum force (N),  $D$  is the shaft displacement (0.0002 m),  $L$  is the shaft length in contact with the sample (0.05 m),  $R_1$  is the shaft radius (0.0045 m), and  $R_2$  is the cylinder inner radius (0.01 m). All determinations were carried out once each on two identical batters.

**Determination of Puncture Test.** The paste resulting from homogenization was stuffed into stainless steel cylinders with screw-on lids and rubber gaskets to provide a hermetic seal. Samples were heated at 90 °C by immersion in a waterbath (UNITRONIC mod. S 320-100, from J.P. SELECTA) for 20 min. They were then stored in a cold room at 4 °C for 24 h before analysis (puncture test). Cylindrical samples (3 cm diameter  $\times$  3 cm height) were removed from the molds and tempered to about 20 °C. Gels were pierced to breaking point using a texturometer (Instron Mod. 4501, Instron Engineering Corp., Canton, MA) with a 5-mm-diameter, round-ended metal probe. Crosshead speed was 10 mm/min, and a 100 N load cell was used. Work of penetration (gel strength) was determined by multiplying maximum breaking force (N) by breaking deformation (mm). All determinations were carried out at least in quadruplicate.

**Determination of Folding Test.** The test piece was a 3-mm-thick slice cut from the cylinders. The evaluation was performed in accordance with a five-point grade system (Suzuki, 1981) as follows: grade 5, no crack when folded into quadrants; grade 4, no crack when folded in half; grade 3, crack develops gradually when folded in half; grade 2, crack develops immediately when folded in half; grade 1, crumbles when pressed by finger.

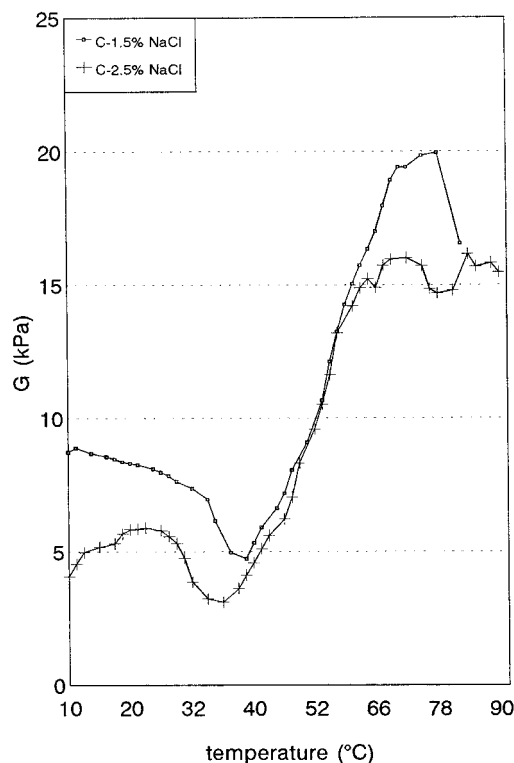
**Statistical Analysis of Data.** The modulus of rigidity ( $G$ ) was the average of two determinations, carried out once on two identical batters.

One-way analysis of variance (ANOVA) was carried out for the different puncture test determinations made on the gels. The difference of means between pairs was resolved by means of confidence intervals using a least significant difference (LSD) range test. The computer program used was Statgraphics (STSC Inc., Rockville, U.S.). The level of significance was set for  $P \leq 0.05$

## RESULTS AND DISCUSSION

Proximate analyses showed that the mantle of giant squid was a muscle with high moisture ( $79.90 \pm 0.16\%$ ), low fat ( $1.43 \pm 0.12\%$ ), and high protein content ( $18.96 \pm 0.15\%$ ). The ash content was  $1.36 \pm 0.05\%$ .

