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Influence of added salt and non-muscle proteins on the rheology and ultrastructure of gels made from minced flesh of sardine (*Sardina pilchardus*)

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A study of rheological and ultrastructural properties of gels made from sardine was undertaken in order to examine the effects of adding non-muscle proteins (egg white, soy protein, casein and gluten) at different gelling temperatures. The highest values for gel strength, breaking force and breaking deformation were obtained with 2.5% salt content, which produced a more homogeneous gel matrix. *Suwari* gels containing soy protein exhibited greater gel strength, while for gels cooked at 90°C the gel strength was highest in those containing egg white and a true three-dimensional network was displayed. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The typical elastic nature of fish *kamaboko* gels is achieved through the formation of a three-dimensional mesh structure which develops when minced muscle, homogenized with NaCl, is heated (Miyake *et al.*, 1971; Lanier & Lee, 1992). By examining the microstructure and the changes occurring within it, data can be derived which bear directly on textural parameters (Stanley & Tung, 1976; Sato & Tsuchiya, 1992). Thus, very elastic gels exhibit a uniform microstructure in which dense regions are prevalent. Gels of low elasticity and gel strength, on the other hand, exhibit numerous cavities of varying sizes, presenting an inhomogeneous overall appearance (Sato & Tsuchiya, 1992). During heating, which is necessary for gel stability, muscle proteins undergo aggregation, the result of which is a mesh whose intervening cavities receive the water expelled as a result of protein syneresis (Hermansson, 1986; Montejano *et al.*, 1984). The extent to which syneresis occurs is directly related to the amount of unbound water present and will determine the size of the cavities (Hermansson, 1986). Cavity size, as noted above, influences gel texture characteristics.

The textural characteristics of gels made from fish mince with added gelling ingredients are determined by such factors as the continuity and properties of the muscle matrix itself, the rheological and hydrodynamic properties of the dispersed ingredient and the extent of interaction between the continuous matrix and the ingredient (Lee & Chung, 1990). By homogenizing minced muscle with NaCl, myofibrillar proteins are dissolved, thus facilitating polymerization. The dissolved myosin combines with actin filaments to form actomyosin. Both myosin and actomyosin play an essential role in gelation of muscle (Niwa *et al.*, 1980). The myofibrillar proteins of some fish species remain soluble at very low ionic strength and as a result can gel with a very low salt concentration, or even without any salt at all (Chung *et al.*, 1993).

The object of this research was to compare the behaviour of different added non-muscle proteins in gelation of sardine mince at a variety of temperatures, and to analyse the influence of NaCl concentration on rheological characteristics and microstructure.

MATERIALS AND METHODS

The fish used in these experiments were sardine of the species *Sardina pilchardus* (Walbaum), caught off Nantes in June 1993. Minced fish flesh was prepared immediately after the fish were brought on board, using the following procedure: sardines were headed, gutted and washed; skin and bones were removed with a Baader

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Model 694 deboning machine (Baader Co., Lübeck, Germany); 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), stirring constantly; the mixture 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 the Instituto del Frío, where they were stored at −80°C in a Revco vertical freezer cabinet in order to minimize alteration during frozen storage for the duration of the experiment.

Homogenization of muscle with NaCl and added non-muscle proteins

The washed sardine mince was semi-thawed and placed in a refrigerated vacuum homogenizer (Model UM5; Stephan u. Söhne, Germany). The muscle was ground for 1 min at high speed (rotor angular velocity 3000 rpm). Sodium chloride (Panreac, Barcelona, Spain) was then added with crushed ice to give the required final gel moisture (75%) and the mixture was further homogenized for 5 min at low speed (1500 rpm) in vacuum conditions. One of the non-muscle proteins was then added (2%) and homogenization continued for a further 5–7 min.

Atomized-dried egg white from Sanofi was used. Soy protein was used in the form of a soy isolate (commercial name PP 500 E, from Protein Technologies International). Atomized-dried sodium caseinate was supplied by La Pilarica. Wheat gluten was supplied by Levantina Agrícola Industrial (LAISA), under the commercial name VITAL 'L' Wheat Gluten.

Heat treatment

The resulting batters were packed into stainless steel cylinders (3 cm×3 cm i.d.) with screw-on lids and rubber gaskets to provide a hermetic seal. At no time during this part of the process did sample temperature exceed 10°C. Samples were heated at 35°C, 50°C, 60°C and 90°C by immersion in a waterbath for 50 min. Samples for prior setting were preincubated at 35°C for 30 min and afterwards heated at 90°C for 50 min. Immediately after heating, the cylinders were placed in recipient vessels containing ice-water for rapid cooling of the gel. They were then stored in a cold room at 4°C for 24 h before analysis.

Rheology: puncture test

Cylindrical samples (3 cm×3 cm) were removed from the moulds and tempered to about 20°C. Gels were pierced to breaking point using a texturometer (Model 4501; Instron Engineering Corp., Canton, MA, USA) with a 5 mm diameter, round-ended metal probe. Crosshead speed was 10 mm min^{-1} and a 100 N load-cell was used. Gel strength was determined by multiplying maximum breaking force (N) by breaking deformation (mm). All determinations were carried out at least in quadruplicate.

