Effect of Ice Storage on the Physicochemical and Dynamic Viscoelastic Properties of Ribbonfish (*Trichiurus* spp) Meat

A.O. DILEEP, B.A. SHAMASUNDAR, P.K.BINSI, F. BADII, AND N.K. HOWELL

ABSTRACT: Changes in physicochemical and dynamic viscoelastic properties of ribbonfish (*Trichiurus* spp) meat during different periods of ice storage were investigated. The differential scanning calorimetry profile of fresh ribbonfish meat revealed transitions at 33.17 °C, 48.85 °C, and 60.96 °C, indicating denaturation temperature of different protein fractions. The effect of cornstarch or tapioca starch at 9% level on the viscoelastic properties of ribbonfish meat stored in ice for different periods was also evaluated. Total volatile basic nitrogen (TVBN) increased significantly ($P < 0.05$) during ice storage for 24 d. However, the myosin heavy chain concentration was unaltered during the ice storage period, as revealed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern. A significant ($P < 0.05$) decrease in protein solubility (in phosphate buffer 50 m*M*, pH 7.5, containing 1 *M* NaCl), calcium-activated adenosine triphosphatase (ATPase) activity, and an increase in reduced viscosity at a protein concentration of 5 mg/mL was observed after 10 d of ice storage indicating protein denaturation and aggregation. The addition of tapioca and cornstarch enhanced storage modulus values of fresh ribbonfish meat. The gelatinization temperature of tapioca starch solution was found to be in the range of 60 °C to 65 °C and for cornstarch 67 °C to 70 °C, as revealed by the differential scanning calorimetry (DSC) profile and dynamic rheological testing. The viscoelastic properties of ribbonfish meat was altered significantly ($P < 0.05$), both due to the addition of starch and ice storage period as revealed by frequency sweep of prepared gels.

Keywords: ice storage, ribbonfish, viscoelastic properties

Introduction

The gel-forming ability of myofibrillar proteins of fish muscle is an important functional property of wide interest because it is responsible for the formation of better texture. Specific proteins responsible for gelation properties are myosin and actomyosin (Ashgar and others 1985). Gelation of myosin molecules involves partial denaturation followed by irreversible aggregation of myosin heads through formation of disulfide bonds and helix-coil transitions of the meromyosin tail region resulting in a 3-dimensional network (Niwa 1992; Stone and Stanley 1992). Various studies carried out on the effect of freshness on the gelling properties of fish muscle proteins have shown that the rate of decrease of gel strength depends upon factors such as species (Matsumiya and others 1985), freshness state of the fish; pre-rigor, rigor, post-rigor states (Park and others 1990; Benjakul and others 2003), and the season of capture (Niki and others 1984). The loss in gelling ability of fish meat or model system is due to denaturation, aggregation, and autolysis of myofibrillar proteins during storage (Haard and Warren 1990; Chalmers and others 1992).

One of the commonly used ingredients to enhance the gel-forming ability of fish meat is starch. Starch is extensively used in surimi (separated fish flesh is water washed, mixed with cryoprotectants, frozen, and frozen stored) based products such as kamaboko, fish sausage, and seafood analogs. Starch can serve several important functions in surimi gels (Ma and others 1996); it can improve gel strength, modify texture, and reduce the cost of production. For the mechanism of interaction between starch and fish protein it has been proposed that the gel-strengthening property might be due to swelling and water uptake of starch during gelatinization upon heating (Lee and Kim 1985). During thermal processing of starch-surimi systems, significant rheological changes due to sol-gel transformation of fish proteins and gelatinization of starch are observed (Belibagli and others 2003). Different botanical sources of starches behave differently with regard to texture of surimi-starch gels (Park 2000). Potato starch, for instance, increases the gel strength of fish paste more than cornstarch because of its ability to bind a larger amount of water or swell to a larger granule size (Lee and others 1992). Rheological monitoring of mixed systems of fish meat and starch during gelation can be valuable in understanding the mechanism of heat set gel formation (Hamann and MacDonald 1992). The texture of these products depends upon the interaction of starch and proteins with water. Hence, from a practical point of view, the rheological characterization of foods and their constituents is very important, particularly in relation to structure and stability. Dynamic measurements involving small deformations under either constant or sinusoidal oscillating stress may give more reliable information on viscoelastic characteristics of the gel compared with large strain test (Oakenfull and others 1997) and could aid in prediction of textural quality for product formulation or in processing (Konstance and others 1995).

Storage of fish in ice is an important pre-processing operation. The rate of deterioration during ice storage of fish is species-specific and depends on the concentration of substrate and metabolite in the tissue of fish, microbial contamination, and conditions after catching

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(Pacheco-Aguilar and others 2000). It is important to understand the various changes that take place in different components of fish meat during ice storage, which have bearing on gelling and other physicochemical properties of proteins. In addition, it is interesting to study the effect of ice storage on the viscoelastic properties of fish meat in combination with starch to predict the textural properties of the heat-induced gel product. Ribbonfish was chosen for the present study because it is a fish of low economic value and contributes to 7.5% of total Indian marine landings (CMFRI 2003). Thus, the potential for the preparation of value-added product from ribbonfish meat appears high. The objective of the present investigation was to study the effect of ice storage on the physicochemical and viscoelastic properties of ribbonfish meat and to evaluate the influence of corn and tapioca starches on the dynamic viscoelastic properties of ice stored ribbonfish meat. In this study, we focus on the use of cornstarch, which is the most widely used ingredient in the food industry and tapioca starch, which is in plentiful supply in the south west coast of India.

