ELSEVIER

Food Chemistry 83 (2003) 99–106

Food Chemistry

www.elsevier.com/locate/foodchem

Hydration of muscle proteins of Bombay duck (*Harpodon nehereus*) during acetic acid-induced gelation and characteristics of the gel dispersion

A. Kakatkar, Arun Sharma, V. Venugopal*

Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

Received 23 October 2002; received in revised form 7 January 2003; accepted 7 January 2003

Abstract

Washed fillets of Bombay duck (*Harpodon nehereus*), a fish having unusually high moisture content (above 90%), were held overnight in cold, 2% (v/w) aqueous acetic acid, which resulted in gelation associated with a 33% increase in weight due to water uptake by the proteins. Gamma irradiation at 3 kGy or repeated freezing and thawing did not affect protein hydration. The presence of sodium chloride in the acid adversely affected protein hydration. The effects of urea, dithiothreitol and *o*-phenanthroline on water uptake suggested that the imbibed water was held by non-covalent interactions with the fish proteins. Water homogenates of the gel had higher apparent viscosities than native muscle. Temperature significantly influenced the apparent viscosity of the gel. Unlike water homogenates of washed fish, heating of the gel dispersion at 100 °C did not cause precipitation of the proteins. However, the stability of the proteins in the dispersion was adversely affected by sodium chloride or by increase in pH above 5.0. The acid-induced gel was very weak; its hardness could be enhanced by incorporation of certain food additives.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Bombay duck (*Harpodon nehereus*); Weak–acid induced gelation; Dispersion; Protein hydration; Solubility

1. Introduction

Gelation of fish muscle structural proteins has been extensively studied because of its importance in the development of surimi and surimi-dependent restructured products. Gelation of myosin molecules involves partial denaturation, followed by irreversible aggregation of myosin heads through formation of disulphide bonds and helix-coil transition of the tail part of the molecules, resulting in a three dimensional network (Lanier & Lee, 1992; Niwa, 1992; Stone & Stanley, 1992). The three-dimensional network has the potential to hold water, fat or other food ingredients in the matrix, facilitating incorporation of additives for the desired texture of the finished products (Hermansson, 1986).

Most of the studies on the gelation of fish proteins have been carried out at near neutral pH conditions (Lanier & Lee, 1992). We have observed that shark muscle structural proteins could form a gel when its pH was lowered to 3.5 by weak organic acids such as acetic or lactic. The process could be monitored by an increase in apparent viscosity of the meat homogenate, the change in viscosity being dependent on its protein concentration and temperature (Venugopal, Doke, & Nair, 1994).

Bombay duck (*Harpodon nehereus*) is a low-valued, seasonal, marine fish, available throughout the west coast of India from September to June. The annual catch of the fish is about 100,000 metric tonnes (Doke, Venugopal & Thomas, 1996). The fish has an unusually high moisture content of more than 90% which, together with the labile nature of the muscle proteins, poses problems in its processing by conventional techniques, including surimi processing (Gopakumar, 1989; Gore & Kumta, 1970). Currently, the fish is dried on the beach and the dried product is consumed in the coastal belt. A process to prepare dehydrated laminates of the fish has been developed to enhance the marketability of the dried fish (Doke, Venugopal, & Thomas, 1996). We have been interested

* Corresponding author. Tel.: +91-22-551-9613; fax: +91-22-550-5151.

0308-8146/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00055-4

in mild acid-induced gelation of fish muscle and its applications for value addition of low cost fish (Venugopal, 1997). Unlike conventional gelation of fish muscle, which requires mild heating of washed meat in the presence of sodium chloride, gelation of proteins under mild acidic conditions offers scope to develop low sodium surimi products. In this paper, we examine weak acid-induced gelation of Bombay duck muscle with particular reference to protein hydration, viscosity and thermostability of proteins in the gel dispersion. The potential of the gel for product development from the fish is also discussed.

2. Materials and methods

2.1. Fish

Fresh Bombay duck were purchased from a local market and brought to the laboratory in ice. The fish were washed in running potable water. The washed fish were beheaded, deskinned and the central bone removed manually. The prepared fish fillets were washed in potable water, drained and chilled to 0–2 °C.