Scanning electron microscopy (SEM)

Cubes of 2–3 mm were cut from inside the gels for microscopic examination. Samples were fixed in 2% glutaraldehyde in phosphate buffer (pH 7.3) and dehydrated in increasing concentrations of acetone (from 40% to 100%). They were then critical-point dried with CO_2 as transition fluid in a Balzer Model CPD030 dryer and mounted on copper sample holders, followed by sputter-coating with gold in a Balzer Model SCD004 metallizer. Samples were kept in a dryer until examination by a Jeol scanning microscope (JSM 6400) at 20 kV.

Statistical analysis of data

Two-way analysis of variance (ANOVA) was carried out for the different samples. The computer program used was STATGRAPHICS (STSC Inc., Rockville, USA). 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 $P \leq 0.05$.

RESULTS AND DISCUSSION

Rheology

Tables 1–3 show gel strength, breaking force and breaking deformation, respectively, of sardine mince gels containing egg white, soy protein, casein or gluten, and made up with 2.5% or 1.5% NaCl, when subjected to different heat treatments. It is worth noting that all gels made at 35°C scored the maximum in the folding test. The highest gel strength was found in the gel containing soy protein and 2.5% salt. The breaking force of this gel was greater than that of the rest, but breaking deformation did not differ significantly from that of equivalent gels containing casein or gluten. This is consistent with the findings of MacDonald *et al.* (1994), who claim that the most important effect of preincubation or setting is to increase the stress/rigidity of gels rather than strain or deformation. Gel strength at this temperature was considerably lower in the gel with egg white than in the gel with soy protein; this could be because egg white begins to gel at temperatures above 62°C (Goldsmith & Toledo, 1985). In any event, *'suwari'* gels were obtained at this temperature. This type of gel has certain peculiar characteristics (high elasticity and translucent appearance) which distinguishes

Table 1. Gel strength of mince gels containing non-muscle proteins, made at various temperatures with 75% moisture and 2.5% NaCl or 1.5% NaCl

Formula	35°C	50°C	60°C	90°C	35°C/90°C
2.5% NaCl					
Egg white	18.31 abc/x	18.94 a/xy	17.50 a/x	24.81 a/y	111.53 a/z
Soy protein	32.89 d/x	4.12 b/y	5.57 b/y	10.57 b/z	75.23 b/u
Casein	19.59 ab/x	—	—	2.79 c/y	52.48 cd/z
Gluten	21.43 b/x	—	—	3.59 c/y	58.67 c/z
1.5% NaCl					
Egg white	15.57 ace/x	11.72 c/xy	12.39 c/x	10.04 b/y	58.83 c/z
Soy protein	16.13 ace/x	3.82 b/y	4.22 b/y	5.23 c/y	71.45 b/z
Casein	12.27 e/x	—	—	3.89 c/y	39.54 e/z
Gluten	14.42 ce/x	—	—	4.06 c/y	46.53 de/z

Significant ($P \leq 0.05$) differences for the same row are indicated by letters (a,b,c,d).
Significant ($P \leq 0.05$) differences for the same column are indicated by letters (x,y).

Table 2. Breaking force of mince gels containing non-muscle proteins, made at various temperatures with 75% moisture and 2.5% NaCl or 1.5% NaCl

Formula	35°C	50°C	60°C	90°C	35°C/90°C
2.5% NaCl					
Egg white	1.67 a/x	1.54 a/y	1.52 a/y	2.10 a/z	7.40 a/u
Soy protein	2.50 b/x	0.65 c/y	0.69 c/y	1.21 b/z	5.60 b/u
Casein	1.59 a/x	—	—	0.57 c/y	3.78 c/z
Gluten	1.74 a/x	—	—	0.66 cd/y	4.37 cd/z
1.5% NaCl					
Egg white	1.54 a/x	1.24 b/y	1.24 b/y	1.29 b/y	5.30 be/z
Soy protein	1.62 a/x	0.62 c/y	0.58 c/y	0.84 e/y	5.39 be/z
Casein	1.25 c/x	—	—	0.71 cde/y	3.17 f/z
Gluten	1.55 a/x	—	—	0.79 de/y	3.74 f/z

Significant ($P \leq 0.05$) differences for the same row are indicated by letters (a,b,c,d).
Significant ($P \leq 0.05$) differences for the same column are indicated by letters (x,y).