Materials and Methods

Materials

Fresh ribbonfish (*Trichiurus* spp) caught by trawl net along the West coast of India, Mangalore, was used for the study. The length of fish used was 90 to 95 cm, weighing 700 to 800 g. Immediately after harvest, the fish were washed in fresh water and iced in the ratio of 1:1 (fish:ice) and transported to the laboratory. Head and entrails were removed manually, and the fish were washed with chilled water (3 °C). For ice storage studies, steaks weighing 100 g to 150 g each was made from the whole fish and packed in polythene bags. The steaks with polythene bags were iced in the ratio 1:1 and stored in insulated boxes, which were kept at ambient temperature (25 °C to 27 °C) and replenished with ice periodically after draining the melted ice water. Corn and tapioca starches were obtained locally in Mangalore, India.

Proximate composition of ribbonfish meat

Meat was separated manually and macerated well using a pestle and mortar. The macerated meat was used for proximate composition. Moisture, crude protein, fat, and ash content in the meat were determined by the AOAC method (Conniff 1995) method.

Total volatile basic nitrogen, tri methyl amine nitrogen, and pH

Total volatile basic nitrogen (TVBN) content and tri-methylamine nitrogen (TMAN) content were determined according to Beatty and Gibbons (1937), using the Conway micro diffusion method. The pH of ribbonfish meat samples was measured using a pH meter (Systronic 324 pH meter, Ahemdabad, India). Five grams of meat was macerated with 45 mL of distilled water, and the pH was measured.

Non-protein nitrogen content

Non-protein nitrogen (NPN) content of ribbonfish meat was determined by the method described by Velankar and Govindan (1958), using TCA extract and was expressed as mg/100 g of meat. About 3.0 g of meat was macerated with 15 mL of 15% TCA for 5 min using a dried pestle and mortar. The homogenate was allowed to stand at 4 °C for 30 min. The slurry was filtered and made up to 50 mL with distilled water and 5 mL of aliquot was taken for nitrogen estimation using the Kjeldahl method.

Solubility of proteins in high ionic strength buffer

Total proteins were extracted using the extraction buffer (EB; phosphate buffer, 50 m*M*; pH 7.5, containing 1 *M* NaCl). The meat:buffer ratio used was 1:10. The meat was homogenized using an Ultra-Turrax homogenizer (Ultra-Turrax, T 25, Janke & Kunkel GMBH & Co., Staufen, Germany) at 9000 rpm for 2 min. The homogenate was centrifuged at 9000 × *g* for 15 min using a refrigerated centrifuge (Intl. Equipment Co., IEC, B22, Needham Heights, Mass., U.S.A.) maintained at 4 °C. The total nitrogen content of the clear supernatant was determined by the Kjeldahl method. The nitrogen value obtained was multiplied by a factor of 6.25 to obtain the protein content and expressed as a percentage of total protein.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970). The concentration of acrylamide was 10%. The thickness of the gel was 0.75 mm. Electrophoresis was carried out at a constant current mode in a vertical slab gel electrophoresis apparatus (Hoefer–Pharmacia Biotech Inc., SE–50, San Francisco, Calif., U.S.A.). A standard marker protein mixture of high range molecular weight obtained from Sigma Chemicals (St. Louis, Mo., U.S.A.) was loaded into a separate well.

Apparent reduced viscosity

Apparent reduced viscosity of total proteins extracted in extraction buffer was measured at 25 °C ± 1 °C using an Ostwald's viscometer. Proteins were extracted with the extraction buffer (EB) as solvent in the ratio 1: 10 (meat:EB). The slurry was homogenized at 9000 rpm for 2 min and centrifuged at 9000 × *g* for 15 min at 4 °C. The clear supernatant obtained was used for viscosity measurements after determining protein concentration by the Lowry method (Lowry and others 1951). The reduced viscosity at different protein concentration was calculated by the method as described by Yang (1961). A plot of apparent reduced viscosity at a single protein concentration of 5 mg/mL (derivative value) during ice storage period was obtained.

Gel filtration

Gel filtration profile of total proteins extracted using EB was carried out on a Sepharose 6B gel packed in a column of 1.5 × 80 cm (diameter × height) at ambient temperature (27 °C). The eluant used was EB. Total bed volume of the column was 150 mL, and the void volume determined by using blue dextran was found to be 50 mL. A protein concentration of 4 mg/mL was loaded onto the column, and elution was carried out at a flow rate of 30 mL/h. Fractions (3 mL) were collected manually, and the concentration of the eluant was determined by measuring the absorbance at 280 nm using a spectrophotometer (Bausch and Lomb, Model 21-UVD, Austin, Tex., U.S.A.).

Calcium activated ATPase activity

Calcium-activated ATPase activity was determined according to the method of Noguchi and Matsumoto (1970) and expressed as microgram of inorganic phosphorus (Pi)/mg protein/min at 27 °C. About 1 g of meat was macerated with 10 mL 50 m*M* glycine-NaOH buffer, pH 9.2. The slurry was filtered through Whatman nr 1 filter paper, and this filtrate was used as an enzyme solution. The reaction mixture containing 0.06 mL of ATP solution (0.05 *M*), 0.4 mL $CaCl_2$ (0.1 *M*), 2 mL buffer (0.05 *M* glycine-NaOH, pH 9.2) and 0.4 mL of meat extract was made up to 4.0 mL with buffer and incubated at 27 °C for 5 min. The reaction was stopped by the addition of 2 mL of 15% TCA. The blank was carried out by adding 15% TCA before the enzyme was added. The mixture was filtered through Whatman nr 1 filter paper and the inorganic phosphorus released was estimated according to the procedure described by Tausky and Shorr (1952).

