Thermally Induced Aggregation of Giant Squid (*Dosidicus gigas*) Mantle Proteins. Physicochemical Contribution of Added Ingredients

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This paper examines the involvement of myofibrillar proteins in the thermal aggregation of giant squid muscle, and the contribution of some nonmuscle proteins (soy protein, casein, and wheat gluten), ι-carrageenan, and starch to the formation of the gel networks at two salt concentrations (1.5% and 2.5% NaCl). For this purpose, rheology, selective protein solubility, SDS−PAGE, and microstructural examination were carried out. The low gel-forming capacity of giant squid mantle coincided with the predominance of hydrophobic interactions at high temperature (<60 °C), mainly involving MHC and paramyosin. The absence of setting coincides with a relatively high incidence of hydrogen bonding at 35 °C. Soy isolate protein and casein showed a great tendency to aggregate by means of hydrophobic interactions when heated. In gels prepared at 90 °C, hydrophobic interactions were more numerous in samples with ingredients than in samples without ingredients; this was reflected by greater work of penetration except in the case of gels containing casein. The low-salt gel with added soy protein showed by SDS−PAGE more polymerization of myosin and paramyosin, which was the main cause for its more uniform microstructural appearance and its higher work of penetration.

Keywords: *Squid; gels; hydrophobic interaction; microstructure; soy; casein*

INTRODUCTION

Giant squid (*Dosidicus gigas*) is a cephalopod of the *Ommastrephes* genus found in abundance off the Pacific coast of Mexico. This species is undervalued because its mantle is too large and too soft, and it can therefore be bought very cheaply. It can, however, be profitable if its muscle is used as the protein base of thermostable gels for the manufacture of squid analogues or other products. However, like all cephalopods, immediately after capture the muscle undergoes intense proteolysis and consequently loses gel-forming capacity (Nagashima et al., 1992; Gómez-Guillén et al., 1996a). On the other hand, giant squid is normally sold in the form of frozen gutted mantles. This means that it is normally purchased in Europe after 1−3 months in frozen storage, by which time it is partially degraded; however, elastic and relatively firm gels have been achieved by adding gelling ingredients; addition of a combination of nonmuscle protein, starch, and ι-carrageenan to giant squid muscle homogenized with 1.5% NaCl (a relatively low salt content) clearly has a positive effect (Gómez-Guillén et al., 1997a; Gómez-Guillén and Montero, 1997). A study of the structure of giant squid muscle gels with these ingredients reported that at moderate temperatures (<60 °C) the starch was included in the gel matrix in the form of granules, whereas at 90 °C it was completely gelatinized (Gómez-Guillén et al., 1996b). The ι-carrageenan, which formed fine networks connecting adjacent structures within the gel (Gómez-Guillén et al., 1996b), was strongly anionic with numerous sulfate groups; it therefore possessed a high capacity to interact with fish proteins (Llanto et al., 1990) and other nonmuscle proteins such as casein (Elfak et al., 1979).

Addition of nonmuscle protein to fish gels could influence the arrangement of the myofibrillar proteins (Ziegler and Foegeding, 1990) and modify gel solubility, as has been reported in sardine gels (Gómez-Guillén et al., 1997b,c). In gels of sardine, as in other fish species, there is a pronounced tendency to setting at below 40 °C and to *modori* (thermal degradation of the gel) at 50−60 °C (Montero and Gómez-Guillén, 1996) not found in giant squid (Gómez-Guillén et al., 1996a) or other cephalopods (Ayensa, 1997). The difference in behavior of muscle of different species during thermal aggregation has been attributed both to variations in the myosin molecule (Niwa et al., 1980) and to differences in capacity for cross-linking (Lee et al., 1990).

The aim of this research was to examine the involvement of the main myofibrillar proteins of giant squid mantle in thermal aggregation through the various types of chemical bonding and to determine in rheological, biochemical, and microstructural terms how nonmuscle proteins (soy protein, sodium caseinate, and gluten), ι-carrageenan, and starch contribute to the formation of muscle networks. Two NaCl concentrations were considered (1.5% and 2.5%).

