ELSEVIER

Food Chemistry 50 (1994) 185–190

0308-8146/94/$7.00

Gelation of shark myofibrillar proteins by weak organic acids

V. Venugopal,* S. N. Doke & P. M. Nair

Food Technology & Enzyme Engineering Division, Bhabha Atomic Research Centre, Bombay-400 085, India

(Received 14 May 1993; revised version received and accepted 2 July 1993)

Washed, collagen-free shark muscle protein dispersions in water formed gels associated with an increase in viscosity when the pH was lowered to 4·5 by either acetic acid or lactic acid, while citric, tartaric and hydrochloric acids were ineffective in causing the gelation and viscosity rises. The increase in viscosity, as measured by the Brabender viscograph, was dependent upon the protein concentration and was a slow process at ambient temperature. Heating of the acidified proteins up to 50°C enhanced the viscosity, while at higher temperatures the gel broke as indicated by a rapid fall in viscosity as well as separation of water. The presence of NaCl, KCl and $CaCl_2$ inhibited the low-pH-induced gelation of the proteins.

INTRODUCTION

Gelation is the cross-linking of randomly dispersed polymer chains in solution to form a three-dimensional network which immobilises liquid in the matrix (Asghar *et al.*, 1985; Smith, 1991; Ziegler & Foegeding, 1991). The sequence of events in gel formation includes initial denaturation to cause protein unfolding, protein–protein interactions and aggregations giving rise to matrices capable of holding water, fat or other components through physico-chemical forces. The forces responsible for the phenomenon include hydrophobic interactions, hydrogen bonding, electrostatic interactions and disulphide cross-bondings (Mulvihill & Kinsella, 1987). Factors which influence gel formation of proteins include concentration (Camou *et al.*, 1989; Foegeding *et al.*, 1991), extractability (Grabowska & Sikorski, 1976), the myosin–actin ratio in the case of muscle proteins (Yasui *et al.*, 1980), heating conditions (Camou *et al.*, 1989; Smith, 1991), pH (Fretheim *et al.*, 1985) and ionic strength (Beas *et al.*, 1988). In muscle systems, myosin is the major gelling protein, while actin can enhance its gelation (Yasui *et al.*, 1980; Foegeding *et al.*, 1991). Gelation has significance in food processing due to opportunities to develop items having varying textural properties (Lanier, 1991).

The ability of fish protein to undergo gelation was recognised mainly with the understanding of the properties of 'surimi', a concentrate of washed fish myofibrillar proteins, which is used for gel products such as shellfish analogues (Lee, 1986; Venugopal, 1992). Sharks, rays and skates are major low-cost fish items belonging to the Elasmobranch family, which forms about 5% of the annual marine landings in India (Govindan, 1972). These items offer large quantities of proteins for the development of value-added products. Shark muscle has been used to prepare kamaboko products based on its gel-forming ability (Suzuki, 1981; Nakamura *et al.*, 1985). For this, the muscle was repeatedly washed to enhance its gelation ability. This paper reports on the gelation of shark myofibrillar proteins using acetic or lactic acid and the effect of temperature on it. The gelation of the protein samples was monitored by measuring viscosity changes using a Brabender viscograph.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Fish

Fresh Indian dog shark (*Scoliodon laticaudus*) was purchased from Bombay market. The fish were beheaded, eviscerated, deskinned and were brought to the laboratory in ice.

Preparation of washed meat dispersion

After washing in potable water, the dressed fish was cut into steaks of 4–5 cm in thickness. Using kitchen scissors, the central bone was removed and the meat was further cut into small pieces, weighing about 4–5 g. The meat pieces (600 g) were held in three times their weight of cold water with gentle stirring for 18 h 0–2°C. The wash water was then decanted and the meat pieces were washed three times in an excess of cold water. The washing step removed significant amounts of

soluble and odour-bearing compounds as well as pigments. The washed meats (200 g batches) were blended in 400 ml of cold water (10°C) in a 'Sumeet' kitchen mixer for 1 min.

Removal of collagen

The meat homogenate was poured into a nylon net (mesh size, 100) and was gently stirred. The collagen was retained on the mesh and the filtrate containing myofibrillar proteins was collected.