Emulsion capacity

The emulsion capacity of ribbonfish meat samples was deter-

Table 1—Proximate composition, non-protein nitrogen (NPN) content, and pH of fresh ribbonfish (*Trichiurus* spp.)[a,b]

Parameters	Values
Moisture (g/100 g meat)	78.0 (1.4)
Fat (g/100 g meat)	1.18 (0.23)
Protein (N × 6.25) (g/100 g meat)	17.9 (0.19)
Ash (g/100 g meat)	3.08 (0.24)
NPN (mg/100 g meat)	672.2 (14)
pH	6.92

[a]Values in parentheses are standard deviation, $n = 5$.
[b]All the values are on wet weight basis.

Table 2—Onset/peak temperature of ribbonfish meat, tapioca, and cornstarch

Starches/fish	Onset point (°C)	Peak T_m (°C)
Tapioca starch	52.32	60.41
Cornstarch	62.60	66.90
Ribbonfish meat	29.84	33.17
	44.09	48.85
	54.94	60.96

mined according to the method described by Swift and others (1961). Meat sample (25 g) was homogenized with 100 mL of chilled EB at 3000 rpm for 2 min. Protein content of the slurry was determined by the Kjeldahl method. To 12.5 g of slurry taken in a beaker, 37.5 mL of chilled EB and 50 mL of refined sunflower oil (ITC, Mumbai, India) were added and mixed thoroughly at 9000 rpm for 10 s using Ultra turrax homogenizer. Homogenization was continued at a high speed (23000 rpm) with the addition of oil at a rate of 0.5 to 0.6 mL/s until phase inversion was observed visually. Emulsion capacity was calculated after considering the initial volume of oil added and expressed as mL oil/mg protein.

Differential scanning calorimetry

The thermodynamic parameters of the ribbonfish meat, corn, and tapioca starch were examined individually using a differential scanning calorimetry (DSC) VII calorimeter (Setaram, Lyon, France). Starches were mixed with water to get a 10% (w/v) solution and loaded to the sample container. Water was kept as reference for all the samples. The heating rate used was 0.5 °C/min from 10 °C to 90 °C. Heat absorbed or released by the sample results in either endothermic or exothermic peaks as a function of temperature. From the thermograms parameters such as T_o (onset temperature), T_m (melting temperature) for fish meat and individual starch were obtained.

Dynamic viscoelastic behavior

Dynamic viscoelastic behavior (DVB) of ribbonfish meat in the temperature range of 30 °C to 90 °C was measured using a Carri Med Controlled Stress Rheometer (CSR-500, Carri-Med, Surrey, U.K.) under oscillatory mode, using a 4 cm parallel plate measuring geometry. Fish meat devoid of connective tissue, fins, and scales was macerated well using pestle and mortar. About 4 g of macerated meat was mixed with 2.5% sodium chloride (w/w) and mixed thoroughly to get a fine ground paste. The fish paste obtained was used for DVB measurement. Starches (9% w/w corn or tapioca) were added to the meat, to study the influence of polysaccharides in enhancing the gelation property of protein. The gap between measuring geometry and peltier plate was adjusted to 2000 μm. The gap was set manually at 80 °C using the micrometer. The applied stress of 500 Pa was within the viscoelastic region. The linear viscoelastic region was determined by a torque sweep with a frequency of 1 Hz. Measurements were made by applying a small amplitude oscillation (0.0005 rad) with a frequency of 1 Hz. A heating rate of 1 °C/min was achieved through peltier plate of rheometer. Applied stress was compared with the resultant strain. The results of such measurement were expressed as the storage modulus (G′) and loss modulus (G″). The dynamic viscoelastic behavior of ribbonfish meat stored in ice for different periods was assessed without and with corn and tapioca starch at periodic intervals. An average of 3 replicates was used for plotting the results.

The gels obtained after temperature sweep were subjected to frequency sweep. Frequency sweep of the gel from ribbonfish meat with and without starch (corn or tapioca starch) was carried out using Control Stress Rheometer at 30 °C. Frequency was varied from 0.5 Hz to 5.5 Hz. Storage and loss modulus (G′ and G″) was obtained as a function of frequency. The frequency sweep was carried out for the gels from ribbonfish meat with and without corn/tapioca starch during different periods of ice storage, and the slope of the regression of ln G′ and ln G″ with change in frequency were obtained to assess the viscoelastic nature of the sample.

The dynamic viscoelastic behavior of corn and tapioca starch was assessed in a temperature range of 45 °C to 90 °C. A suspension of 10% (w/v) starch solution in water was used for the experiment. The gap between Peltier plate and measuring geometry was set at 500 μm. The storage modulus (G′) values as a function of temperature sweep was obtained to determine the temperature of gelatinization.

Statistical analyses

Data were analyzed to evaluate the correlation between the different parameters and ice storage period and significance of Karl Pearson correlation coefficient was determined using student *t* test at a significance level of 5%. Comparison of Dynamic Viscoelastic Behavior of ribbonfish as a function of ice storage period and with the addition of starches were carried out using 1-way analysis of variance as described by Snedecor and Cochran (1962).