Evolution of modulus of rigidity of giant squid (*D. gigas*) homogenized with 1.5% and 2.5% NaCl with respect to temperature is shown in Figure 1. It differed from the typical curves described by Montejano et al. (1983) in Alaska pollack surimi or Kim et al. (1987) in croaker surimi, essentially in that rigidity values dropped



**Figure 1.** Modulus of rigidity of giant squid muscle homogenized with 2.5% and 1.5% NaCl, at 78% moisture.

considerably between 25 and 40 °C. Falling rigidity values between 18 and 35 °C have been reported in meat (Montejano et al., 1984; Patana-Anake and Foegeding, 1985), although these authors attributed it largely to increased viscosity of the system resulting from melting fats. This is unlikely in giant squid, as the mantle contains only 1.43% lipids. In the present case, these low rigidity values could be attributed to multiple factors, such as differences in the myosin molecule (Niwa et al., 1980; Taguchi et al., 1986), the presence of paramyosin in the squid muscle (Sano et al., 1989), proteolysis, which is high in cephalopod muscle in this temperature range (Kolodziejaska et al., 1987; Nagashima et al., 1992; Konno and Fukazawa, 1993), H-bond breaking (Niwa, 1992), or melting of connective tissue remaining from the removed inner fascia. This finding is worth taking into account when selecting an appropriate heat treatment with which to make gels from this species, since it means that a setting stage is not necessary.

From 40 °C upward, the modulus of rigidity underwent a rapid increase up to 78 °C in the batter with 1.5% salt. Peak gelation in the sample with 2.5% salt occurred somewhat earlier, at 69 °C, and values of  $G$  were lower. The lower rigidity observed in the 2.5% salt batter with respect to the 1.5% salt batter at low temperatures suggests that greater solubilization of myofibrillar proteins and dissolution of myofibrils causes a more fluid sol to form (Burgarella et al., 1985a). This would favor the production of less rigid but more elastic gels at temperatures in the range 69–80 °C, by the monomer form of myosin predominant in the higher-salt batter (Wicker et al., 1986).

After reaching the highest peak, a pronounced drop was observed in all gelled batters. Reduction in gel rigidity at high temperatures has been documented (Young et al., 1992; Srinivasan and Xiong, 1996) and ascribed to precipitation/coagulation processes of myo-

**Table 1. Folding Test, Breaking Force, Breaking Deformation, and Work of Penetration of Giant Squid Muscle Gels, Made with 1.5% or 2.5% NaCl, at 78% Moisture<sup>a</sup>**

formula	folding test	breaking force (N)	breaking deformation (mm)	work of penetration (N mm)
1.5% NaCl control	2			
CR	2			
ST	3			
EW	2			
SO	2			
CA	1			
GL	1			
CR-EW	5	1.91 <sup>a</sup>	8.33 <sup>a</sup>	15.80 <sup>a</sup>
CR-SO	4	1.51 <sup>b</sup>	5.65 <sup>b</sup>	8.56 <sup>b</sup>
CR-CA	5	2.10 <sup>a</sup>	8.28 <sup>a</sup>	17.22 <sup>a</sup>
CR-GL	3	1.94 <sup>a</sup>	5.88 <sup>b</sup>	11.43 <sup>c</sup>
CR-ST-EW	5	1.66 <sup>c</sup>	9.66 <sup>a</sup>	16.00 <sup>a</sup>
CR-ST-SO	5	1.82 <sup>d</sup>	8.48 <sup>a</sup>	15.44 <sup>a</sup>
CR-ST-CA	5	2.00 <sup>a</sup>	8.51 <sup>a</sup>	16.87 <sup>a</sup>
CR-ST-GL	5	1.71 <sup>c</sup>	8.72 <sup>a</sup>	14.99 <sup>a</sup>
2.5% NaCl control	2			
CR-ST-EW	5	1.26 <sup>e</sup>	6.02 <sup>b</sup>	7.59 <sup>b</sup>
CR-ST-SO	5	1.27 <sup>e</sup>	7.52 <sup>c</sup>	9.53 <sup>b,c</sup>
CR-ST-CA	5	1.01 <sup>f</sup>	8.02 <sup>a,c</sup>	8.13 <sup>b</sup>
CR-ST-GL	5	1.40 <sup>e</sup>	7.07 <sup>c</sup>	11.35 <sup>c</sup>