MATERIALS AND METHODS

The species used was *Dosidicus gigas*. The time elapsing from capture to arrival (frozen at −20 °C) at the laboratory

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Table 1. Chemical Interactions (Expressed as Selective Protein Solubility) and Work of Penetration of Gels Made at Different Temperatures with 2.5 and 1.5% NaCl[a]

	chemical interactions (% soluble protein)				work of penetration (N mm)
sample	nonspecific associations	ionic bonds	hydrogen bonds	hydrophobic interactions	
2.5% NaCl					
35 °C	10.73a/x	6.37b/x	21.60c/x	6.56b/x	0.17x
50 °C	10.46a/x	3.38b/y	17.42c/y	18.38c/y	3.29yz
60 °C	1.32a/y	1.18a/z	8.17b/z	32.93c/z	6.81v
90 °C	<1.0a/y	1.66b/z	3.54c/v	45.01d/v	7.77w
1.5% NaCl					
35 °C	10.77a/x	4.54b/v	26.89c/w	14.95d/y	0.37x
50 °C	9.53a/z	5.35b/xv	18.30c/xy	41.30d/v	2.02z
60 °C	<1.0a/v	1.04a/z	6.61b/z	51.66c/w	4.42y
90 °C	<1.0a/v	<1.0a/z	6.53b/z	18.68c/y	6.54v

[a] Different letters a, b, c, ..., in the same row indicate significant differences in protein solubility; different letters v, w, x, ..., in the same column indicate significant differences among gels.

was estimated as between 1 and 1.5 months. Frozen mantles were cut into small portions (about 3×3 cm^2), thus ready for homogenization, which were placed in polyethylene bags in lots of 300 g and stored at −80 °C to maintain stability over the experimental period.

NaCl was supplied by PANREAC, Montplet & Esteban S. A. Barcelona (Spain). Soy protein was used in the form of a soy isolate (product name PP 500 E) from Protein Technologies International (Gerona, Spain). Atomized-dried sodium caseinate was supplied by La Pilarica, S. A. (Valencia, Spain), and wheat gluten by Levantina Agrícola Industrial, S. A. (LAISA) (Barcelona, Spain), product name VITAL "L" Wheat Gluten. CLEARAM CH20 starch from Roquette Freres was supplied by Levantina Agrícola Industrial. 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. All other chemicals used were of reagent grade.

Homogenization of muscle with NaCl (2.5% and 1.5% on finished product weight basis) and added ingredients (non-muscle protein, 2%; starch, 5%; and ι-carrageenan, 2%) was carried out according to Gómez-Guillén et al. (1996b).

The resulting batters were stuffed into stainless-steel cylinders (inner diameter 3 cm, height 3 cm) with screw-on lids and rubber gaskets to provide a hermetic seal. At no time during this part of the process did the sample temperature exceed 10 °C. Samples were heated at 35, 50, 60, and 90 °C by immersion in a waterbath for 20 min. Immediately after heating, the cylinders were placed in recipients containing ice water (0 °C) for rapid cooling of the gel.

Protein solubility in 5% NaCl and apparent viscosity, as the protein functionality index, were carried out according to Ironside and Love (1958) and Borderías et al. (1985), respectively.

The folding test was carried out according to a 5-point grade system (Suzuki, 1981). The puncture test (cross-head speed, 10 mm/min; 100-N load-cell) and scanning electron microscopy (SEM) were carried out as described previously (Montero and Gómez-Guillén, 1996). Work of penetration was determined by multiplying the maximum breaking force (N) by the breaking deformation (mm).

Gel solubility studies commenced immediately after the newly made gels were sufficiently cool. According to Matsumoto (1980) and Montero and Gómez-Guillén (1996), gels were treated with chemicals selected for their capacity to cleave certain kinds of bonds: 0.05 M NaCl (S1), 0.6 M NaCl (S2), 0.6 M NaCl + 1.5 M urea (S3), and 0.6 M NaCl + 8 M urea (S4). Proteins were partially solubilized with these solutions in order to determine the existence of nonspecific associations (protein solubilized in S1), ionic bonds (difference between protein solubilized in S2 and protein solubilized in S1), hydrogen bonds (difference between protein solubilized in S3 and protein solubilized in S2), and hydrophobic interactions (difference between protein solubilized in S4 and protein solubilized in S3). Two grams of chopped gel was homogenized

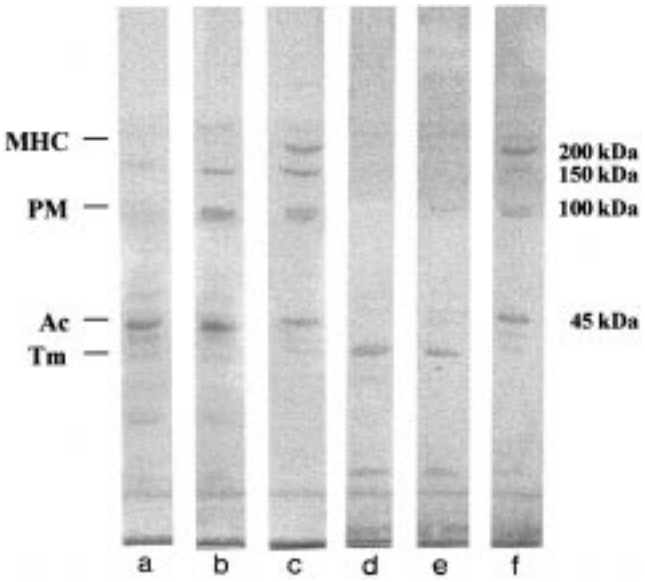

Figure 1. SDS−PAGE of proteins soluble in S1 (0.05 M NaCl), S2 (0.6 M NaCl), and S4 (0.6 M NaCl + 8 M urea). Gels made at 35 and 90 °C, with 1.5% NaCl. (a) S1, 35 °C; (b) S2, 35 °C; (c) S4, 35 °C; (d) S1, 90 °C; (e) S2, 90 °C; (f) S4, 90 °C.