2.2. Processing treatments

Two fish fillets were each sealed in polyethylene pouches. The pouches, held in ice, were subjected to gamma irradiation at a dose of 3 kGy in a ^{60}Co Gamma cell at a dose rate of 12 Gy/min. After irradiation, the packages were held in ice until next day. Some of the fresh fillets were also subjected to freezing in liquid nitrogen, followed by thawing under running tap water. The freeze–thaw cycle was thrice repeated.

2.3. Gel formation

The fillets were washed by soaking in cold water (<10 °C) for 18 h in a cold room (2–3 °C) to remove soluble components of the muscle. The washed fillets were then drained and transferred to equal amounts of cold aqueous solutions of 2% (v/v) acetic acid. The samples were held for 18 h in the cold room, which resulted in gelation of the meat and uptake of water. In some of the samples containing fish fillets in dilute acetic acid, chemicals, including sodium chloride, urea and dithiothreitol were incorporated at varying concentrations After incubation, the gel fillets were drained.

2.4. Hydration of fillets during gelation

Hydration of meat proteins during gelation was determined by noting the weight of the fillets before and after soaking overnight in the aqueous acetic acid.

2.5. Water holding capacity of gel

An amount of 10 g of homogenized gel was centrifuged at 12,100$\times g$ for 20 min in a Sorvall RC2 refrigerated centrifuge and the volume of water released was noted. In order to determine the influence of heat and salt on water-holding capacity, the gel was heated at 80 °C for 10 min in the presence of sodium chloride at concentrations ranging from 0 to 2% (w/w) of the gel. The treated gel was cooled to room temperature and subjected to centrifugation as mentioned earlier.

2.6. Determination of hardness

The gel was homogenized in a 'Sumeet' kitchen mixer and the gel mince was collected. The homogenized gel was filled into aluminium trays ((9.0$\times$6.5$\times$2.0 cm), and subjected to steaming for 10 min under atmospheric pressure in a kitchen cooker. After heat treatment, the trays were cooled to ambient temperature (20–21 °C) and stored in a cold room (0–2 °C) until used. In order to improve the texture of the gel, additives, including potato starch, egg white, gum acacia, carboxy methylcellulose, propylene glycol and also shark meat gel were incorporated in the gel at various concentrations. The homogenized gel, containing the additives, was subjected to heating and cooling, as mentioned above. Shark meat gel was prepared from washed shark meat as described by Venugopal, Doke, Kakatkar, Alur, and Bongirwar (2002a).

The hardness of the gel before and after cooking was determined in terms of shear force by penetrating it to a breaking point with a laboratory-fabricated five-blade (3$\times$1 cm, each 3 mm apart) metallic probe attached to a Universal Testing Machine (Table model TM Instron Corporation, Canton, MA), using a load cell size CCTM (0–100 kg range). The scale was calibrated to 2 kg $\pm$0.5%. Cross head speed was 10 mm per min. Hardness (N/cm^2) was taken as the force required to break the gel and was considered as the maximum height of the peak. All determinations were carried out in quadruplicate.

2.7. Viscosity measurement

The gelled meat was homogenized in cold water in various proportions by homogenizing it in a kitchen homogenizer to get protein dispersions. The viscosity of the dispersion (200 ml) taken in a 250-ml beaker, held in an ice water bath was measured using a Brookfield synchro-lectric viscometer model RVT(Cooksville, Ontario, Canada) as described by Venugopal and Shahidi (1994). The viscosity was measured using spindles, No. 2, 3 or 4 at a speed of 50 rpm. The values were recorded after rotation of the spindle for 30 s. Variations in the viscosity of a given sample measured by two spindles at the

same speed were within 10% and average values were used. The apparent viscosity values were obtained using a conversion factor provided by the manufacturer and were expressed in Pascal seconds (Pa s). The average of two independent experiments were used.

2.8. Proximate composition

Protein and moisture of the fish were determined according to AOAC (1990). The protein content was determined by measuring nitrogen (Kjeldahl method), using Kjelplus digestion and distillation system (Pelican Instruments, Madras, India) and was expressed as N×6.25.

3. Results

3.1. General

Evisceration, beheading, and deskinning of whole Bombay duck gave fillets, with a yield of 87%. The beheaded and eviscerated fish muscle had a proximate composition of 93.2±0.1% moisture and 5.8% protein. Washing of the fillets by overnight soaking in cold water resulted in removal of odour bearing compounds. There was about a 5% increase in weight of the fish as a result of the washing treatment.