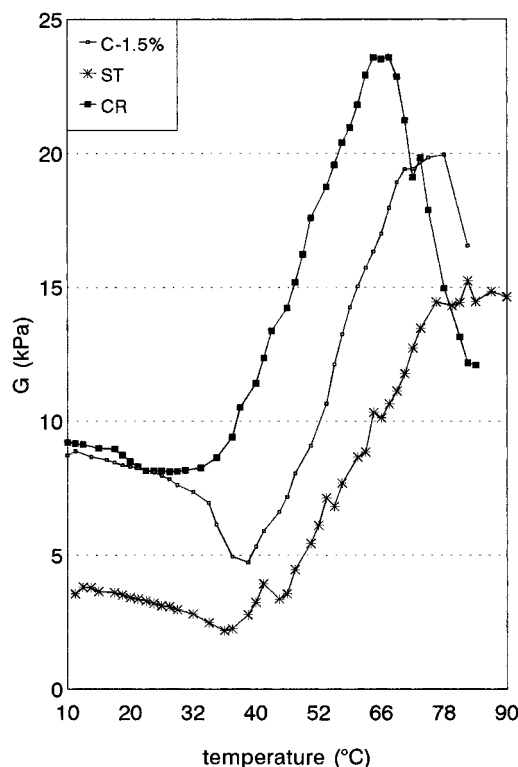
<sup>a</sup> CR = *l*-carrageenan, ST = starch, EW = egg white, SO = soy protein, CA = casein, GL = gluten. Different letters (a–f) in the same column indicate significant differences ( $P < 0.05$ ) among formulas.

fibrillar proteins (Liu et al., 1982) and also to kinetic constraints in myosin gel network formation (Wu et al., 1991). Barbut and Mittal (1990) reported that, when a relatively unstable batter is monitored by TSRM, structural breakdown after gelification was associated with the forces applied to the sample. Another reason may be that, with the gelling mechanism virtually complete, subsequent heating leads to a reduction in hydrogen bonds and hence to a decrease in rigidity.

Analyses of breaking force and deformation in the cooked gels were tried, but it was very difficult to determine the breaking point, given that folding test scores were also very low (Table 1). This is why data have not been included. Moreover, these data are completely irrelevant when compared to data of gels with maximum folding test score.

**Effect of Adding Hydrocolloids.** Throughout the experimental temperature range, rigidity was lower than in the control (muscle with 1.5% NaCl) when starch was added (Figure 2). With added *l*-carrageenan, peak gelation occurred at lower temperature (64–68 °C), but although rigidity was greater than the control in absolute terms, there was a sharp drop at higher temperatures, denoting a certain degree of instability in the gelled batter. This effect was consistent with the low value scored in the folding test by the corresponding cooked gel (Table 1). A second peak at 75 °C, which was much less pronounced than the first, could reflect complete gelation of the carrageenan (Foegeding and Ramsey, 1987).

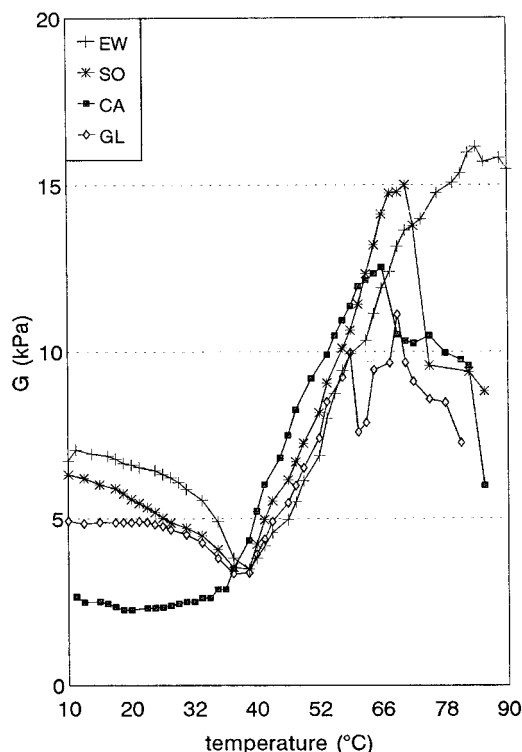
The mechanisms whereby starch and carrageenan act are different. Starch shows little affinity for myofibrillar protein during heating and thus does not interact with the protein matrix. According to Ziegler and Foegeding (1990), starch acts as a "simple" or "passive filler". With regard to carrageenan, some authors have reported that *l*-carrageenan (Llanto et al. 1990) and  $\kappa$ -carrageenan (Niwa, 1992) bind the water in the