Table 3. Breaking deformation of mince gels containing non-muscle proteins, made at various temperatures with 75% moisture and 2.5% NaCl or 1.5% NaCl

Formula	35°C	50°C	60°C	90°C	35°C/90°C
2.5% NaCl					
Egg white	10.91 ab/x	12.25 a/x	11.46 a/x	11.84 a/x	15.12 a/y
Soy protein	13.13 c/x	6.29 b/y	8.12 bc/z	8.70 b/z	13.40 bc/x
Casein	12.33 c/x	—	—	6.77 cd/y	13.85 c/x
Gluten	12.29 c/x	—	—	5.47 e/y	13.42 bc/x
1.5% NaCl					
Egg white	10.09 bd/x	9.45 c/x	9.95 ab/x	7.74 bd/y	11.09 d/x
Soy protein	9.84 bd/x	6.11 b/y	7.19 c/z	6.20 ce/y	13.18 bc/u
Casein	9.76 bd/x	—	—	5.46 e/y	12.43 be/z
Gluten	9.29 d/x	—	—	5.09 e/y	12.41 be/z

Significant ($P \leq 0.05$) differences for the same row are indicated by letters (a,b,c,d).
Significant ($P \leq 0.05$) differences for the same column are indicated by letters (x,y).

it from gels cooked at high temperature (Montejano *et al.*, 1984).

At 50°C and 60°C, temperatures associated with the occurrence of *modori*, there was a pronounced drop in gel strength, breaking force and breaking deformation in gels with soy protein. Deterioration of texture was so great in gels with casein or gluten that the puncture test could not be performed. Only the gels with egg white retained comparable gel strength to that obtained at 35°C (folding test score: 5) with either NaCl concentration. This may be attributed to a possible inhibitory effect produced by egg white on the muscle proteases that induce *modori*, by means of a sulphydryl–disulphide interchange reaction between albumin and muscle protease (Niwa *et al.*, 1975; Chang-Lee *et al.*, 1989; Akazawa *et al.*, 1993), or else to the stabilizing effect of the non-muscle protein on the pre-gel forming at low temperature, which thus prevents degradation.

Of gels cooked directly at 90°C, only the one containing egg white and 2.5% salt exhibited higher gel strength and breaking force than gels cooked at lower temperatures. Values for the other formulae were

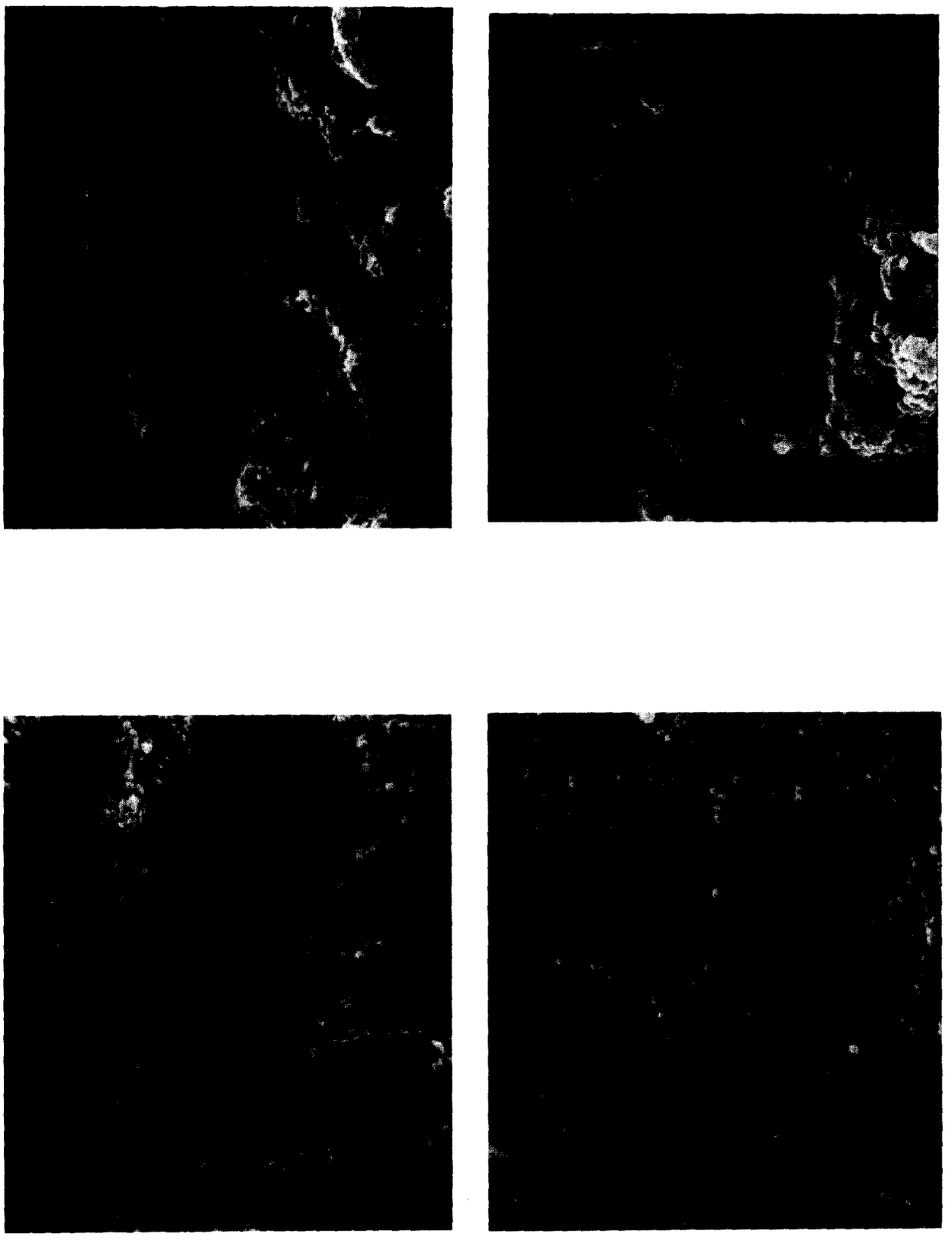

Fig. 1. Scanning electron micrographs (×6000) of mince gels containing egg white and 2.5% NaCl (A: top left), egg white and 1.5% NaCl (B: top right), soy and 2.5% NaCl (C: bottom left), soy and 1.5% NaCl (D: bottom right).