Gel formation experiments

The myofibrillar protein fraction (500 ml) was poured into the stainless-steel cup (capacity, 500 ml) of a Brabender viscoamylograph, which is essentially a torsion viscometer (Kent Jones & Amos, 1967). The slurry was stirred by a stainless-steel spindle consisting of seven blades, suspended in the cup. Glacial acetic acid was added dropwise in order to reduce the pH from an initial value of 6·4. The pH was measured using a Cole–Palmer pH wand. Viscosity changes as a result of gel formation were recorded by a pen attached to the spindle of the viscograph. The gel formation experiments were conducted either at ambient temperature or under conditions when the temperature of the slurry was raised constantly at a rate of approximately 1·5°C/min by the heating system of the equipment. The viscosity was expressed arbitrarily on the Brabender scale.

Gels were also prepared by incubation of the acetic acid-added dispersions (pH 4·5) in a water bath at temperatures ranging from 30 to 70°C for 15 min. Gels prepared by heating either in the water bath or viscograph were subjected to centrifugation at ambient temperatures in a Sorvall centrifuge at 12 000 × g in order to determine their stability, which was calculated as

$$\frac{\text{Weight of water separated}}{\text{Weight of initial gel}} \times 100$$

The influence of salts on gelation was examined by incorporation of NaCl, KCl, $CaCl_2$ and sodium acetate at concentrations ranging from 4 to 20 mM into the dispersion (500 ml) taken in the viscograph. Acetic acid was then added to lower the pH to 4·5 followed by heating up to 50°C.

Protease assay

The protease activity of the gel was determined using haemoglobin as the substrate, as described by Sherekar *et al.* (1986).

Chemical analyses

Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Urea was determined colorimetrically according to the AOAC (1990) procedure.

RESULTS

Preliminary studies showed that washing the pieces of shark meat overnight in cold water removed significant amounts of soluble components including pigments and urea. The washed meat had a urea content of only 42 mg% as compared with 622 mg% in unwashed meat (wet wt basis). Even though the soluble components were not completely removed by the washing procedure, their presence did not interfere with gel formation. It was observed that, if the whole shark meat was initially blended in water, the myofibrillar proteins formed a dispersion, and hence washing of the proteins was difficult, although centrifugation was not attempted. Homogenisation of the washed meat with cold water in the ratio of 1 : 2 was found ideal for extraction of the myofibrillar proteins which formed a dispersion in water. Collagen of the washed, deboned meat, could be easily removed by passing the homogenate through a nylon net. At higher meat to water ratios, separation of collagen from the homogenate was also difficult.

The effect of the addition of glacial acetic acid (HAc) on pH and viscosity of the homogenate taken in the Brabender viscograph at ambient temperature is shown in Fig. 1. The homogenate (protein content, 23 mg/ml) had an initial pH of 6·4. Dropwise addition of HAc lowered the pH. At a pH of about 5·8, the viscosity of the homogenate tended to increase. With increasing amounts of HAc, the viscosity recorded a further rise. It was observed that a pH of 4·5 gave a marked change in viscosity of the homogenate. A maximum viscosity of 65 units on the Brabender scale was observed after the addition of 0·8 ml of HAc.

In the above experiment, the viscosity and pH values were measured 1 min after the addition of HAc. It was, however, found that when the pH of the homogenate was brought to 4·5, the viscosity continued to increase at ambient temperature (Fig. 2). The rate of increase was dependent on protein concentration (see below). It is interesting to note that no increase in viscosity

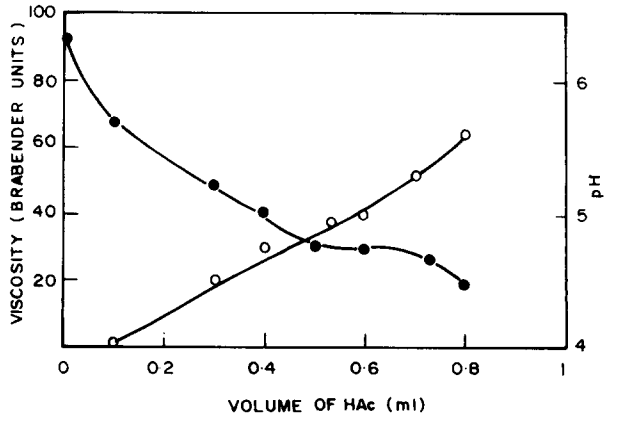

Fig. 1. Effect of acetic acid on viscosity of washed, collagen-free shark myofibrillar proteins. The acid was added dropwise to 500 ml of the collagen-free shark myofibrillar protein dispersion (protein content, 23 mg/ml) taken in a Brabender viscograph. (●) pH; (○) viscosity.