with 10 mL of each solution in an Omni-Mixer, Model 17106, homogenizer (OMNI International, Waterbury, CT) for 2 min at setting 5. The resulting homogenates were stirred at 4−5 °C for 1 h and then centrifuged for 15 min at 20000*g* in a Cryofuge 20-3 centrifuge (Heraeus CHRIST GmbH, Germany). The protein concentration in the supernatants was determined following the method of Lowry et al. (1951) using a commercial preparation, DC Protein Assay Reagent S no. 500-0116 (BIO-RAD Laboratories, Hercules, CA). Results are averages of two determinations and are expressed as percent protein solubilized with respect to total protein in the gel, which was previously determined by the Kjehldahl method.

For the electrophoretic study (SDS−PAGE), supernatants obtained with S1, S2, and S4 solutions were treated according to Hames (1985) with a solution composed of 5% β-mercaptoethanol, 2.5% SDS, 10 mM Tris-HCl, 1 mM EDTA, and 0.002% bromophenol blue. They were adjusted to a final average concentration of 1 mg/mL and then heated at 100 °C for 5 min. Electrophoresis was carried out on a Phast-System horizontal apparatus (PHARMACIA LKB Biotechnology AB, Uppsala, Sweden) using 12.5% polyacrylamide gels (PhastGel, Pharmacia LKB Biotechnology). Electrophoresis conditions were 10 mA, 250 V, and 3.0 W, at 15 °C. The protein bands were stained with coomassie brilliant blue (PhastGel Blue R, Pharmacia LKB Biotechnology). As a reference for molecular masses a standard high molecular mass reference kit (Pharmacia LKB Biotechnology) was used: ferritin half-unit, 220

Table 2. Chemical Interactions (Expressed as Selective Protein Solubility) and Work of Penetration of Gels Made with Added Ingredients at Different Temperatures with 2.5 and 1.5% NaCl[a]

sample	chemical interactions (% soluble protein)				work of penetration (N mm)
	nonspecific associations	ionic bonds	hydrogen bonds	hydrophobic interactions	
ιCR−ST−SOY with 2.5% NaCl					
35 °C	7.16a/xy	14.61b/x	20.38c/x	10.71d/x	0.49x
50 °C	6.17a/y	2.20b/y	7.71a/y	40.01c/y	4.33y
60 °C	2.75a/zv	4.11b/z	1.81a/z	56.52c/z	5.72y
90 °C	2.39a/z	1.77b/y	1.76b/z	57.54c/z	8.13z
ιCR−ST−SOY with 1.5% NaCl					
35 °C	7.28a/xy	9.30b/v	13.06c/v	28.24d/v	0.98x
50 °C	7.57a/x	<1.0b/w	9.58c/w	37.43d/y	4.15y
60 °C	3.28a/v	1.08b/w	4.15a/u	50.56c/z	4.8y
90 °C	2.18a/z	2.34a/y	1.42b/z	54.50c/z	14.21v
ιCR−ST−CAS with 2.5% NaCl					
35 °C	9.63a/w	12.55b/u	17.24c/r	40.27d/y	0.65x
50 °C	6.83a/y	4.06b/z	6.96a/y	44.06c/y	3.31u
60 °C	6.03a/y	2.42b/y	6.09a/y	48.65c/yz	3.39u
90 °C	5.32a/u	1.17b/w	2.67b/s	51.38c/z	5.65y
ιCR−ST−CAS with 1.5% NaCl					
35 °C	11.61a/r	12.18ab/u	18.89c/x	13.76b/x	0.71x
50 °C	7.93a/x	4.34b/z	7.66a/y	47.83c/yz	3.99yu
60 °C	7.41a/xy	2.39b/y	4.18a/u	47.81c/yz	3.89yu
90 °C	7.56a/x	<1.0b/w	3.72c/s	60.32d/v	7.93z
ιCR−ST−GLU with 2.5% NaCl					
35 °C	7.03a/xy	10.95b/u	20.69c/x	8.39d/x	0.43x
50 °C	1.57a/z	1.01a/w	6.27b/y	20.53c/u	3.89yu
60 °C	1.36a/z	<1.0a/w	5.04b/yu	38.47c/y	4.65y
90 °C	1.48a/z	<1.0a/w	3.76b/s	29.48c/v	8.01z
ιCR−ST−GLU with 1.5% NaCl					
35 °C	7.84a/x	10.51b/u	16.17c/r	15.16c/xu	0.99x
50 °C	3.71a/v	<1.0b/w	6.95c/y	42.61d/y	3.87yu
60 °C	2.03a/z	1.27a/w	5.43b/yu	35.06c/y	4.26y
90 °C	2.48a/z	1.53a/wy	<1.0b/z	36.81c/y	9.41z