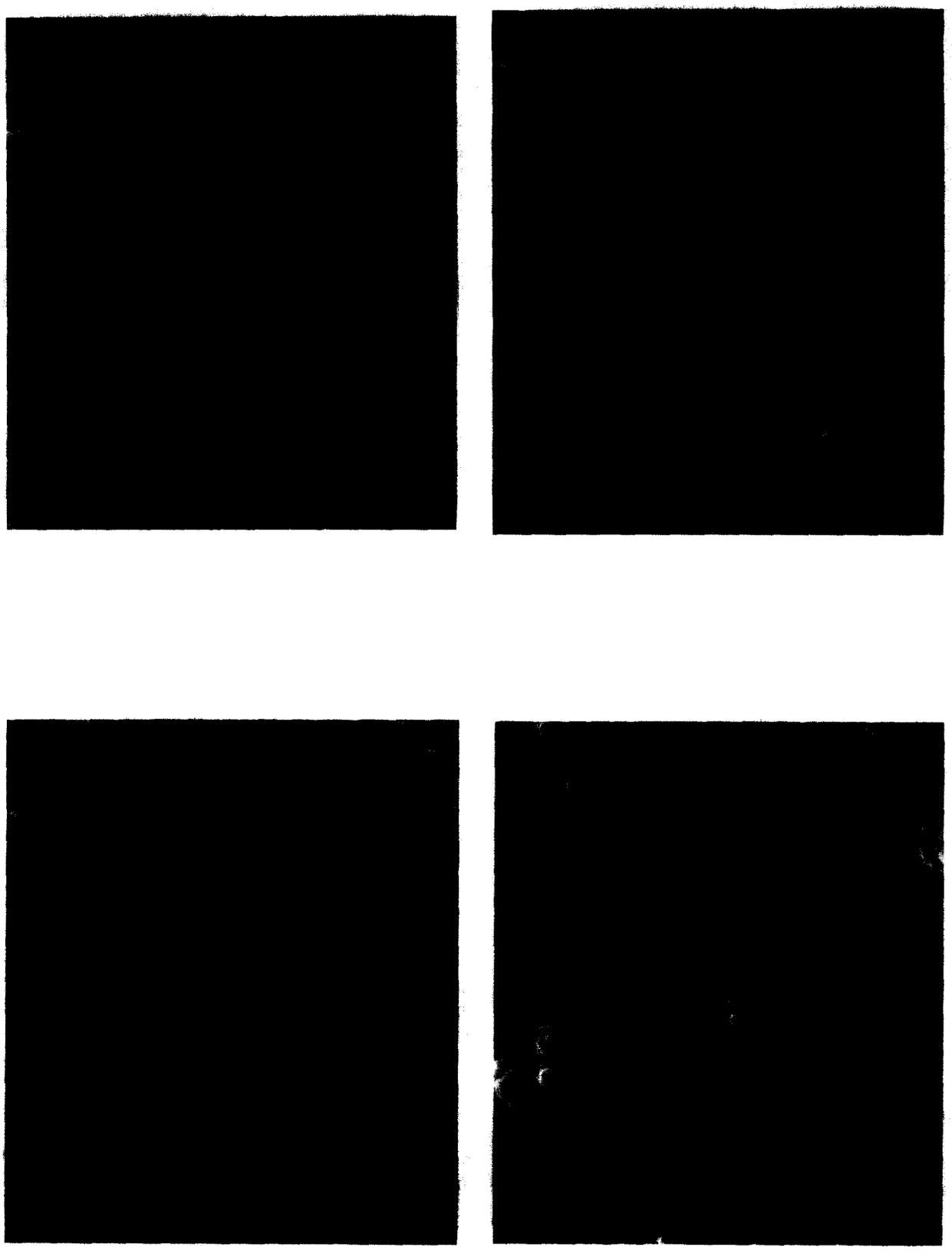

Fig. 1. Casein and 2.5% NaCl (E: top left), casein and 1.5% NaCl (F: top right), gluten and 2.5% NaCl (G: bottom left), and gluten and 1.5% NaCl (H: bottom right), made at 35°C.