Holding the washed meat in dilute acetic acid resulted in gelation of the meat proteins. While the washed fillets were opaque in appearance, the acetic acid-treated fillets were translucent. There was significant swelling of the fillets as a result of uptake of water during the gelation process. Table 1 shows hydration of washed fillets when incubated in different concentrations of acetic acid. While, at 0.5% acetic acid, there was not much hydration, at higher acetic acid concentrations, there were significant amounts of water uptake; the washed meat absorbed only 7.0±2.7% water, whereas the extent of water absorbed increased to 25.2±7.2 and 32.2±1.6% when the fillets were held overnight in 1 and 2% aqueous acetic acid, respectively. Unwashed fillets, when incubated in 2% acetic acid, imbibed only 5% water. On a dry weight basis, the water retained, per 100 g washed dry fillet after the treatments increased from 934 to 1109, when the acid concentration increased from 0 to 2%. Extent of hydration was not affected by gamma irradiation or repeated freeze–thaw treatment of the fillets.

It was of interest to examine the effects of certain agents on water uptake. Hydration of proteins was adversely affected by the presence of NaCl in the 2% aqueous acetic acid. Thus, while the water uptake was 1109 ml per 100 g dry fish, it was only 74.5 ml, when 0.5% NaCl was present in the dilute acid. At 1% acetic acid, there was loss of 104 ml from the initial moisture content, calculated on 100 g dry fish, presumably due to an osmotic effect (Table 2). The presence of dithiothreitol, urea or *o*-phenanthroline in the acetic acid medium adversely affected water uptake.

3.2. Apparent viscosity of gel dispersions

Fig. 1 shows the apparent viscosity of water homogenates of gel as well as non-gelled fish fillets, as a function of protein concentrations. At the same protein levels, the gel showed a higher apparent viscosity than non-gelled meat homogenates.

The influence of temperature on the apparent viscosity of dispersions having protein contents of 1.5 mg and 1.2 mg per ml is given in Fig. 2. In the case of both samples, the viscosity initially decreased with increase in temperature. However, at 50 °C, there was increase in the viscosity, followed by decrease at higher temperatures, finally giving a dispersion of the fish structural proteins in water. Cooling of the heated dispersions resulted in at least partial regaining of the viscosity. Thus, when chilled to 5 °C, the samples had apparent viscosities of 0.26 and 0.14 Pa s in the case of samples having protein contents of 1.5 and 1.2 mg per ml, respectively.

The stability of the proteins in homogenates of washed fish meat as well as gel dispersions is shown in Table 3. Heating at 100 °C for 10 min resulted in significant precipitation of proteins in the case of

Table 1
Hydration of washed Bombay duck fillets during gelation in dilute aqueous acetic acid

Acetic acid concentration (%)	Water uptake (%, wet/wt. basis)	Water content per 100 g dry meat
Nil	7.03±2.70	934
0.5	7.13±2.85	934
1.0	25.23±7.42	1015
2.0	32.17±1.62	1109

Fillets of the washed fish were soaked overnight in the above solutions maintained at 2–3 °C. Initial moisture content of 100 g dry fish, 900 (90% wet wt. basis).

Table 2
Influence of certain additives on the hydration of Bombay duck fillets in 2% (v/v) aqueous acetic acid

Additives	Water content per 100 g dry fillet after the treatment
Acetic acid alone	1109
Sodium chloride (0.5% w/v)	74.5
Sodium chloride (1.0% w/v)	−104
Urea (5 mM)	749
Urea (10 mM)	509
Dithiothreitol (2 mM)	484
Dithiothreitol (5 mM)	425
o-phenanthroline (5 mM)	525

Fillets of the washed fish were soaked overnight in the earlier solutions maintained at 2–3 °C and the water uptake was noted.