[a] ι-CR, ι-carrageenan; ST, starch; SOY, soy protein; CAS, sodium caseinate; GLU, gluten. Different letters a, b, c, ..., in the same row indicate significant differences in protein solubility; different letters r, s, t, ..., in the same column indicate significant differences among gels.

kDa; albumin, 67 kDa; catalase subunit, 60 kDa; lactate dehydrogenase subunit, 36 kDa; and ferritin subunit, 18.5 kDa.

Two-way analysis of variance (ANOVA) was carried out. The computer program used was Statgraphics (STSC Inc., Rockville, MD). The difference of means between pairs was resolved by means of confidence intervals using a least significant difference (LSD) range test. The level of significance was set for $p \leq 0.05$.

RESULTS AND DISCUSSION

Giant squid as a raw material habitually exhibits some deterioration on arrival because a month or more may go by from the time it is caught until it reaches points of sale in Europe in the frozen state. But despite this, the quality was judged acceptable on the basis of the protein functionality indexes compiled for this research (protein solubility in 5% NaCl: 61.15 ± 0.1%; apparent viscosity: 3285 ± 55 cP).

At 35 °C, the muscle homogenized with NaCl remained semiviscous and showed no sign of setting. This finding agrees with Ayensa (1997), who likewise detected no setting in fresh muscle of other cephalopod species (*Todaropsis eblanae*). At 90 °C, gels were very weak, scoring "2" in the folding test. Table 1 shows (i) protein solubility in a number of solutions selected for their capacity to disrupt certain kinds of bonds and (ii) work of penetration of giant squid gels made at different temperatures, with high- and low-salt content. The results of work of penetration have been previously described and discussed in another paper (Gómez-Guillén et al., 1996a) and here are included only to help to interpret the present work and to compare with gels with added ingredients. The results for selective protein solubility revealed a drastic decrease in the number of nonspecific associations and ionic and hydrogen bonds in gels at 60 and 90 °C with either salt concentration. This was a consequence of protein denaturation and subsequent aggregation, which reduce protein solubility and extractability through the interaction of certain proteins (Li-Chan et al., 1985; Camou et al., 1989). The formation of hydrogen bonds in thermal aggregation of giant squid muscle was particularly important at moderate temperatures (35 °C). Their effect was about 3 times greater than in frozen minced sardine, which is well-known as a species that produces setting, in the same conditions (Montero and Gómez-Guillén, 1996). On the other hand, at setting temperatures there were 2−3 times less hydrophobic interactions in giant squid than in the sardine mince referred to (Montero and Gómez-Guillén, 1996). The differences in setting behavior of the different species may therefore be attributed not only to variations in the myosin molecule (Niwa et al., 1980; Taguchi et al., 1986) and the presence of paramyosin (Sano et al., 1986), but also to differences in cross-linking capacity (Gill and Conway, 1989; Lee et al., 1990). Hydrophobic interactions increased progressively with augmented gelling temperature and clearly predominated at high temperatures (50, 60, and 90 °C), varying in degree according to salt content. The fraction insoluble in S4 corresponds to proteins aggregated by stronger bonding than hydrophobic interactions, such as covalent bonds. The decrease in hydrophobic interactions observed in the gel with 1.5% NaCl at 90 °C could be due, therefore, to extensive covalent cross-