Results and Discussion

The proximate composition of fresh ribbonfish showed that the fat content was less than 2%, indicating that they are lean fishes (Table 1). Differential scanning calorimetry of ribbonfish meat and of tapioca and cornstarch 10% (w/v) solution with water has been carried out. The onset and peak endotherm temperatures are given in Table 2. The thermogram of ribbonfish meat showed 3 transitions. The peak temperature (T_m) of the transitions was 33.17 °C, 48.85 °C, and 60.96 °C. The 1st transition is due to the denaturation of myosin, the 2nd transition is assigned to water-soluble sarcoplasmic proteins, and the 3rd transition is due to the denaturation of actin (Badii and Howell 2002). DSC thermogram of arrowtooth flounder showed that the onset of myosin denaturation at 25 °C with 2 maximum transition temperatures at 30 °C and 36 °C (Visessanguan and others 2000). The gelatinization peak temperatures for tapioca and cornstarch were at 60.41 °C and 66.90 °C, respectively. The gelatinization peak for cornstarch has been reported to be at 68.4 °C (Chung and others 2004). The gelatinization endotherm observed in DSC profile is due to granular disruption brought about by the co-operative combination of increasing solvent and thermal plasticisation (Perry and Donald 2002). The gelatinization temperature of tapioca and cornstarch as revealed by dynamic rheological testing was found to be at 65.2 °C and 70.1 °C, respectively (Table 3). The gelatinization temperature from rheological study of the starch were found to be slightly higher than that obtained in the DSC endotherm.

The changes in VBN and TMA content of ribbonfish meat during

Table 3—Dynamic rheological characteristics of corn and tapioca starch in the temperature range of 45 ° to 80 °C[a]

Temperature (°C)	Cornstarch (G′ values in Pa)	Tapioca starch (G′ values in Pa)
45.2	3.73	0.78
55.1	7.14	1.13
65.2	8.07	841.4
70.1	2340	775.4
75.0	2275	662.7
80.0	1903	481.3

[a]The concentration of starch used was 10% (w/v) solution.

different periods of ice storage are given in Figure 1a and 1b. The values for VBN and TMA increased with storage and were within the limits prescribed for fresh fish up to 10 d of storage. The normal limit prescribed for VBN and TMA is 40 mg/100 g and 5 mg/100 g, respectively (Connell 1995). The rapid increase in VBN and TMA content after 15 d of ice storage is likely to be influenced by psychrotropic or psychrotolerant bacteria and enzymatic activity (Sasajima 1975; Gokodulu and others 1998). The increase in pH value (Figure 1c) by 0.5 units during ice storage was likely to be induced by the increase in VBN content.

The non-protein nitrogen (NPN) content of fresh ribbonfish (Figure 2a) accounted for 23% of total nitrogen. The contribution of higher NPN content could arise from free amino acids, low-molecular-weight peptides, urea, and trimethylamine oxide. The NPN content in fish meat is due to trimethylamine oxide and other energy-rich compounds such as creatine phosphate (Ikeda 1979). The NPN content decreased to 8.7% of total nitrogen at the end of 24 d of ice storage. This decrease could be attributed to a leaching effect as a result of continuous replenishment of ice during storage. However, an increase in NPN value between 10 and 15 d of ice storage was recorded. This increase is likely to have occurred due to the release of NPN constituents either due to breakdown of compounds such as tri-methyl amine oxide (TMAO) or hydrolysis of proteins by microbial enzymes.

The changes in protein solubility of ribbonfish meat in extraction buffer as a function of ice storage period is given in Figure 2b. There was significant decrease ($P < 0.05$) in soluble protein from an initial value to 72% to 32.65% of total protein during the 20 d of ice storage. The reasons for reduction in solubility are mainly attributed to aggregation of myofibrillar proteins (Tsuchiya and others 1980; Reddy and Srikar 1991). The solubility in high ionic strength buffer is taken as an index of denaturation of muscle proteins (Owasu and Hultin 1992; Lin and Park 1998). In the present study, the decrease in solubility was gradual up to 20 d of ice storage and the decrease was due to extensive denaturation of proteins.

The SDS-PAGE pattern of total proteins from ribbonfish revealed higher concentrations of myosin heavy chain (205 kDa) and bands in a molecular weight range of 45 to 14 kDa were also present (Figure 3). There was no change in the concentration of myosin heavy chain during 20 d of ice storage. The concentration of low-molecular-weight compounds (less than 36 kDa) increased, possibly due to proteolysis. Increase in low-molecular-weight components in SDS PAGE pattern as a function of ice storage of prawn (*Macrobrachium rosenbergii*) have been reported (Kye and others 1988). The de-