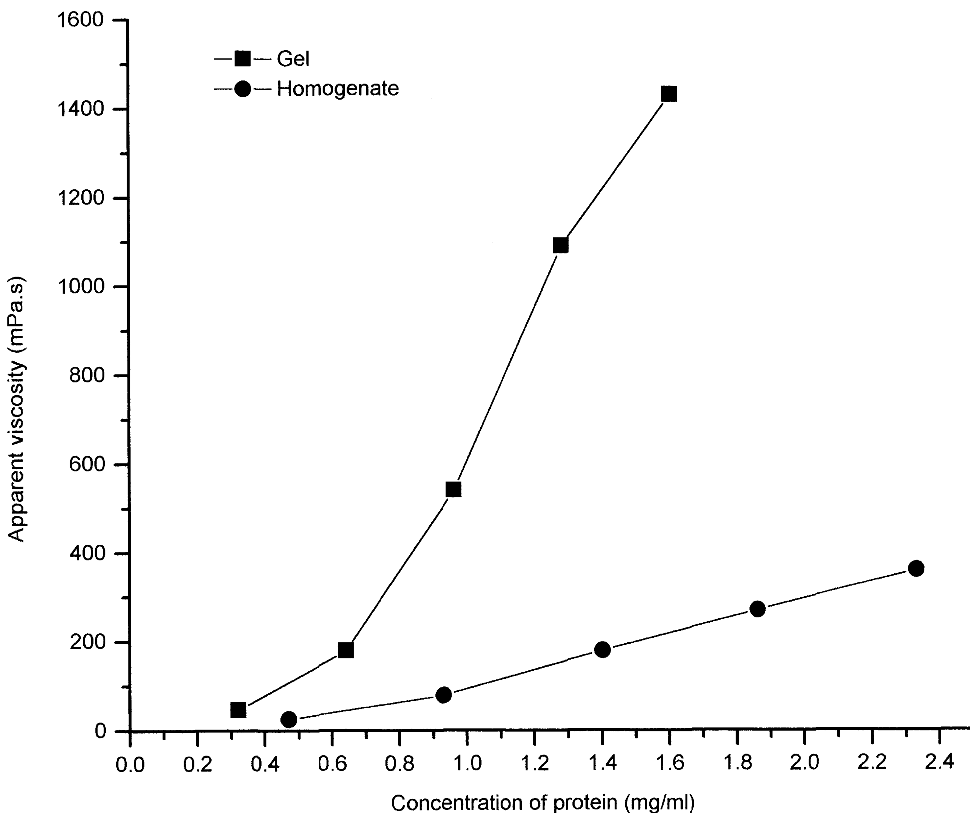

Fig. 1. Apparent viscosity of gel and washed homogenate of Bombay duck muscle as a function of protein concentration.

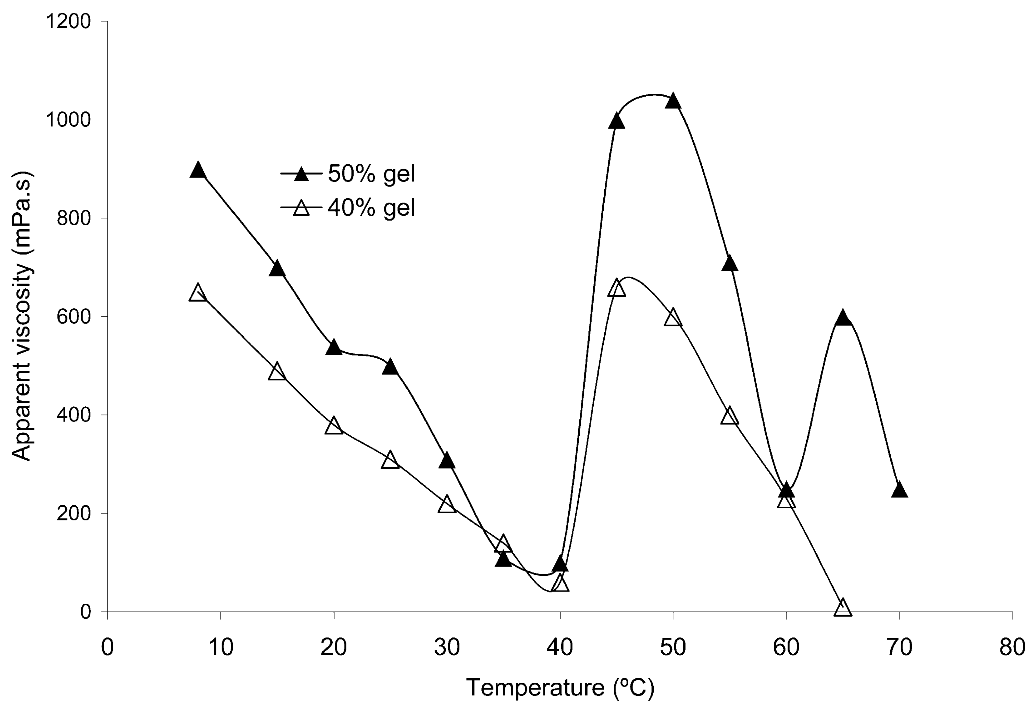

Fig. 2. Apparent viscosity of gel of Bombay duck as a function of temperature.