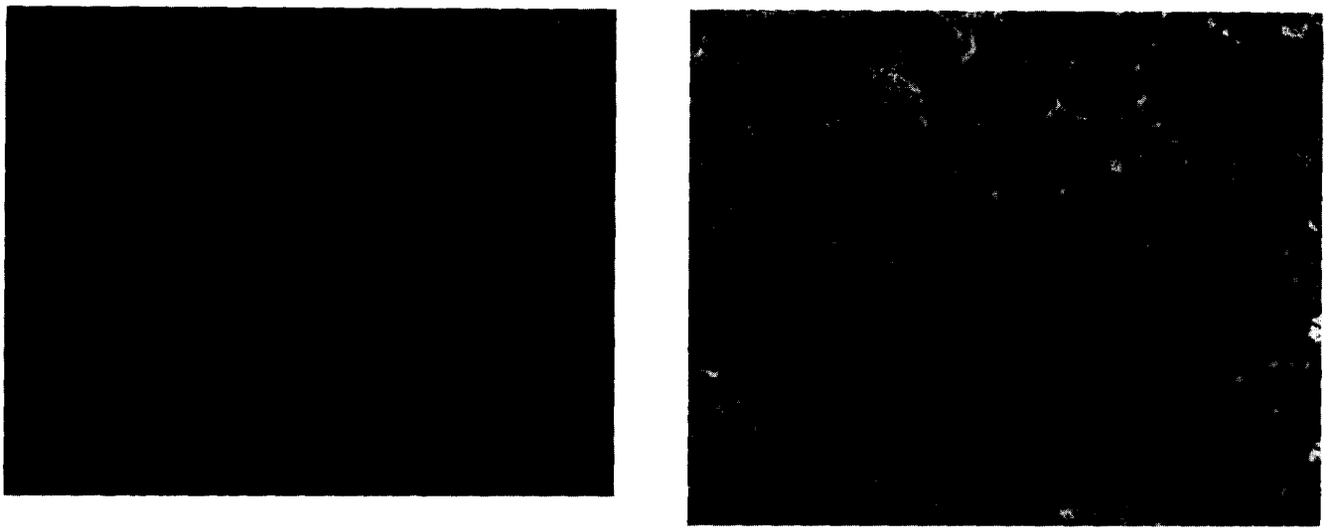

Fig. 2. Scanning electron micrographs (×500) of mince gels containing egg white (A: left) and soy (B: right) made at 35°C with 2.5% NaCl.

significantly lower. According to Lee & Chung (1990), this is because there is extensive protein syneresis and proteins do not unfold properly, resulting in a less stable matrix (Foegeding *et al.*, 1986; Saliba *et al.*, 1987). Addition of egg white thus prevents excessive, uncontrolled muscle protein aggregation.

Where pre-incubation or setting was carried out at 35°C prior to final cooking at 90°C, however, all gels scored the maximum in the folding test and gel strength increased significantly with respect to the other treatments assayed. The highest values were given by the gel with egg white and 2.5% NaCl. Increased gel strength was due to a very sharp rise in breaking force in all cases, but particularly in the gel with egg white. Breaking deformation in gels with soy protein, casein or gluten did not vary significantly with respect to gels made at 35°C. With 1.5% NaCl, on the other hand, the gel with soy protein attained greater gel strength than the others. Breaking force of this gel was significantly higher than that of the gels containing casein or gluten, and similar to that of the gel with egg white. In this latter gel, however, breaking deformation was significantly lower than in the rest. The setting phase involves slower, more progressive heating, giving the proteins more time to unfold and interact; the result is a more orderly matrix and hence a stronger, more elastic, gel (Hermansson, 1979).

Ultrastructure

Figure 1 shows SEM images at ×6000 magnification of gels made at 35°C (*suwari*), with each of the experimental non-muscle proteins and with 2.5% or 1.5% NaCl. In general, the appearance of the matrices was more irregular in gels made with the lower salt concentration than with the higher salt concentration and the muscle protein appeared much more compact, which caused the formation of large cracks and cavities of uneven size. With the higher salt concentration, there is more extensive solubilization of myofibrillar proteins, thus favouring the monomer form of myosin (Burgarella *et al.*, 1985). Gel microstructures are thus more uniform (Wicker *et al.*, 1986; Sato & Tsuchiya, 1992). The structure of the matrix in gels containing egg white or soy protein and 2.5% NaCl appeared to be a relatively homogeneous three-dimensional network. Micrographs taken at ×500 magnification (Fig. 2) show the matrix of the gel containing soy protein as better formed and more uniform than that of the gel with egg white, which would explain why gel strength, breaking force and breaking deformation were significantly higher in the former case. The same finding was reported by Hermansson (1979) and Beveridge *et al.* (1984). With 1.5% NaCl, egg white and soy protein underwent considerably more aggregation, thus contributing to lower gel strength values, especially in the gel with soy protein. On microscopic examination, the matrix of gels containing casein or gluten (Fig. 1) appeared far more compact; the muscle protein was condensed in large, amorphous, structureless masses, with no sign of the formation of a true three-dimensional network. Casein and gluten were arranged in randomly distributed clusters, which were of considerable size in the case of gluten.