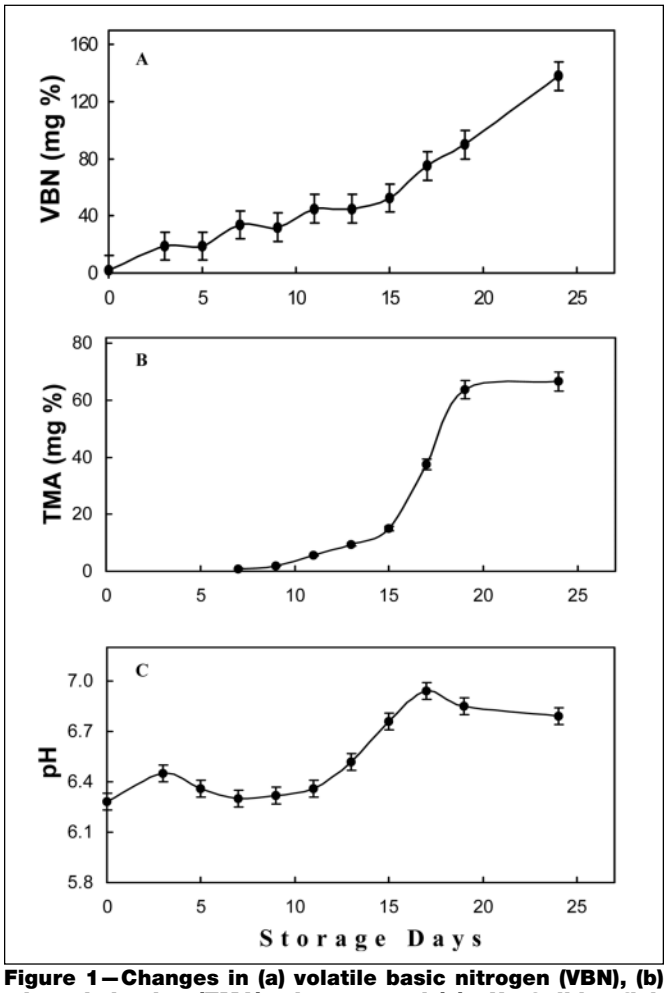

Figure 1—Changes in (a) volatile basic nitrogen (VBN), (b) tri-methylamine (TMA), nitrogen and (c) pH of ribbonfish stored in ice for different periods

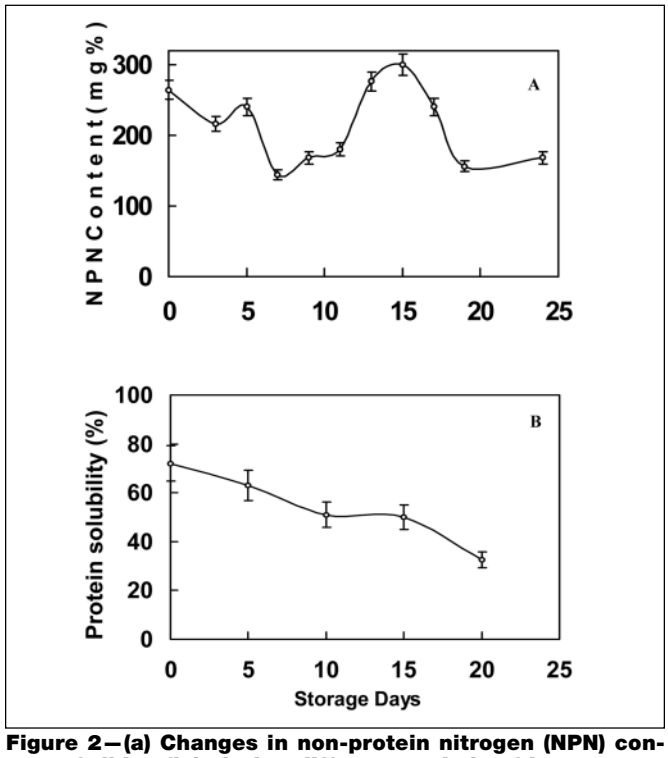

Figure 2—(a) Changes in non-protein nitrogen (NPN) content of ribbonfish during different periods of ice storage; (b) changes in protein solubility of ribbonfish during different periods of ice storage

crease in myosin heavy chain content of lizardfish and Pacific whiting during ice storage has been reported (Benjakul and others 1997; Benjakul and others 2003). The likely cause for the increase in low-molecular-weight components in the present study is due to proteolytic enzymes present in the muscle system.

The apparent reduced viscosity of total protein from ribbonfish at a concentration of 5 mg/mL as a function of ice storage is given in Figure 4. A sharp increase in the viscosity during the 20th day of ice storage indicated a change in protein conformation. Viscosity measurement is considered to be a more reliable index of quality of proteins from fish than either protein solubility or emulsifying capacity (Colmenero and others 1988). When myofibrillar protein molecules undergo denaturation resulting from association or dissociation, the viscosity of the protein solution changes because of the decrease of particle axis ratio (Suzuki 1981). Changes in apparent viscosity of muscle homogenate are generally related to changes in conformation of actomyosin (Borderias and others 1985). Chalmers and others (1992) reported that the changes in values of apparent viscosity of cod stored in ice were not significant. Similarly, Yasui and others (1987) found that changes in viscosity values in lizardfish stored in ice for 15 d were minimal. In the present study, the apparent reduced viscosity values showed a steep increase after 15 d of ice storage, indicating that ribbonfish actomyosin during ice storage is unstable compared with that of cod and lizardfish. The results of viscosity are in agreement with those of the solubility study in which a decrease was significant during 20 d of ice storage.

Gel filtration profile of total proteins from fresh ribbonfish showed 3 peaks eluting at an elution volume of 59 mL, 117 mL, and 160 mL, 1 high-molecular-weight fraction and 2 low-molecular-weight fractions (Figure 5). The high-molecular-weight fraction is actomyosin complex. The concentration of low-molecular-weight components increased at the end of 20 d of ice storage, whereas, the concentration of high-molecular-weight components was almost constant during the storage period. The increase in low-molecular-weight components possibly would have resulted from proteolysis occurring in the muscle system during ice storage. This was in conformity with the SDS PAGE result in which an increase in low-molecular-weight components less than 36 kDa was observed.