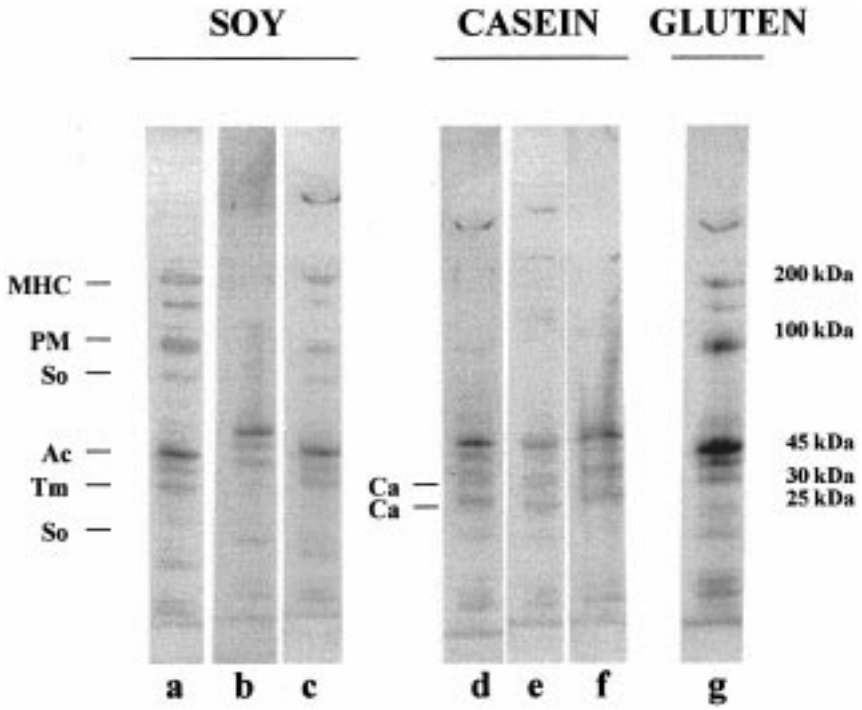

Figure 2. SDS−PAGE of proteins soluble in S1 (0.05 M NaCl) and S4 (0.6 M NaCl + 8 M urea). Gels with added *ι*-carrageenan, starch, and nonmuscle proteins made at 35 and 90 °C, with 1.5% NaCl. (a) S4, 35 °C; (b) S4, 90 °C; (c) S4, 90 °C, 2.5% NaCl; (d) S4, 35 °C; (e) S4, 90 °C; (f) S1, 90 °C; (g) S4, 90 °C.

linking; however, it did not coincide with an increased work of penetration.

For the fraction soluble in S1 (0.05 M NaCl) of muscle homogenized with 1.5% NaCl and heated at 35 °C (Figure 1a), the electrophoresis profile showed bands of molecular masses consistent with actin, tropomyosin, and proteins of lower molecular mass. This would indicate that a considerable portion of these proteins were weakly linked to the gel matrix by nonspecific associations and hence were readily extracted. Upon solubilization at higher ionic strength (S2: 0.6 M NaCl) (Figure 1b), a new band appeared with a molecular mass similar to that described for paramyosin (Sano et al., 1986). Thus, at 35 °C this protein, or at least a considerable part of it, interacted by means of relatively weak links, such as ionic bonds. In the same solubilization conditions, an appreciable amount of a polypeptide of about 150 kDa was found. According to Kolodziejska et al. (1992), this could be C protein, although it could also be a subfragment of the MHC produced by proteolytic activity (Niwa et al., 1993; Asakawa and Azuma, 1990), which is known to be very intense in squid (Nagashima et al., 1992). The MHC band was only visible in the fraction soluble in S4 (0.6 M NaCl + 8 M urea) (Figure 1c). This would indicate that MHC was the protein mainly involved in both hydrogen bonding and hydrophobic interactions, given that neither paramyosin nor the 150-kDa protein were more intensely visible in this fraction than in the lower ionic strength (S2) fraction. In the gels made at 90 °C, the principal myofibrillar proteins of giant squid (MHC, paramyosin, and actin) appeared mainly in the profile of the fraction soluble in S4 (0.6 M NaCl + 8 M urea) (Figure 1f), but traces were detected in the fraction solubilized with S2 (0.6 M NaCl) (Figure 1e). This means that at a high temperature (90 °C), MHC, paramyosin, and actin became highly aggregated through hydrophobic interactions. In most species, however, MHC is completely polymerized, mainly through disulfide bonds, and hence does not appear in electrophoresis profiles of protein extracts in similar conditions (Montero and Gómez-Guillén, 1996; Sano et al., 1990a,b; Taguchi et al., 1987). Tropomyosin was the predominant protein in fractions solubilized with S1 and S2 at 90 °C (Figure 1d,e) and was therefore the main factor in the low percentage of nonspecific associations and ionic bonds. Tropomyosin has also been found to contribute little to gel formation in other species such as sardine, hake, or chicken (Montero and Gómez-Guillén, 1996; Jimenez-Colmenero et al., 1994; Shiga et al., 1988), possibly due to its high heat resistance (Samejima et al., 1982).