Figure 3 shows micrographs (×500) of gels cooked at 90°C, with both salt concentrations. Only in gels containing egg white did the matrix have a reticulate appearance, with round, evenly sized pores. The egg white was evenly distributed throughout the matrix, in the form of small, light-coloured nuclei. In the gel with 1.5% NaCl, the mesh was rather more irregular and the pore size noticeably larger. Where the myofibrillar protein had not been fully solubilized, the aggregation occurring during heating was accompanied by more extensive protein syneresis. As water was expelled, the muscle protein retracted and a number of holes or

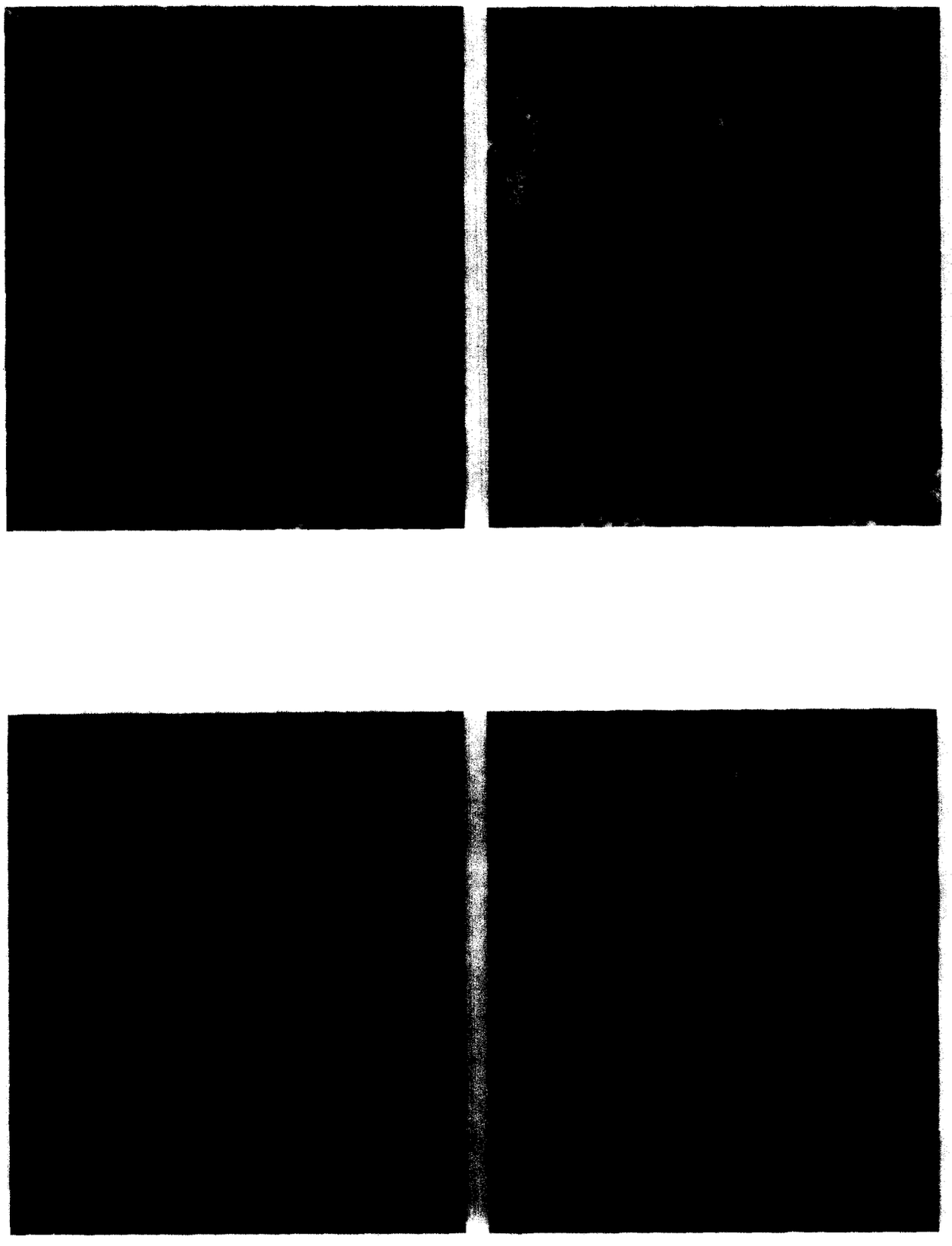

Fig. 3. Scanning electron micrographs (×500) of mince gels containing egg white and 2.5% NaCl (A: top left), egg white and 1.5% NaCl (B: top right), soy and 2.5% NaCl (C: bottom left), soy and 1.5% NaCl (D: bottom right).