The ATPase enzyme activity of ribbonfish meat (Figure 6a) extract in glycine-NaOH buffer showed a decreasing trend as a function of ice storage period. The initial activity of 0.135 μg P_i /mg protein/min was taken as 100% activity index. At the end of 20 d of ice storage, the activity index reduced to less than 20%, indicating significant loss in activity ($P < 0.05$). The loss of ATPase activity may be due to alteration in the conformation of enzyme or due to aggregation of myosin molecule. A decrease in myofibrillar ATPase activity of sardine has been reported as a direct function of pH (Kamal and others 1991). ATPase

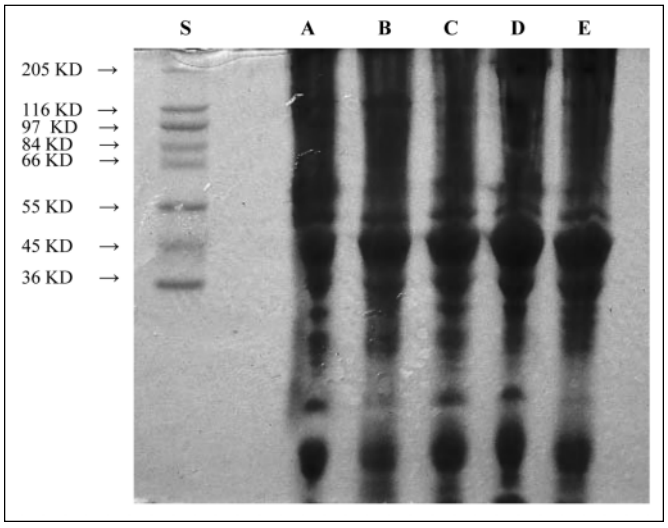

Figure 3—Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of total proteins from ribbonfish as a function of ice storage period S = standard markers; ribbonfish stored in ice for A = fresh, B = 5 d, C = 10 d, D = 15 d, E = 20 d.

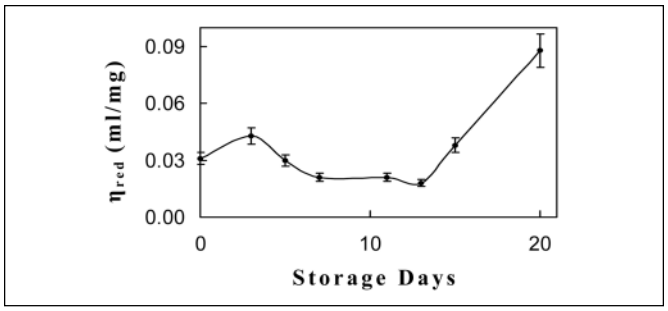

Figure 4—Apparent reduced viscosity at a protein concentration of 5 mg/mL as a function of ice storage period

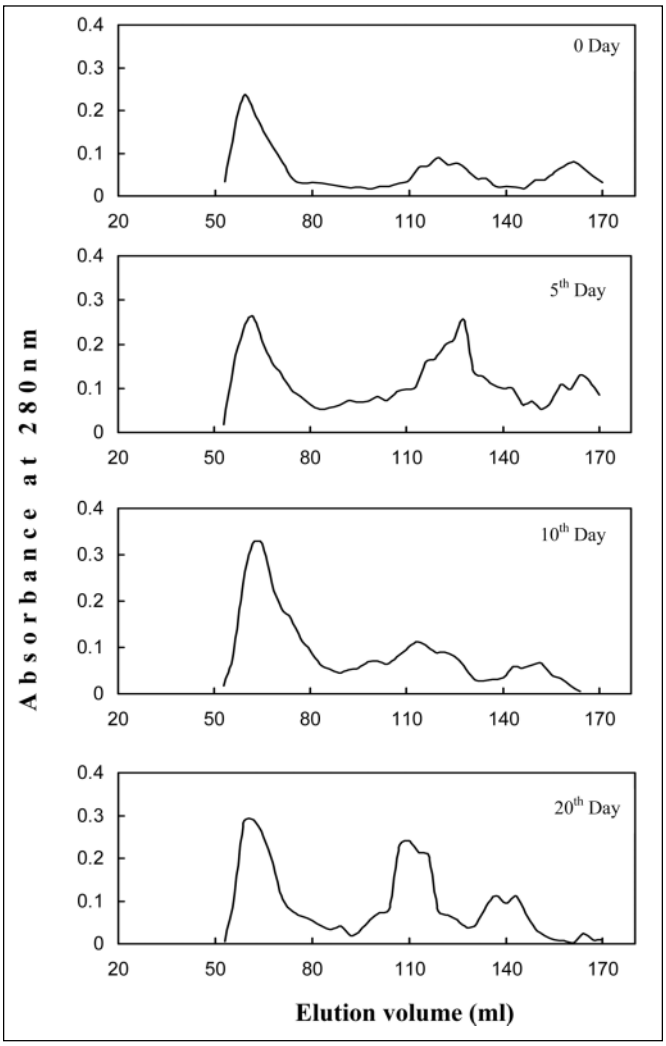

Figure 5—Gel filtration profile of total proteins from ribbonfish as a function of ice storage period

activity began to decrease when pH declined to 6.4. The loss in calcium activated ATPase was possibly associated with the proteolysis of myosin (Quali and Valin 1981) and indirectly reveals changes in the myosin-actomyosin system. Calcium-activated ATPase has been used as an indicator of myosin integrity (Benjakul and others 1997). In the present study, loss in solubility, increase in reduced viscosity, and reduced Ca^{++}ATPase enzyme activity indicated aggregation/denaturation of the muscle proteins.

Emulsion capacity of ribbonfish meat during different periods of ice storage is given in Figure 6b. The initial value of 0.36 mL oil/mg protein reduced to 0.26 mL oil/mg protein at the end of 15 d of ice storage and increased to 0.29 mL oil/mg protein. The reduction in emulsion capacity was found to be significant ($P < 0.05$) both at 15 and 20 d of ice storage. A significant reduction in emulsion capacity of shark meat during ice storage has been reported (Sijo Mathew and Shamasundar 2002). This reduction in emulsion capacity is likely to be associated with loss of solubility during ice storage. The ability of protein molecules to emulsify a given volume of oil mainly depends on the surface hydrophobic group, which orient toward the oil phase and hydrophilic groups, which can orient readily in the aqueous phase, thus lowering the interfacial free energy (Kato and Nakai 1980).