Final gels (made at 90 °C) scoring maximum (5) in the folding test can be obtained by adding a combination of *ι*-carrageenan, starch, and a nonmuscle protein (soy protein, casein, or gluten) at low salt concentration (Gómez-Guillén and Montero, 1997). Formulations of this kind were therefore used to determine the involvement of nonmuscle proteins in chemical interactions during gel formation at various different heating temperatures (Table 2). The most pronounced changes in work of penetration occurred in the jump from 35 to 50 °C as the homogenate of muscle with salt and ingredients changed from sol (35 °C) to gel (50 °C). Changes in the jump from 60 °C (folding test 3) to 90 °C (folding test 4−5) were caused by complete gelation of muscle, nonmuscle proteins, and *ι*-carrageenan, and also by gelatinization of the starch (Gómez-Guillén et al., 1997a). As observed previously (Gómez-Guillén and Montero, 1997), the incorporation of gelling ingredients was essential to improve the folding test at 90 °C although not a great increase in work of penetration was observed with respect to the gel without ingredients. As regards NaCl concentration, work of penetration was higher at 90 °C in gels with low salt content (maximum folding test), especially those containing soy protein.

In analysis of the contribution of the various types of bonding to gelation (Table 2), the most important aspect

generally was the major contribution of hydrophobic interactions, particularly at more than 50 °C, as was the case in muscle without ingredients (Table 1). Moreover, the gels containing soy protein or casein exhibited a considerably higher proportion of hydrophobic interactions than muscle without ingredients. This is directly related to the intrinsic tendency of these nonmuscle proteins to aggregate by means of this type of interaction (Rha and Pradisapena, 1986; Kinsella et al., 1989). However, there is no clear relationship between this and texture at 90 °C; although work of penetration was higher in gels with soy protein than in muscle without ingredients (especially with 1.5% NaCl), work of penetration was nonetheless significantly lower in gels containing casein, which had a comparable number of hydrophobic interactions, than in gels containing soy protein. Unlike gels containing soy protein or gluten, gels containing casein all had a considerably higher proportion of nonspecific associations at all heating temperatures. This was attributed to the fact that casein in the form of sodium caseinate is highly soluble. However, the fact that there was no great temperature-related reduction in the number of nonspecific associations (especially at 90 °C) suggests that the binding of the gel structure was weaker than in gels made with added soy protein or gluten, and this did coincide with lower values of work of penetration. As for the role of ionic bonds in the various different gels with ingredients, we should note that at 35 °C and with either salt concentration, the proportion of ionic bonds was approximately double that found in the sample without ingredients. This effect was attributed to the presence of *ι*-carrageenan, which is strongly anionic and reacts with the positive charges of the proteins (Tolstoguzov, 1986). However, at higher temperatures (50−90 °C), this type of bonding played no significant part in the build-up of the gel. The same was true of hydrogen bonding.

The electrophoresis profiles of the most interesting solubilized fractions were analyzed (Figure 2). In the case of the gel containing soy protein (combined with *ι*-carrageenan and starch) and 1.5% NaCl and heated at 35 °C (not true gel), the profile of the fraction solubilized with S4 (0.6 M NaCl + 8 M urea) (Figure 2a) resembled that of muscle without ingredients in terms of myofibrillar proteins (Figure 1c). There were new bands corresponding to the various constituent proteins of the soy isolate. At 90 °C (Figure 2b), unlike the gel without gelling ingredients (Figure 1), neither MHC nor paramyosin was visible in the fraction solubilized with S4, but most of the soy isolate proteins were visible. This confirmed the involvement of soy protein in aggregation through hydrophobic interactions. In this case, the polymerization of MHC and paramyosin through bonds stronger than hydrophobic interactions may have been due to interaction with *ι*-carrageenan by way of the sulfate groups (Llanto et al., 1990), which could explain why work of penetration was higher in these low-salt gels (Table 1). At 90 °C with 2.5% NaCl (lower work of penetration), however, myosin and paramyosin appeared in the electrophoresis profile (Figure 2c), suggesting less involvement of these proteins in strong covalent bonding. The higher salt concentration could have weakened the capacity of *ι*-carrageenan and soy protein to interact with the myofibrillar proteins at high temperature.