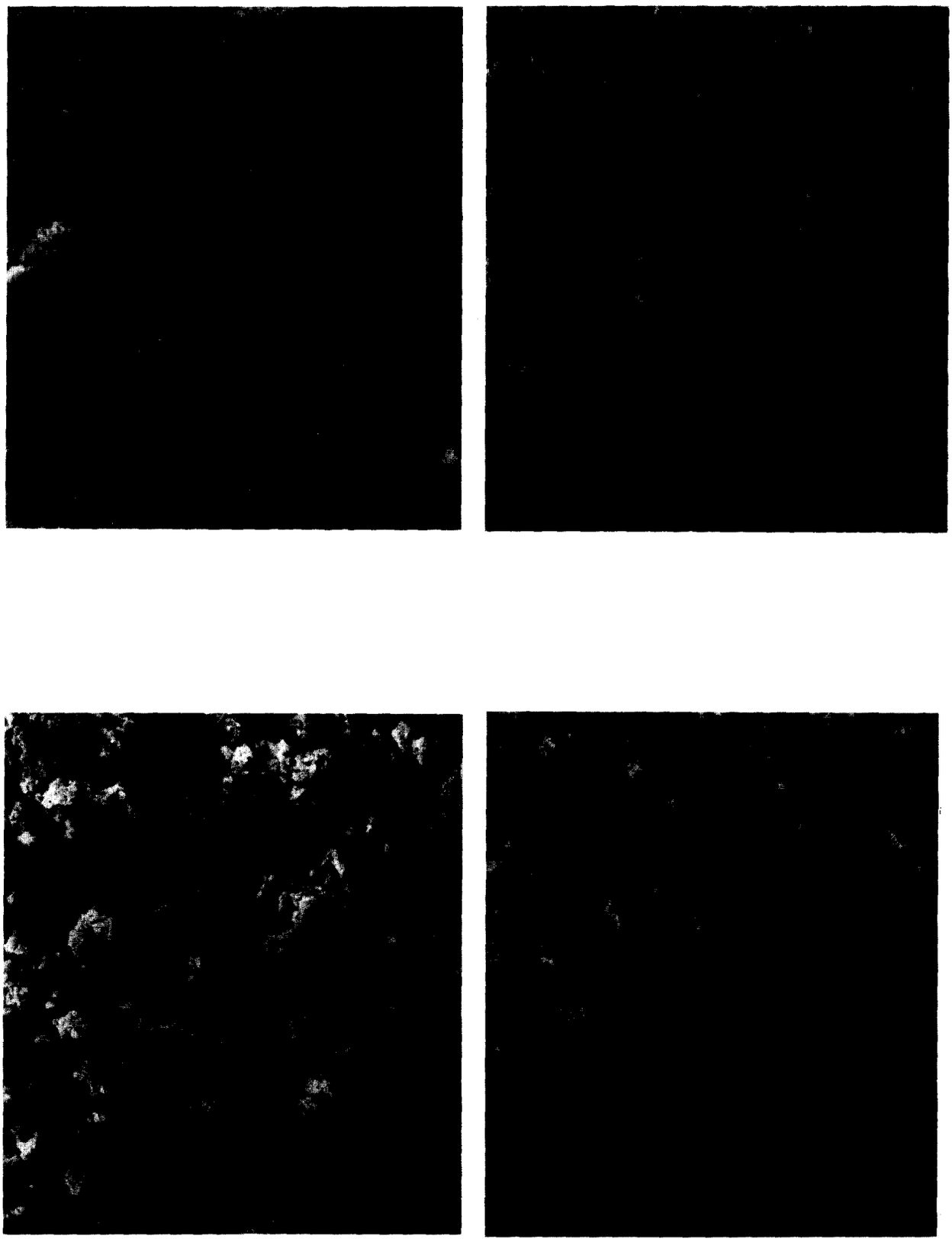

Fig. 3. Casein and 2.5% NaCl (E: top left), casein and 1.5% NaCl (F: top right), gluten and 2.5% NaCl (G: bottom left), and gluten and 1.5% NaCl (H: bottom right), made at 90°C.