Table 3
Influence of heating on the stability of protein in washed homogenates and gel dispersions of Bombay duck muscle

Homogenate		Gel	
Initial protein (mg/ml)	Protein retained in supernatant after treatment (%)	Initial protein (mg/ml)	Protein retained in supernatant after treatment (%)
0.5	9.4	0.2	72.6
0.9	16.7	0.3	90.1
1.4	9.8	0.5	82.6
1.9	8.9	0.6	90.0

The dispersions of washed meat homogenate and gel having different protein concentrations were held in a boiling water bath for 10 min, cooled to room temperature and centrifuged at 12,100×*g* for 10 min. Protein content of the supernatant was determined.

unacidified fish homogenates, containing native proteins. However, at least 72–90% of the proteins were retained in solution after heat treatment of the gel dispersions.

3.3. Water holding capacity of gel

The imbibed water was strongly held in the protein gel, since no water was separated when the gels were centrifuged at 12,100×*g* for 10 min. However, significant amount of water was separated when, prior to centrifugation, the pH of the gel was raised to 5.0 and above, as shown in Fig. 3. Fig. 4. shows the effect of salt on the water-holding capacity. Incorporation of salt resulted in release of water. The water release was further enhanced by heating of the salted gel.

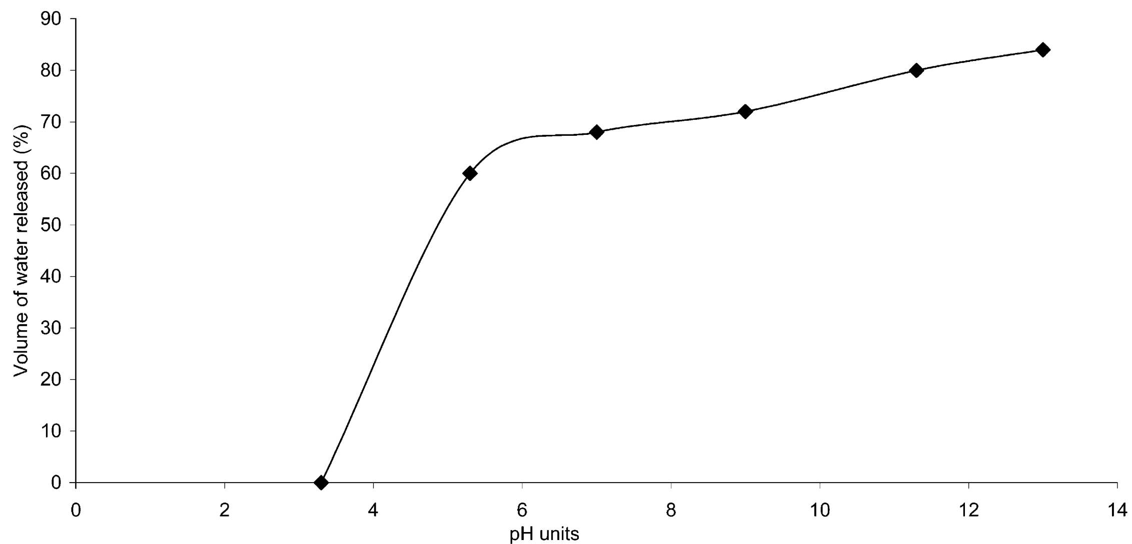

Fig. 3. Influence of pH on water-holding capacity of gel. The pH of the gel was raised using dilute NaOH.