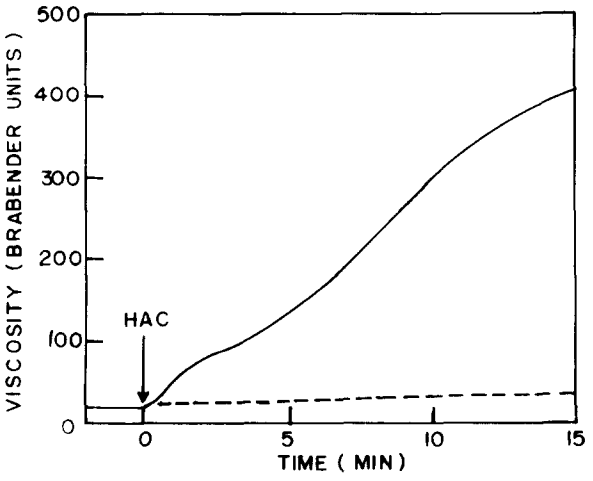

Fig. 2. Brabender viscosity of washed, collagen-free shark myofibrillar proteins (protein, 23 mg/ml) after lowering the pH to 4·5 by acetic acid. (——) HAc; (– – –) no HAc.

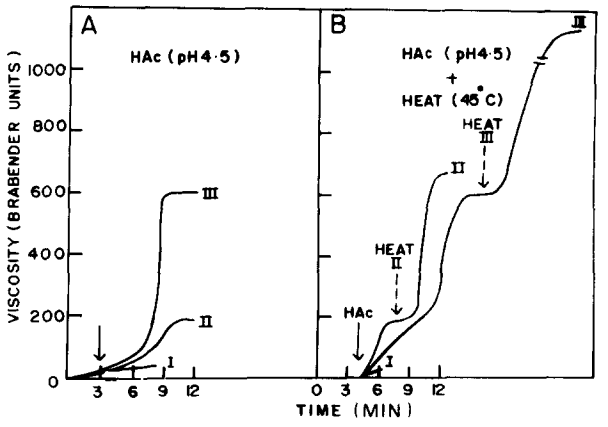

Fig. 4. Effect of protein concentrations on Brabender viscosity after (A) lowering the pH to 4·5 by HAc only, and (B) HAc addition (pH 4·5) followed by heating up to 47°C. The continuous arrow indicates HAc (1·5 ml) addition, while the broken arrow indicates switching on of heating. (I) 8 mg/ml; (II) 16 mg/ml; (III) 23 mg/ml.

was observed at the protein concentration when the homogenate was subjected to heating alone up to 65°C without initial pH reduction.

The influence of heat on HAc-induced viscosity increase of shark myofibrillar proteins was then examined. The pH of the homogenate was brought to 4·5 by HAc and then the temperature of the homogenate was raised at a rate of about 1·5°C/min. It can be seen that the heating caused further increase in viscosity. The viscosity increase continued up to 50°C and then dropped rapidly at higher temperatures. However, when the heating was discontinued and the temperature was maintained at 47°C, no fall in viscosity was observed, as shown in Fig. 3.