The DVB of fresh ribbonfish indicated moderate gel-forming ability as revealed by storage modulus (G′) values (Figure 7a). The maximum G′ value of 175 kPa was attained at 90 °C. The loss modulus (G″) value also increased during heating; however, the magnitude was less than that of G′ values. This is an indication of the formation of a viscoelastic gel network. The maximum rate of increase in G′ values was found to be in the temperature range of 56.8 ° to 63.3 °C. The higher rate of increase in G′ indicates that proteins underwent ordered aggregation and formation of a 3-dimensional network with entrapment of water in the matrix. The forces responsible for gelation have been found to be hydrophobic interactions, disulphide cross bridges, and hydrogen bonds (Hamann 1992). The gelation of muscle proteins results from the transformation of an amorphous viscous solution to a 3-dimensional elastic network.

The dynamic rheological test or small strain or gel rigidity test has been used widely to study the heat-induced gelation of myofibrillar proteins (Hamann 1987; Visessanguan and others 2000). Changes in storage modulus (G′) have been used to monitor gelation of proteins including structural proteins (Venugopal and others 2002). Because G′ is a measure of the energy recovered per cycle of sinusoidal shear deformation, its increase indicates rigidity of the sample associated with formation of elastic gel structure. The loss modulus is a measure of the energy dissipated or lost as heat per cycle of sinusoidal strain when different systems are compared at the same strain amplitude. The loss factor (G″) indicates extent of viscous element in the sample. In the present study, the G″ values were less compared with G′ values, indicating the viscoelastic nature of the gel. The gelation process was also monitored by measuring changes in stress-strain phase angle during oscillatory test and indicates the temperature at which transition from sol-gel took place. The tan δ values were obtained by taking a ratio of G″/G′ during isothermal heating. This transition for fresh ribbonfish meat occurred at 36.7 °, 63.3 °, and 70 °C (Figure 8a).

The DVB profile of fresh ribbonfish meat with added cornstarch at 9% level (w/w) revealed considerable enhancement of gel-forming ability. The maximum G′ value (353.9 kPa) was recorded at 90 °C (Figure 7b). Similarly, tapioca starch at 9% (w/w) increased the G′ values by 2.3 times compared with DVB profile of meat alone (Figure

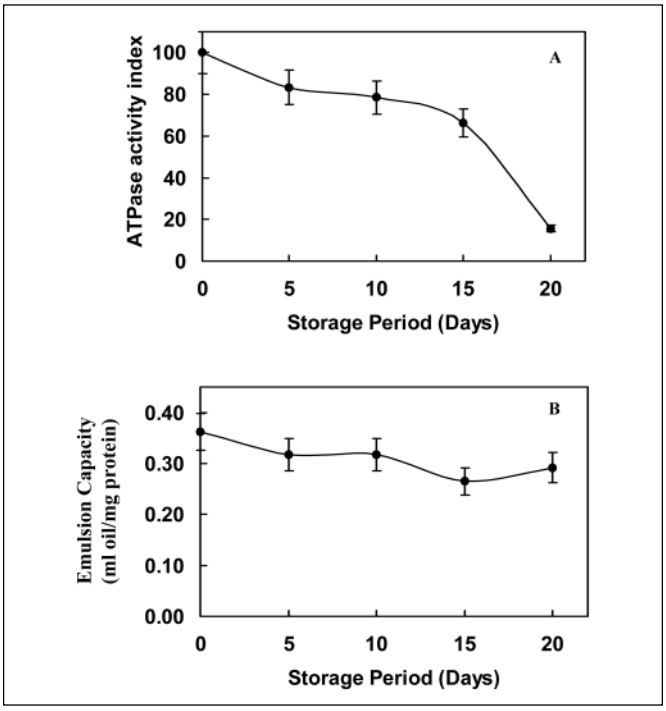

Figure 6—(a) Calcium ATPase activity during ice storage of ribbonfish; (b) emulsion capacity of protein from ribbonfish during different period of ice storage.