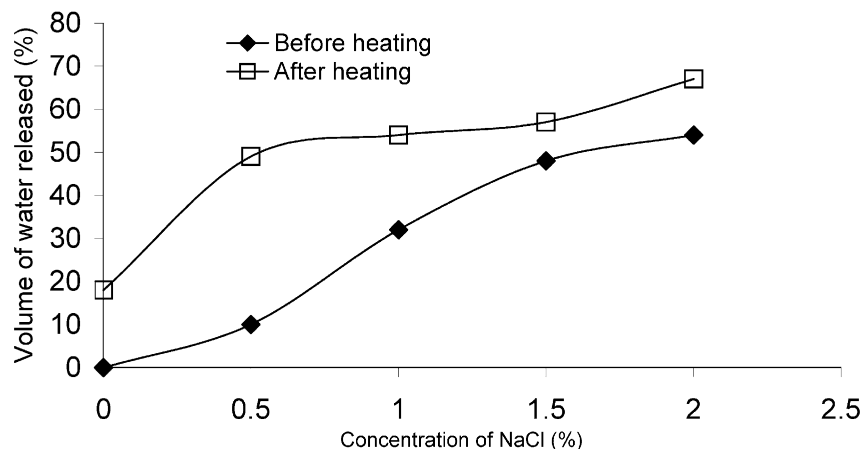

Fig. 4. Influence of salt and heating (70 °C, 10 min) on the water-holding capacity of gel.

Table 4
Influence of certain food additives on hardness of acetic acid-induced Bombay duck gel

Additives	Breaking strength (% of control)
Bombay duck gel alone	100
Gum acacia, 1% + potato starch, 1% + egg white, 1%	100
Propylene glycol, 0.5%	115
Xanthan gum, 1%	115
Egg white, 2%	125
Gum acacia, 1%	131
Carboxymethyl cellulose, 0.5%	162
Xanthan gum, 0.5% + soy flour, 1%	227
Casein, 1%	230
Sodium alginate, 1%	269
Bombay duck gel, 75% + shark gel, 25%	276
Bombayduck gel, 50% + shark gel, 50%	393
Xanthan gum, 0.5% + soy flour, 1% + sodium alginate, 0.5%	493
Bombay duck gel, 25% + shark gel, 75%	593
Bombay duck gel, 0% + shark gel, 100%	1523

3.4. Hardness of the gel

Table 4 shows the hardness of the gel as determined by Instron measurements. Because of the significant uptake of water, the gel obtained was very weak. However, the breaking force could be enhanced by the incorporation of ingredients. Gelled shark meat, which has a significantly hard texture (Venugopal et al., 2002) could be incorporated in the Bombay duck gel. The composite gels of both Bombay duck and shark meat proteins had a hardness that depended on the proportions of both gels (Table 4).

4. Discussion

Previous studies conducted in our laboratory showed that the high moisture content and formation of excessive drip during storage hampered attempts to extend the refrigerated shelf life of the fish by low dose gamma irradiation. A dip treatment in 10% aqueous solution of sodium tripolyphosphate or sodium chloride was required to reduce drip formation in irradiated fish during storage (Gore & Kumta, 1970). The present results show that, in spite of high moisture content, the washed fish muscle could still imbibe a further amount of water during gelation under mild acidic conditions. Washing was essential for the process, since unwashed fillets imbibed much less water. The ability of the fillets to take up water was not affected by gamma irradiation or by repeated freeze–thaw operations. Gelation and water uptake required holding the fillets in cold acetic acid for several hours. However, the gel was weak, unlike shark meat gel, as observed by the hardness test (Venugopal et al., 2002). The low hardness of the gel has limitations in the development of texturized products such as surimi from the gel. Nevertheless, there is potential to develop restructured products by enhancing the gel strength by the incorporation of additives such as starch and carrageenan, as reported by Gao, Piggot, and Reine (1999) in the case of surimi.

Water-holding capacity is an important functional property of muscle foods (Ashgar, Samejima, & Yasui, 1985). The present results suggest that weak acid induced gelation of fish muscle is associated with significant hydration of the muscle structural proteins. While the native meat imbibed less water upon overnight soaking in dilute acetic acid (5% on wet weight basis), washing of the meat enhanced its ability to imbibe water. In the native muscle, several low molecular weight compounds and enzymes adhere to myosin and actomyosin, which hinder their interactions with a solvent (water) and hence result in poor solubility of the structural proteins. Washing of the meat, as is done in the production of surimi, removes the adhering soluble components, liberating polar sites for interactions with water (Lanier & Lee, 1992; Niwa, 1992; Stone & Stanley, 1992). In surimi, it has been verified by electrostatic measurements that the total amount of bound water increases as the polar amino acids are dispersed more homogeneously in the surimi gel (Niwa, 1992). The tendency of a protein to form either a coagulum or a translucent-type gel is related to its molecular structure (Damodaran, 1996). Hwang and Damodaran (1997) prepared a fish protein hydro-gel having the ability to hold significant amounts of water by chemical modification which introduced a large number of hydrophilic groups in the protein. Fink, Calciano, Goto, and Palleros (1990) suggested that lowering of pH-induced confomational changes in protein molecules, influencing their α-helix content. The water uptake by the fish gel was sensitive to ionic strength. In the present case, the influence of different additives, including urea, dithiothreitol and *o*-phenanthroline suggested that the water