The effect of protein concentrations of 8, 16 and 23 mg/ml, on gelation by HAc and heat is given in Fig. 4. The pH of the homogenates taken separately in the viscograph was lowered to 4·5. The acidified homogenates were then subjected to heating at a rate of 1·5°C/min. At 8 mg/ml protein concentration, addition

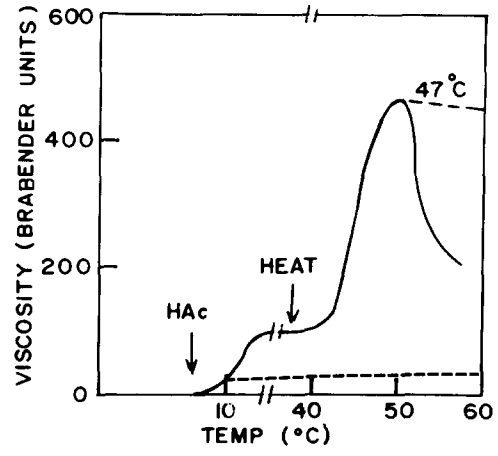

Fig. 3. Brabender viscosity changes of washed collagen-free shark myofibrillar protein (protein, 16 mg/ml) after lowering pH by HAc followed by heating. The broken line at the top represents viscosity of the gel when heating was stopped at 47°C. The broken line at the base shows viscosity of heated sample in the absence of HAc.

of HAc did not cause any change in viscosity, while at higher protein levels, addition of HAc enhanced the viscosities. When the temperature of the dispersion was raised, further increase in viscosity was noted at protein concentrations of 16 and 23 mg/ml. At 8 mg/ml protein, precipitation was observed with no increase in viscosity. The increases in viscosity of dispersions of higher protein concentrations continued up to 50°C.

The viscosity curve of the protein was characterised by a steep fall after reaching a maximum at 50°C, suggesting breaking of the gel at higher temperatures. In order to examine whether this is due to the mixing action of the viscograph, a parallel experiment was conducted where acetic acid-treated (pH 4·5) dispersions (16 mg protein/ml) were incubated for 15 min in water baths at temperatures ranging from 30 to 70°C. The gels were then cooled and were centrifuged. It was observed that water was separated from gels heated at 50°C and above in the water bath as well as the Brabender viscograph. The water separated from the water bath-incubated gels were 15, 22·5 and 30% for gels treated at 50, 60 and 70°C, respectively, while 20% water was separated from Brabender samples heated to 50°C. No water was separated from gels from either the water bath or the viscograph when subjected to heat treatments at lower temperatures.

The influences of different acids on the proteins in combination with heat are given in Fig. 5. Gelation of the proteins was observed when lactic acid (1·5 ml) was used instead of acetic acid to lower the pH. Heating further increased the viscosity of the lactic acid-treated homogenate. At temperatures above 50°C, the gel broke as in the case of the HAc-treated sample as indicated by a rapid fall in viscosity. No gel was formed when citric acid (2M) or tartaric acid (2M) was used to lower the pH of homogenate to 4·5. Although when 6 M hydrochloric acid was used, a slight increase in viscosity was observed at ambient temperature, no further increase in viscosity was observed as a result of heating. On the other hand, heating of the

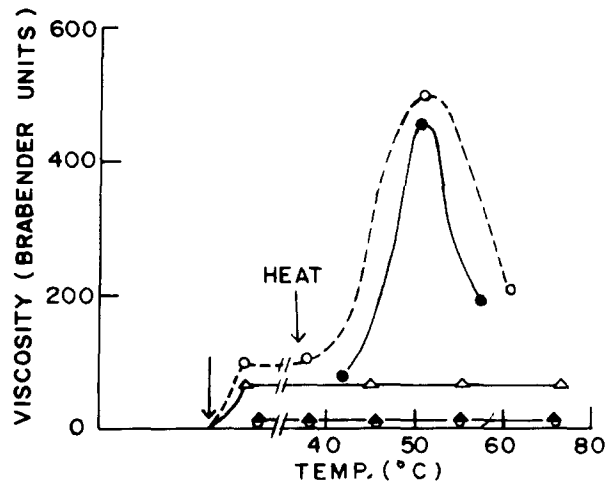

Fig. 5. Effect of different acids on gelation of collagen-free shark myofibrillar proteins (protein, 16 mg/ml) as measured by viscosity changes in a Brabender viscograph. (▲) Citric acid; (○) tartaric acid; (Δ) HCl; (●) lactic acid; (– –○– –) acetic acid.

homogenates in the presence of these acids caused precipitation of the proteins.