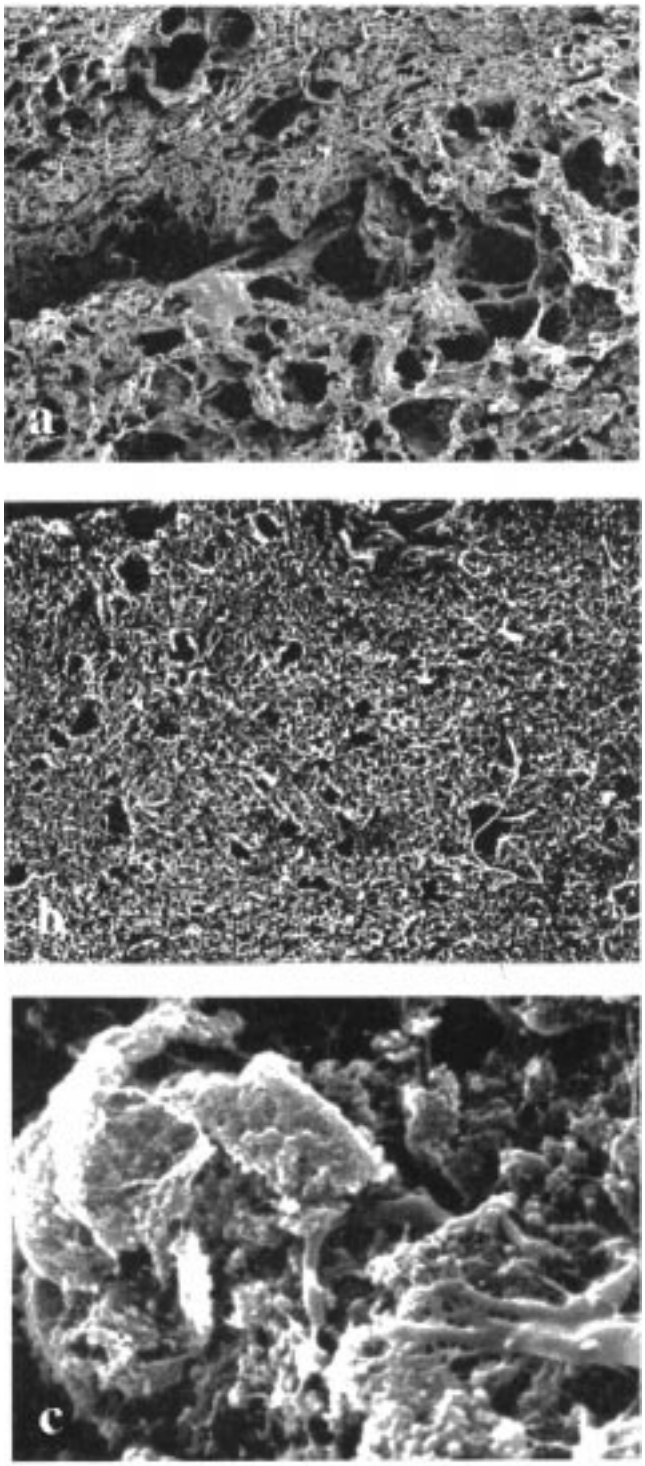

Figure 3. Scanning electron microscopy of gels made at 90 °C with added *ι*-carrageenan, starch, and soy protein, with different salt content. (a) 1.5% NaCl (29×); (b) 2.5% NaCl (29×); (c) 1.5% NaCl (4980×).

The S4-solubilized fraction of muscle homogenized at 35 °C with 1.5% NaCl and *ι*-carrageenan, starch, and casein (Figure 2d) exhibited no bands for MHC or paramyosin, which was consistent with the low percentage of hydrophobic interactions found in this sample (Table 2). At this temperature, the bands chiefly involved in hydrophobic interactions were α- and *β*-casein along with actin, tropomyosin, and troponins. The most characteristic feature of the corresponding gel made at 90 °C was that α-casein (30 kDa) was clearly visible in

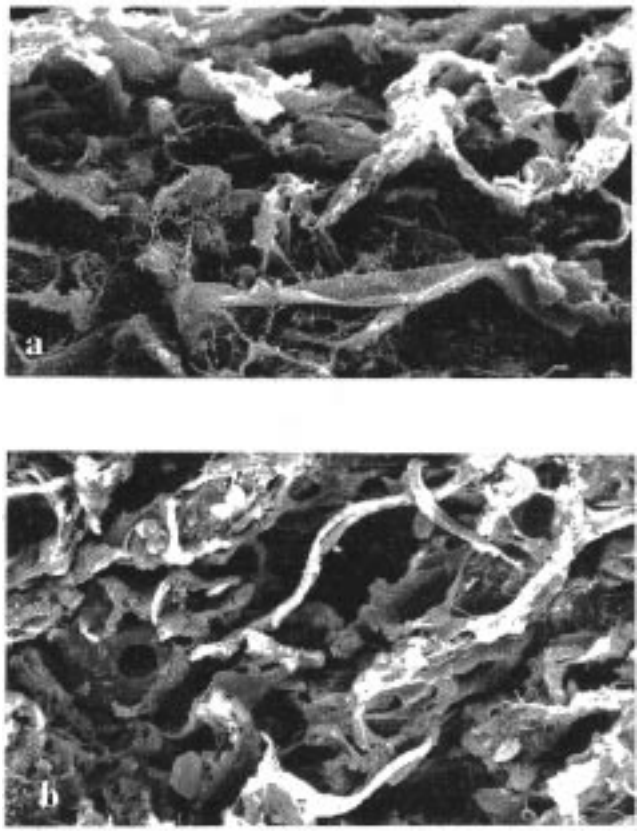

Figure 4. Scanning electron microscopy of gels made at 35 and 90 °C with added *ι*-carrageenan, starch, and casein, with 1.5% NaCl. (a) 35 °C (380×); (b) 90 °C (380×).