was held by non-covalent interactions in the proteins. However, the water could not be separated from the gel, even by centrifugation at an appreciable centrifugal speed. Further, the proteins from the gel dispersions were also not precipitated, even after heating up to 100 °C, suggesting a high water-holding capacity of the gel proteins. It is likely that, apart from water, other low molecular weight compounds, including food flavours, could be incorporated in the gel matrix to make desired products (Damodaran, 1996; Hermannson, 1986).

The presence of sodium chloride adversely affected water uptake by the fillets. At a 1% salt concentration, the fillets, lost water, presumably due to osmotic effects. Increase of pH and/or the addition of salt affected the water holding capacity. Several factors, such as pH, the presence of salts, temperature and comminution influence the ability of myofibrils to hold water and the resultant swelling (Ofstad, Kidman, Myklebust, Olsen, & Hermansson, 1995, 1996; Wilding, Hedges, & Lilliford, 1986; Hermansson, 1986). Both pH and ionic strength affect the volume of the myofibrils, either by an electrostatically or by an entropically driven swelling/shrinkage mechanism, which may affect the liquid loss on heating (Honikel, 1989). Weinberg et al. (1984) showed that expressible moisture content of cod is affected by both the ionic strength and the specific ion. Similar results have also been reported in the case of trout (Regenstein, Jauregui, & Baker, 1984). In the case of conventional surimi, when fish is comminuted with salt at neutral pH, the muscle cell wall, which sheathes the myofibrils is no longer maintained. The proteins dissolve in the salt, resulting in a myofibrillar system that is transformed from one of limited swelling to one of unlimited swelling, accompanied by increased liquid holding capacity (Ofstad et al., 1995, 1996). However, in the case of weak acid-induced gel, a small amount of salt is required for optimum gel strength and WHC. The capacity of the myofibrils to take up water was greatly affected by the presence of salt. The results are also in concurrence with Cecilia and Cristina (1998), who reported the adverse effect of salts on WHC of heat-induced soy protein gel, presumably due to their influence on gel matrix formation.

Temperature has a profound influence on water-holding capacity of processed muscle products in the neutral pH range. Water release in comminuted cod muscle was very low, between 5 and 30 °C, which increased at higher temperatures (Ofstad et al., 1995, 1996). In the present case, temperature influenced the apparent viscosity of the gel. The increase in apparent viscosity at 50 °C suggested some degree of interactions among the proteins at this temperature. However, at higher temperature, a low viscous thermostable dispersion of the proteins was obtained, from which no water could be separated. A shift in the pH or the presence of salts affects the balance of the electrostatic forces, causing aggregation and precipitation of the protein molecules and release of the trapped water, as suggested by Venugopal (1997). According to Kocher and Foegeding (1993), in a protein gel, water could be mechanically trapped and not chemically bound within the matrix. Although the molecular changes associated with mild pH-gelation of Bombay duck meat proteins have yet to be studied, the phenomenon in the case of meat from threadfin bream was associated with the formation of covalent bonds and the degradation of heavy chain myosin (Chawla, Venugopal, & Nair, 1996). Making use of the thermostability of proteins, products, such as spray-dried protein powders, biofilms and texurized products, have been developed from Bombay duck muscle. These studies will be reported elsewhere.

References

Ashgar, A., Samejima, K., & Yasui, T. (1985). Functionality of muscle proteins in gelation mechanisms of structured meat products. *Critical Reviews in Food Science and Nutrition*, *22*, 27–106.

Association of Official Analytical Chemists (AOAC). (1990). *Methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists.

Cecilia, P. M., & Cristina, A. M. (1998). Water holding capacity of heat-induced soy protein gel prepared with NaCl and $CaCl_2$. *Journal of Agricultural and Food Chemistry*, *46*, 3583–3589.