Salts are known to enhance the gel-forming capacity of fish protein (Roussel & Cheftel, 1990). Therefore, it was of interest to examine the influence of some salts on HAc-induced gel formation. For this purpose, the salts were incorporated at the concentrations mentioned in the 500 ml slurry (protein, 23 mg/ml) taken in the Brabender cup. HAc (1·5 ml) was added followed by heating up to 50°C. The results are presented in Table 1. It was found that, in the presence of NaCl or KCl, HAc addition resulted in only a marginal increase in the viscosity at ambient temperature. Heating of the homogenate containing the salts and HAc also gave significantly less increase in viscosity. The reduction in viscosity was proportional to the concentration of the salts used. At 8 mM NaCl, the viscosity was reduced to 77% of that of control (heated sample). A similar effect was observed when KCl (20 mM) was used. $CaCl_2$ at 10 mM caused 33% reduction in viscosity as compared with the control (treated with HAc ± heat).

The acid protease activity of the dispersion at pH values of 3·8 and 6·0 were 13·6 and 15·3 units, respectively, which decreased to 8·5 units when the pH was lowered to 4·5 by the addition of HAc.

Table 1. Influence of salts on HAc and HAc ± heat-induced viscosity changes of shark myofibrillar proteins[a]

Salt	Viscosity reduction (%)		
	Concentration (mM)	HAc alone	HAc ± heat (50°C)
NaCl	4	2	13
	8	100	77
KCl	20	100	94
$CaCl_2$	10	0	23
	20	60	41
Na acetate	10	0	20

[a] The values are expressed as a percentage of control (no salt addition).

DISCUSSION

The salient observations of this study include the following: (i) washed, collagen-free shark myofibrillar protein dispersions can undergo gelation at a concentration as low as 16 mg/ml in the presence of weak acids such as acetic or lactic acid; (ii) the HAc-induced gelation was a slow process at ambient temperature, the rate of which can be enhanced by mild heat treatment of the acidified protein while, at the protein concentrations used, heat alone did not induce gelation; (iii) the low-pH gelation could be negatively influenced by the presence of salts; and (iv) gelation can be monitored continuously in terms of viscosity using a Brabender viscometer.

The gel formation of fish proteins has been the subject of several studies because of the possibilities in the development various texturised products (Niwa, 1992; Stone & Stanley, 1992). Gelation of muscle proteins has been suggested to take place in two steps: an initial unfolding (denaturation) followed by aggregation of the proteins into a three-dimensional network. In the case of myosin, tail portions of the molecules are involved in the aggregation, essentially through hydrophobic interactions (Niwa, 1992). In the present study, the weak organic acids could favour unfolding of the proteins facilitating the gelation process. Fretheim *et al.* (1985) reported that gel formation of myosin was enhanced by slow lowering of pH and that heating was not essential for the process. The authors observed a maximum gel strength at pH 4·5 and suggested the involvement of conformational changes of the protein during gelation. In the case of shark myofibrillar proteins, a certain minimum concentration of protein was required for low-pH gel formation. At lower concentration (e.g. 8 mg/ml), small aggregates were formed giving a turbid solution, as observed by Fretheim *et al.* (1985). Fish myofibrillar proteins have been reported to form gel at a minimum protein concentration of 0·7% when extracted at a pH of 5·6 (Grabowska & Sikorski, 1976). Similarly, natural actomyosin gels, made from hake, had a maximum rigidity at a pH of 6·0 (Beas *et al.*, 1988). The inability of strong acids to cause gelation of proteins could be due to their drastic lowering of pH which resulted in random denaturation and precipitation of the proteins.