**Figure 2.** Modulus of rigidity of giant squid muscle with added hydrocolloids and 1.5% NaCl, at 78% moisture. CR = *l*-carrageenan; ST = starch; C-1.5% = control (1.5% NaCl).

system without any linkage to the myofibrillar protein. In previous studies our group found that *l*-carrageenan formed fine mesh structures irrespective of experimental temperature, which were more or less appreciable depending upon the degree of overall aggregation of the matrix; these networks provide connections between adjacent structures within the gel and may serve a supporting function (Gómez-Guillén et al., 1996b), which could be the reason for the high rigidity values.

**Effect of Adding Nonmuscle Proteins.** Modulus of rigidity of homogenized muscle batters containing the various experimental nonmuscle proteins and 1.5% salt is shown in Figure 3. Modulus of rigidity of batters with nonmuscle proteins was lower than the control. Similarly, folding test values of cooked gels produced the same or inferior scores (Table 1). This may be connected with the "diluting" effect undergone by myofibrillar protein upon addition of nonmuscle proteins (Burgarella et al., 1985b), which appear to lack any capacity to improve muscle gelation. As in the control sample and in the batters with starch or *l*-carrageenan, when nonmuscle proteins were added, there was also a drop in rigidity between 25 and 40 °C, with the sole exception of the sample with casein, which registered very low rigidity values from the beginning of the heating regime (10 °C), thus hindering the detection of possible changes. The explanation for this lower rigidity at low temperatures could lie in the fact that sodium caseinate thickens the batter less because of its lower water binding ability. Rigidity peaks in batters with nonmuscle proteins occurred at lower temperature (60–70 °C) than in the control, with the exception of batters with egg white: these gave greater rigidity beyond 80 °C, which is the gelling temperature of this protein (Beveridge et al., 1984; Burgarella et al., 1985a). Maximum rigidity values in batters with casein or gluten were considerably lower than in batters with egg white or soy protein.



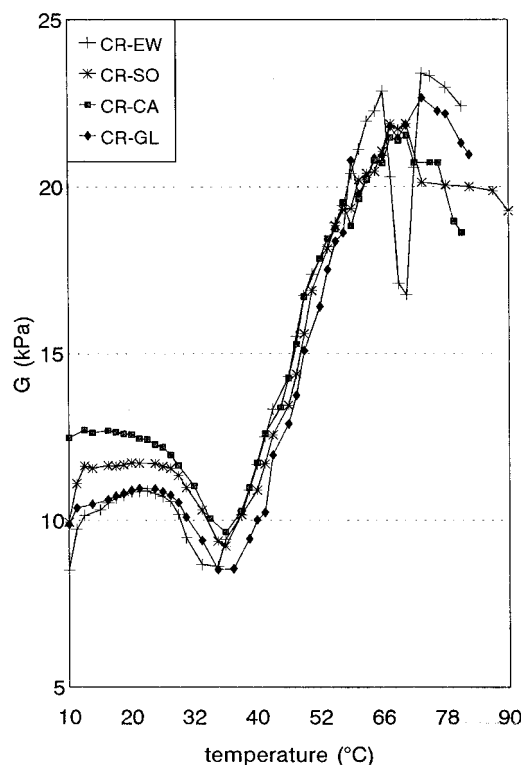
**Figure 3.** Modulus of rigidity of giant squid muscle with added nonmuscle protein and 1.5% NaCl, at 78% moisture. EW = egg white; SO = soy protein; CA = casein; GL = gluten.

Data of breaking force, breaking deformation, and work of penetration are not shown, since these measurements were not reliable because of the weakness of the cooked gels.