Chawla, S. P., Venugopal, V., & Nair, P. M. (1996). Gelation of proteins from washed muscles of threadfin bream under mild acidic conditions. *Journal of Food Science*, *61*, 362–366.

Damodaran, S. (1996). Functional properties. In S. Nakai, & H. W. Modler (Eds.), *Food proteins: properties and characteristics* (pp. 167–234). New York: VCH Publ.

Doke, S. N., Venugopal, V., & Thomas, P. (1996). Innovative method for dehydrated laminates of Bombay duck. *Infofish International*, *6*, 49–51.

Fink, A. L., Calciano, L. J., Goto, Y., & Palleros, D. R. (1990). Acid denatured states of proteins. In J. J. Vilafranca (Ed.), *Protein chemistry: techniques, structure and function* (pp. 417–424). New York: Academic Press.

Gao, J. C., Piggot, G. M., & Reine, B. (1999). Gel forming additives effects or properties of thermally induced fish gel. *Journal of Food Science*, *64*, 414–417.

Gopakumar, K. (1989). *Indian food fishes: biochemical composition*. Cochin: Central Institute of Fisheries Technology.

Gore, M. S., & Kumta, U. S. (1970). Studies on textural stability of irradiated Bombay duck. *Food Technology*, *24*(3), 286–289.

Hermansson, A. M. (1986). Water and fat holding. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional properties of food macromolecules* (pp. 273–314). London: Elsevier.

Honikel, K. O. (1989). The meat aspects of water and food quality. In T. M. Harmann (Ed.), *Water and food quality* (pp. 277–304). London: Elsevier.

Hwang, D.-C., & Damodaran, S. (1997). Synthesis and propertis of fish protein-based hydrogel. *Journal of the American Oil Chemists, Society*, *74*, 1165–1171.

Kocher, P. N., & Foegeding, E. A. (1993). Microcentrifuge based method for measuring water holding capacity of protein gels. *Journal of Food Science*, *58*, 1040–1045.

Lanier, T. C., & Lee, C. M. (1992). *Surimi technology*. New York: Marcel Dekker.

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

Ofstad, R., Kidman, S., Myklebust, R., Olsen, R. L., & Hermansson, A. M. (1995). Liquid holding capacity and structural changes in comminuted salmon (*Salmo salar*) muscle as influenced by pH, salt and temperature. *Lebensmitted-Wissenschaft + Technologie*, *28*, 329–339.

Ofstad, R., Kidman, S., Myklebust, R., Olsen, R. L., & Hermansson, A. M. (1996). Factors influencing liquid-holding capacity and structural changes during heating of comminuted cod (*Gadus morhua* L.) muscle. *Lebensmitted-Wissenschaft + Technologie*, *29*, 173–183.

Regenstein, J. M., Jauregui, C. A., & Baker, R. C. (1984). The effect of pH, polyphosphates and different salts on water retention properties of ground trout muscle. *Journal of Food Biochemistry*, *8*, 123–131.

Stone, D. W., & Stanley, A. P. (1992). Gelation mechanisms of fish muscle proteins. *Food Research International*, *25*, 381–388.

Venugopal, V. (1997). Functionality and potential applications of thermostable water dispersions of fish meat. *Trends in Food Science and Technology*, *8*, 271–276.

Venugopal, V., Doke, S. N., Kakatkar, A., Alur, M. D., & Bongirwar, D. R. (2002). Restrctured steaks from shark meat gel. *Lebensmitted-Wissenschaft + Technologie*, *35*, 185–190.

Venugopal, V., Doke, S. N., & Nair, P. M. (1994). Weak acid induced gelation of shark meat. *Food Chemistry*, *50*, 185–190.

Venugopal, V., & Shahidi, F. (1994). Thermostable water dispersion from Atlantic herring. *Journal of Food Science*, *59*, 265–268.

Weinberg, Z. G., Regenstein, J. M., & Baker, R. C. (1984). Effects of different salts on water retention and heat initiated binding properties of communited cod muscles. *Journal of Food Biochemistry*, *8*, 215–227.

Wilding, P., Hedges, N., & Lilliford, P. (1986). Salt-induced swelling of meat: the effect of storage time, pH, ion-type and concentration. *Meat Science*, *18*, 55–75.