The acetic or lactic acid-induced gelation, which was a slow process at ambient temperatures, was enhanced by heating the dispersion to 50°C. Yano (1990), who explained the relationship between heating condition and gel strength, assumed four different states in the gelation of fish proteins. Thus, the native state of the protein could be changed into a gelation-possible state which further changed into a gelation-impossible or gelled state depending upon the environment. It is likely that the presence of acetic or lactic acid favoured

a gelation-possible state which is converted into a gel by mild heat. Low-pH-induced conformational change in myosin has been suggested (Fretheim *et al.*, 1985). Xiong and Brekke (1991) studied the temperature requirements for protein unfolding, protein–protein interactions and subsequent gel formation. They observed that these processes occurred between 32 and 48°C with the rigidity development associated with gelation of myosin generally beginning between 40 and 50°C and being maintained up to 70°C. The exact transition within the temperature range depended upon the compositional variations in myofibrillar proteins. Our studies also indicate comparable temperature ranges for low pH gel formation of shark myofibrillar proteins. Kaminishi *et al.* (1990) showed that gel formation reactions of smooth muscle paste of dog fish can be divided into two temperature ranges. While aggregation reactions proceeded below 50°C, gelation took place above 50°C. The turbidity associated with gelation increased over the range of 40–50°C, but decreased with further temperature rise.

The viscosity of the gel decreased rapidly above 50°C and was associated with the separation of water. This was true both in the case of gels heated in the Brabender viscograph as well as in the water bath, suggesting that the phase separation was not due to the mixing effect of the viscometer. This is comparable to surimi pastes which undergo thermal shrinkage at higher temperatures associated with liberation of water together with development of heterogeneity in the dispersion (Niwa, 1992). Hermansson (1986) observed a moisture loss of 5% in blood plasma and 12% whey protein gels when heated above 75°C, giving about 50% water separation at 95°C. The phenomenon was attributed to random aggregation of proteins at elevated temperatures.

The presence of NaCl has been reported to enhance the gel strength of several fish proteins. Adequate gel strength required about 1·7–3·5% of the salt (Grabowska & Sikorski, 1976; Roussel & Cheftel, 1990). In contrast, salts did not favour low-pH gelation of the shark proteins. Similarly, increasing moisture losses of blood plasma, whey protein and soya protein gels were observed with increasing salt concentration (Hermansson, 1986). Hennigar *et al.* (1988) showed that gels from a number of fish species could be made in the absence of salt if the muscle was washed prior to gelation. In the case of shark, the washing step also removed soluble components which included urea, responsible for the characteristic odour of the fish and also significant quantities of acid proteases usually present in the intact muscle (Sherekar *et al.*, 1986), which would have affected the textural quality of the gel.

Gelation is usually studied in terms of rigidity, elasticity, storage and loss moduli (Ziegler & Foegeding, 1991). The present results suggest scope for continuous monitoring of gelation through viscosity changes in the Brabender viscograph. It would be of interest to examine the influence of food additives such as polysaccharides as well as other proteins on the gelation profiles of shark myofibrillar proteins, with a view to developing texturised products. We have made use of the present observations to develop a spray dried functional protein powder from shark. This will be described elsewhere.

ACKNOWLEDGEMENT

Thanks are due to Dr F. Shahidi of the Biochemistry Department, Memorial University of Newfoundland, for critical reading of the manuscript.

REFERENCES

AOAC (1990). *Official Methods of Analysis* (15th edn), ed. K. H. Elrich. Association of Official Analytical Chemists, Virginia, USA.

Asghar, A., Samajima, K. & Yasui, T. (1985). Functionality of muscle proteins in gelation mechanisms of structural meat products. *CRC Crit. Rev. Food Sci. Nutr.*, **22**, 27–106.

Beas, V. E., Crupkin, M. & Trucco, R. E. (1988). Gelling properties of actomysin from pre and post spawning hake (*Merluccius hubbsi*) *J. Food Sci.*, **53**, 1322–6.

Camou, J. P., Sebranek, J. C. & Olson, D. G. (1989). Effect of heating rate and protein concentration on gel strength and water loss of muscle protein gels. *J. Food Sci.*, **54**, 850–4.

Foegeding, E. A., Brekke, C. J. & Xiong, Y. L. (1991). Gelation of myofibrillar proteins. *Interaction of Food Proteins* (ACS Symposium Series No. 454). ed. N. Parris & R. Q. Barford. American Chemical Society, Washington, DC, USA, pp. 257–67.

Fretheim, K., Egelandsdal, B., Harbitz, O. & Samejima, K. (1985). Slow lowering of pH induces gel formation of myosin. *Food Chem.*, **18**, 169–78.