**Effect of Simultaneous Addition of *l*-Carrageenan and Nonmuscle Protein Together.** Addition of *l*-carrageenan along with nonmuscle proteins produced higher rigidity than in the control throughout the experimental temperature range (Figure 4). Batters with *l*-carrageenan and egg white exhibited two peaks at 66 and 75 °C coinciding with the temperatures at which the batter containing only added carrageenan peaked. However when egg white was included the second peak, which was ascribed to complete gelation of carrageenan (at 75 °C), appeared more pronounced. This effect suggests some kind of interaction or positive action between carrageenan and egg white and seems consistent with the findings of Bullens et al. (1990), who reported synergy between carrageenan and a nonmuscle protein (serum protein) in surimi gels. Batters with soy, casein, or gluten peaked once at 72–75 °C, coinciding with gelation of both myofibrillar protein and *l*-carrageenan.

Absolute values of  $G$  (kPa) were very similar from one sample to another and also to that of the sample containing only *l*-carrageenan. This suggested that the latter contributes chiefly to formation of a more rigid gel of minced giant squid muscle, particularly at high temperatures, considering that, when only proteins were added, rigidity curves were lower than control.

In cooked gels, folding test scores increased considerably, with work of penetration significantly higher in the gels containing egg white or casein (Table 1). The data for breaking force and breaking deformation suggested that lower work of penetration in gels with soy and gluten was mainly due to lower deformation (Table 1). However, these results were not consistent with rigidity peaks values determined by the TSRM. Ac-



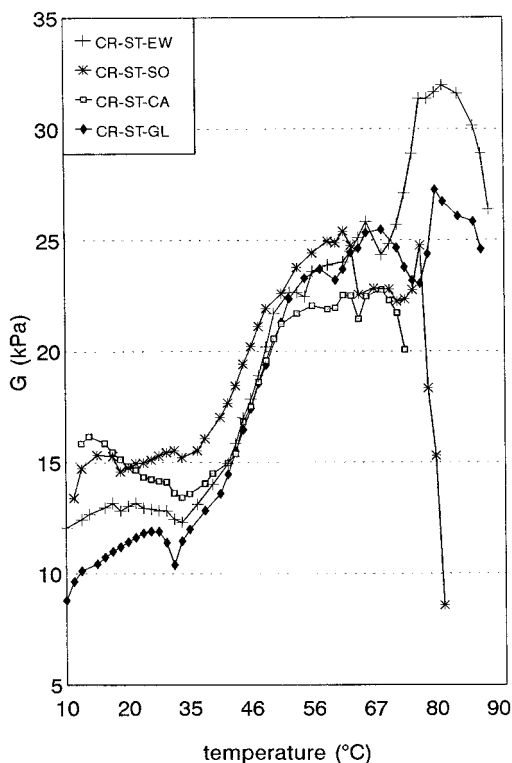
**Figure 4.** Modulus of rigidity of giant squid muscle with added *l*-carrageenan and nonmuscle protein, with 1.5% NaCl and 78% moisture. CR = *l*-carrageenan; EW = egg white; SO = soy protein; CA = casein; GL = gluten.

ording to Hamann (1987), small-strain gel rigidity has been shown in several studies not to correlate well with rupture strength.

**Effect of Adding Starch, *l*-Carrageenan, and Nonmuscle Protein.** Addition of starch along with *l*-carrageenan and nonmuscle protein, without modifying final moisture (78%), produced the highest rigidity values throughout the experimental temperature range (Figure 5), even though muscle protein concentration was considerably lower than control (9.5% versus 18%). The effect was however less pronounced in gels containing casein.

The appearance of a number of peaks in the graph of the heat gelation process was related to the presence of the various ingredients. The first peak appearing at temperatures between 60 and 65 °C could be related to an earlier peaking of myofibrillar protein gelation due to the dilution effect reported by Burgarella et al. (1985a). The appearance of peaks around 70 °C may be attributed to the presence of carrageenan as noted earlier (Foegeding and Ramsey, 1987). The final peak at 80 °C in batters with added egg white was especially pronounced, marking both gelation of egg white (Burgarella et al., 1985a; Beveridge et al., 1984) and complete gelatinization of starch. Addition of starch to sample containing gluten also produced a pronounced peak at 80 °C, which suggests that, when combined with gluten, starch particularly contributes to the formation of a more rigid gel.