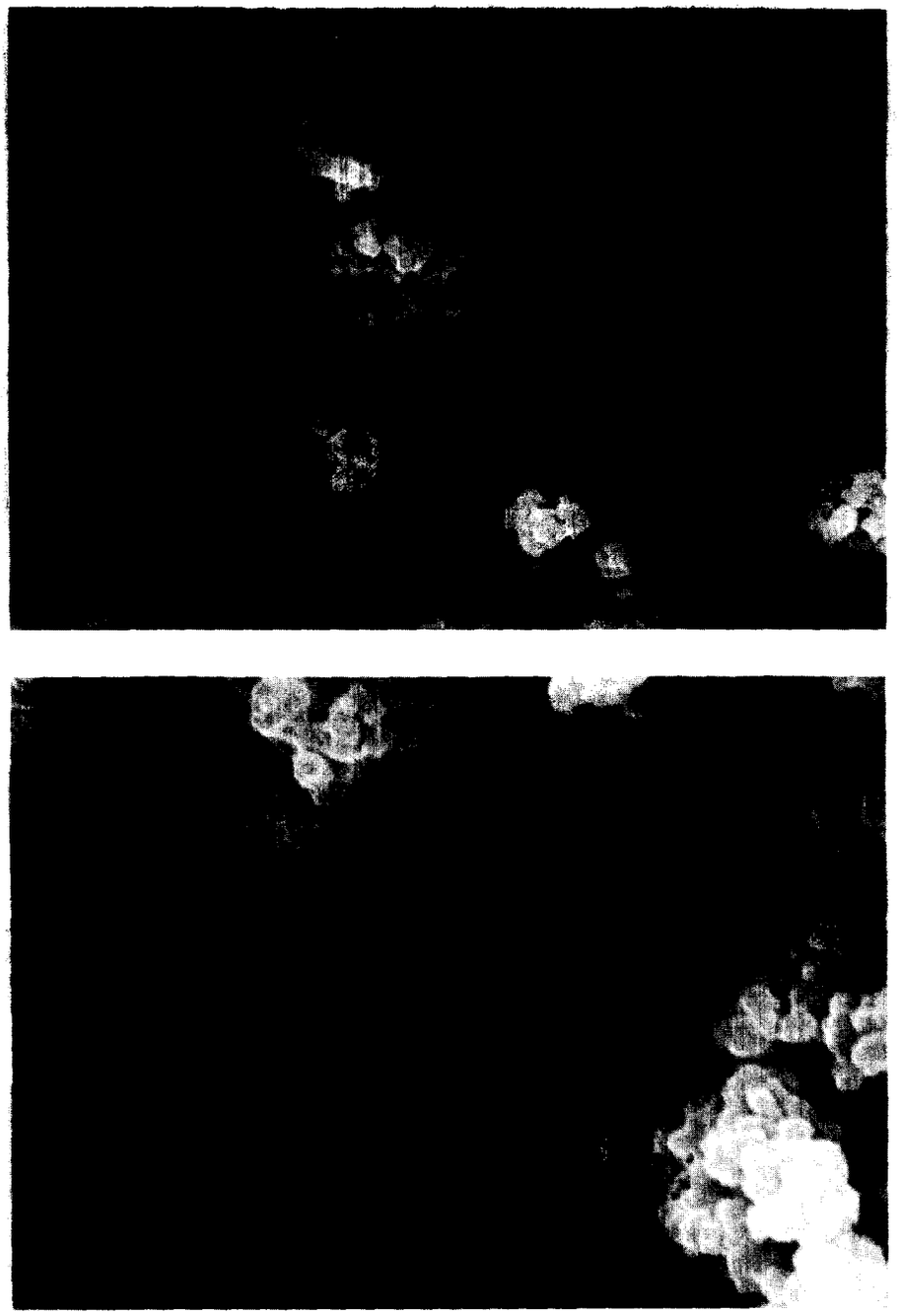

Fig. 4. Scanning electron micrographs (×6000) of mince gels containing casein (A: top) and gluten (B: bottom) made at 90°C with 2.5% NaCl.

cavities appeared, their size being determined by the amount of water expelled (Hermansson, 1986). There was a negative relationship between the size of these cavities and gel strength, which was lower in the gel with less salt. Egg white too was visibly more aggregated in the gel with 1.5% NaCl, where it appeared in the form of clusters of larger size than in the gel with 2.5% NaCl.

Gel with soy protein cooked at 90°C with 2.5% NaCl lost the typically reticular structure it displayed at 35°C. Muscle protein exhibited an aggregated morphology, with deep cracks and unevenly sized cavities in between. There was some lengthwise fibrillar orientation, however, which was not apparent in equivalent gels containing casein or gluten. This would explain why gel strength was significantly higher in gels with soy protein than in gels with casein or gluten. In any event, direct cooking of these gels at 90°C clearly produced excessive and haphazard protein aggregation, giving rise to a less stable structure. Microscopic examination (×6000) of the gel with casein and 2.5% NaCl made at 90°C (Fig. 4) showed large white-coloured clusters formed by aggregation of small, spherical particles of low electronic density, which were attributed to casein. According to Kinsella *et al.* (1989), α- and β-casein are highly amphipathic molecules with a strong tendency to associate in the form of micelles by means of hydrophobic interactions

and hydrogen bonds. This propensity to self-interaction of casein interferes with cross-linking of myofibrillar proteins, chiefly myosin, to form an adequate gel network. Examination (×6000) of the gel containing gluten with 2.5% NaCl and cooked at 90°C showed a very loose reticular structure, and other thick, uneven structures in which the gluten again appeared highly aggregated, in large, lighter-coloured clusters (Fig. 4).

As regards the influence of NaCl content, the difference was very marked in gels with soy protein, which displayed a much denser, more aggregated morphology with 1.5% NaCl than with 2.5% NaCl, gel strength being lower in the former. With casein or gluten, on the other hand, the appearance of gels with either salt concentration was very similar and there were no significant differences in gel strength.

CONCLUSIONS

When gels made from minced sardine flesh were fabricated with low salt concentration (1.5% NaCl), their gel matrix exhibited a less uniform and more aggregated morphology than with 2.5% salt, and consequently lower gel strength. With the four non-muscle proteins assayed, gelation at 35°C produced *suwari* gels according to rheological tests. At the microscopic level, however, only with the addition of egg white or soy protein was there evidence of formation of a true three-dimensional network at that temperature. At 90°C, only the gels containing egg white exhibited a genuine reticular structure. With the other non-muscle proteins, the matrix presented a very disorganized appearance, with a good deal of compacting. This is consistent with the gel strength findings.

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