Govindan, T. K. (1972). The cartillagenous fishes of India. *Seafood Exper. J.*, **4**(12), 19–22.

Grabowska, J. & Sikorski, Z. E. (1976). The gel forming capacity of fish myobribrillar proteins. *Lebensm. wiss. u. Technol.*, **9**, 33–7.

Hennigar, C. J., Buck, E. M., Hultin, H. O., Peleg, M. & Vareltzis, K. (1988). Effect of washing and sodium chloride on mechanical properties of fish muscle gels. *J. Food Sci.*, **53**, 963–4.

Hermansson, A. M. (1986). Water- and fatholding. In *Functional Properties of Food Macromolecules*, ed. J. R. Mitchell & D. A. Ledward. Elsevier Applied Science Publishers, London, UK, pp. 273-314.

Kaminishi, Y., Mike, H., Isohata, T. & Nishimoto, J. (1990). Effect of temperature on reactions of heat-induced gel formation in smooth dog fish muscle. *Bull. Jap. Soc. Sci. Fish.*, **56**, 1385–92.

Kent Jones, D. and Amos, A. J. (1967). *Modern Cereal Chemistry*. Food Trade Press, UK, p. 321.

Lanier, T. C. (1991). Interactions of muscle and non-muscle proteins affecting heat set gel rheology. In *Interactions of Food Proteins*. (ACS Symposium Series No. 454), ed. N. Parris & R. Barford. American Chemical Society, Washington, DC, USA, pp. 268–74.

Lee, C. M. (1986). Surimi manufacturing and fabrication of surimi based products. *Food Technol.*, **40**(3), 115–24.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Mulvihill, D. & Kinsella, J. E. (1987). Gelation characterisation of whey proteins and β-lactoglobulins. *Food Tech.*, **41**(9), 102–11.

Nakamura, K., Eide, H., Nakamura, L. & Ishikawa, S. (1985). Studies of utilization of shark meat. II. Qualities of products prepared from shark meat. *Bull. Tokai Reg. Fish. Lab.*, **115**, 23–8.

Niwa, E. (1992). Chemistry of surimi gelation. In *Surimi Technology*, eds. T. C. Lanier & C. M. Lee. Marcel Dekker Inc., New York, USA, pp. 389–427.

Roussel, H. & Cheftel, J. C. (1990). Mechanisms of gelation of sardine proteins. Influence of thermal processing and of various additives on the texture and proteins solubility of kamaboko gels. *Int. J. Food Sci. Technol.*, **25**, 260–70.

Sherekar, S. V., Doke, S. N., Gore, M. S. & Ninjoor, V. (1986). Proteinases of tropical fish and their role in autolysis. *Ind. J. Exper. Biol.*, **24**, 440–3.

Smith, D. M. (1991). Factors influencing heat induced gelation of muscle proteins. *Interactions of Food Proteins.* (ACS Symposium Series No. 454), ed. N. Parris & R. Barford. American Chemical Society, Washington, DC, USA, pp. 243-56.

Stone, A. P. & Stanley, D. W. (1992). Mechanisms of fish muscle gelation. *Food Res. Int.*, **25**, 381–8.

Suzuki, T. (1981). *Fish and krill protein processing technology.* Elsevier Applied Science, London.

Venugopal, V. (1992). Mince from low cost fish species. *Trends in Food Sci. Technol.*, **3**, 2–5.

Xiong, Y. L. & Brekke, C. J. (1989). Changes in protein solubility and gelatin properties of chicken myofibrils during storage. *J. Food Sci.*, **54**, 1141–5.

Yano, T. (1990). Kinetic study on gelation of fish meat solution. *Nippon Shok Kogyo Gak.*, **37**, 220–3.

Yasui, T., Ishiorishi, M. & Samejima, K. (1980). Heat-induced gelation of myosin in the presence of actin. *J. Food Biochem.*, **4**, 61–5.

Ziegler, G. R. & Foegeding, A. (1991). The gelation of proteins. In *Advances in Food and Nutrition Research* (Vol. 34), ed. J. E. Kinsella. Academic Press, PA, USA, pp. 203–60.