the profiles of the fractions solubilized with S4 (Figure 2e) and with S1 (Figure 2f). This indicates that the α-casein was partially aggregated by hydrophobic interactions but also remained largely associated with the gel matrix nonspecifically. This is why despite the fact that both MHC and paramyosin were aggregated by bonds stronger than hydrophobic interactions, the gel containing casein exhibited lower work of penetration than the gel containing soy protein and at the same time had a higher proportion of nonspecific associations. As to gels with added gluten, because of the high molecular weight of its constituent, its high insolubility, and its tendency to form large aggregates (Chung and Lee, 1991), gluten did not appear in any of the profiles of the various solubilized fractions. What was found, however, was that at high temperature (90 °C) gluten favored aggregation of the principal myofibrillar proteins (MHC, paramyosin, and actin). This took place chiefly through hydrophobic interactions and hydrogen bonds, judged by its appearance in the profile of the S4-solubilized fraction of the gel made at 90 °C (Figure 2g).

When the microstructures of gels containing soy protein and 1.5%/2.5% NaCl prepared at 90 °C were compared by SEM (Figure 3a,b), the overall appearance (29×) of the high-salt gel was spongy, with large heterogeneous cavities. The appearance of the low-salt gel was much more uniform, which coincided with more cross-linking or polymerization of myosin and paramyosin as observed by SDS−PAGE (Figure 1c); this greater polymerization was the main reason work of penetration was higher in the gel with 1.5% NaCl. Pictures at 4980× magnification showed small networks (Figure 3c), attributed chiefly to *ι*-carrageenan, which helped make the muscle matrix more open and structured.

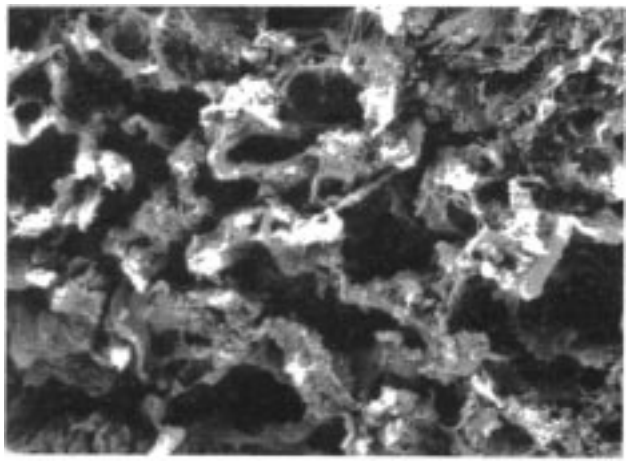

Figure 5. Scanning electron microscopy of gels made at 90 °C with added *ι*-carrageenan, starch, and gluten, with 1.5% NaCl.

Such a reticular arrangement of *ι*-carrageenan in giant squid gels, and also of starch being granulated at 35 °C and gelatinized at 90 °C, had been reported previously in gels containing egg white as nonmuscle protein (Gómez-Guillén et al., 1996b). Soy protein was found adhering to the matrix in the form of discrete irregular clusters, largely hydrophobic in nature (Figure 3c). In the "gel" heated at 35 °C, the casein was distributed fairly evenly through the matrix as a discontinuous mesh with little aggregation (Figure 4a). When samples were heated at 90 °C, the casein clusters were considerably denser and more compact than at 35 °C (Figure 4b), which would confirm the tendency of casein to aggregate by means of hydrophobic interactions when heated (Figure 2); this would further hinder interaction with the medium and favor nonspecific associations reducing work of penetration (Table 2). Gluten was distributed very loosely about the matrix in highly compact clusters of varying sizes, resulting in numerous irregular cavities and cracks providing low values of work of penetration (Figure 5).

CONCLUSIONS

In giant squid mantle, low gel-forming capacity coincided with the predominance of hydrophobic interactions at high temperature (<60 °C), mainly involving MHC and paramyosin. Unlike other species, MHC is not completely polymerized by covalent bonding (disulfide or other kinds). The absence of setting in this species coincides with a relatively high incidence of hydrogen bonding at 35 °C accompanied by linkage of MHC by hydrophobic interactions and of paramyosin by ionic bonding. In gels prepared at 90 °C, hydrophobic interactions were more numerous in samples with ingredients than in samples without ingredients; this was reflected by greater work of penetration except in the case of gels containing casein, where there were more nonspecific associations due to the tendency of casein to aggregate and not to interact with the muscle matrix.

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