Work of penetration and folding test scores also increased in gels containing soy and gluten along with the two hydrocolloids (Table 1). There were no significant differences among work of penetration data of gels containing the different proteins plus *l*-carrageenan and starch. In the case of gels with carrageenan plus egg white and carrageenan plus casein, the inclusion of starch had no effect on work of penetration; however,

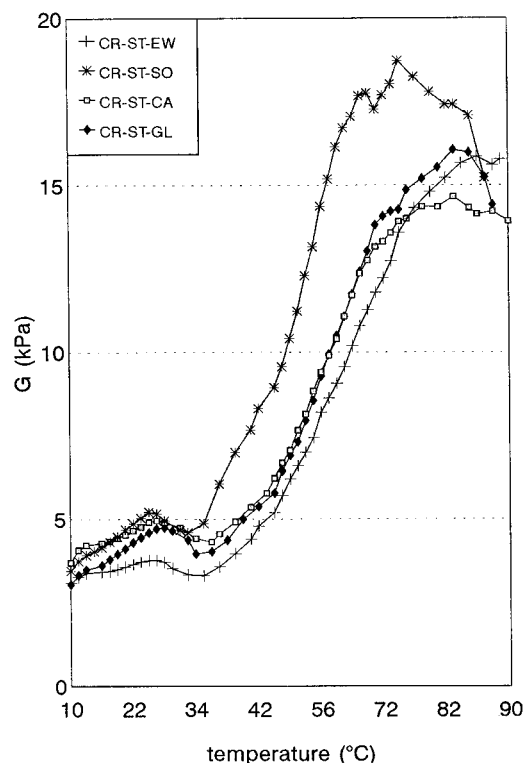


**Figure 5.** Modulus of rigidity of giant squid muscle with added *ι*-carrageenan, starch, and nonmuscle protein, with 1.5% NaCl and 78% moisture. CR = *ι*-carrageenan; ST = starch; EW = egg white; SO = soy protein; CA = casein; GL = gluten.

this does not mean that starch is of no interest, since muscle content, which is the main cost item in the formula, can be considerably reduced without detriment to textural properties.

Formulas containing *ι*-carrageenan, starch, and nonmuscle protein were also checked with 2.5% NaCl. Modulus of rigidity of these formulas are shown in Figure 6. At all events rigidity was considerably lower than in equivalent formulas made with 1.5% NaCl. So a marked difference cannot reasonably be attributed to the difference in muscle protein concentration (about 1%). Work of penetration values (Table 1) were low as compared to the gels with 1.5% NaCl, reflecting lower values for both breaking force and breaking deformation. A similar effect, related to the fact that lower salt concentration gives more gel strength, has been reported in muscle of other species by Burgarella et al. (1985b), Wu et al. (1985), and Kim et al. (1986). Folding test data showed, as in the 1.5% NaCl formula, a dramatic increase with respect to the control, revealing the importance of adding these hydrocolloids together with a nonmuscle protein to obtain more elastic gels.

**Conclusions.** Giant squid muscle, which has very poor gel forming capacity in the conditions which usually can be purchased by the European markets, could serve as a basis for the manufacture of gel products when *ι*-carrageenan, starch, and a nonmuscle protein are added together. *ι*-Carrageenan is the main contributor to rigidity, but at high temperature, the batter needs the additional action of starch and protein to achieve more rigidity. This suggests considerable synergy among these ingredients, which has also been observed in cooked gels. Formulas made with 1.5% NaCl produced higher rigidity in batters as well as higher work of penetration in cooked gels.



**Figure 6.** Modulus of rigidity of giant squid muscle with added *ι*-carrageenan, starch, and nonmuscle protein, with 2.5% NaCl and 78% moisture. CR = *ι*-carrageenan; ST = starch; EW = egg white; SO = soy protein; CA = casein; GL = gluten.

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