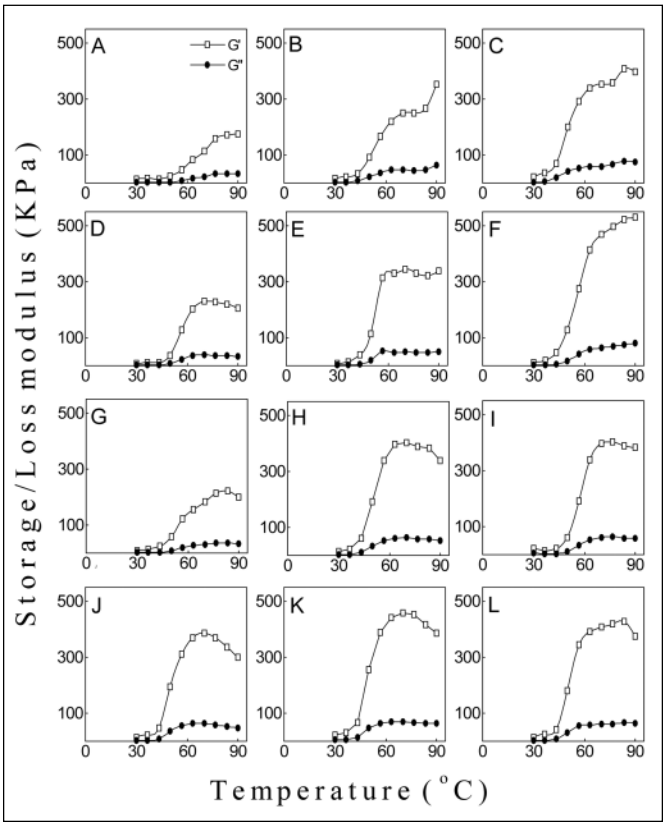

Figure 7—Effect of starch (corn/tapioca) at 9% w/w level on the dynamic viscoelastic behavior (DVB) profile of ribbonfish meat as a function of ice storage days. Day 0: (a) meat alone; (b) meat + cornstarch; (c) meat + tapioca starch. Day 5: (d) meat alone; (e) meat + cornstarch; (f) meat + tapioca starch. Day 10: (g) meat alone; (h) meat + cornstarch; (i) meat + tapioca starch. Day 20: (j) meat alone; (k) meat + cornstarch; (l) meat + tapioca starch.

7c). The sol-gel transition temperature for the cornstarch-ribbonfish mixture and tapioca starch-ribbonfish mixture was at 36.7 °C and 83.4 °C, and 36.7 °C and 69.9 °C, respectively (Figure 8b and 8c). During heating of fish mince/surimi-starch systems, significant rheological changes due to sol-gel transformation of fish proteins and gelatinization of starch are observed (Wu and others 1985). In the present investigation, 3 transitions for fish meat and 2 transitions for fish mince-starch mixture system were observed during heating. The 1st transition (36.7 °C) in all 3 samples is due to myosin molecule. The 2nd transition in the fish-starch mixture is due to gelatinization of starch molecule. Gelatinization of starch upon heating does not occur instantaneously at a specific temperature and depends on botanical source and availability of water. The gelatinization temperature for corn and tapioca starch at 6% level in water has been found to be 67 °C and 65 °C, respectively (Park 2000). Starch gelatinization temperature will be shifted to higher temperature when mixed with fish paste that included salt (Belibagli and others 2003). Two possible mechanisms have been proposed by Lee and Kim (1986) for how swelling of starch makes surimi gels more cohesive and firmer: (1) an increased density of protein matrix resulting from the transfer of moisture from the matrix to the swelling starch granules, and (2), formation of large number of elastic starch globule through gelatinization. The ratio of amylose and amylopectin will have a bearing on gelatinization of starch granule. Konoo and Ogawa (1998) evaluated the effects of the amylose/amylopectin ratio and degree of pregelatinization of starch on Alaska Pollock surimi gels and pointed out that for native starch, the amylose/amylopectin ratio did not significantly affect breaking strength (force). The amylose content of native cornstarch and tapioca starch used in the present study was 26% and 17%, respectively (Park 2000), and the DVB profile of fish meat-starch mixture revealed that both corn and tapioca could increase G′ values significantly, and between 2 starches there was no significant ($P < 0.05$) difference in gel-enhancement ability. The 1st transition in the thermogram of ribbonfish is well correlated with the transition from DVB measurement as per the tan δ values. The transitions were irreversible. Hence, it is evident that the transitions at temperatures above 70 °C in the DVB measurements are due to the combined effect of denaturation of different myofibrillar protein fraction as well as the gelatinization of starches.

The viscoelastic nature of the gel obtained from the ribbonfish meat and in presence of corn or tapioca starch was assessed by frequency sweep at 30 °C. The frequency sweep of the sol (fish meat + 2.5% NaCl) with and without starch has also been assessed. The results are given in Figure 9. The slope of the regression of G′ and G″ as a function of frequency is taken as index of viscoelastic nature of the material. The increase in storage modulus coupled with low phase angle with increase in frequency is indicative of weak gel network (Hudson and others 2000). The frequency sweep of acetic acid–induced shark meat gel indicated a steep increase in G′ values, with an increase in frequency indicating weak gel structure, further confirmed by a large strain test (Venugopal and others 2002). In the present study, the slope of the G′ value of the gel obtained from ribbonfish meat and ribbonfish meat + corn/tapioca starch revealed significant changes ($P < 0.05$) in the value (Table 4). Among the 3 samples, the slope of the G′ value of the gel obtained from cornstarch with fish meat mixture was minimum. It is evident from the frequency sweep that the viscoelastic nature of the ribbonfish meat could be modified significantly ($P < 0.05$) by the addition of cornstarch.

The DVB profile of ribbonfish meat as a function of the ice storage period is given in Figure 7d through l. The DVB was assessed without and with corn and tapioca starch. The profile of ribbonfish meat alone during different periods of ice storage is given in Figure 7d, g, and j. The storage modulus increased with the storage period and at the end of 25th day, the maximum G′ value obtained was 358.8 kPa at a temperature of 70 °C. Although an increase in the elastic modulus on heating is often considered a useful functional property of proteins (in gelled products), an increase in the elastic modulus of raw materials such as fish fillets is related to protein-protein and protein-lipid interactions leading to aggregation into undesirable, tough products (Badii and Howell 2002). The gel-forming ability of whole lizardfish (*Saurida tumbil*) as measured by large strain test decreased with the increase in the ice storage period because of denaturation of myosin (Benjakul and others 2003). In the present study, the physicochemical properties of proteins as revealed by solubility behavior in high ionic strength buffer (extraction buffer), gel filtration profile, Ca^{++} ATPase enzyme activity, reduced viscosity profile, and emulsion capacity pattern from ribbonfish revealed that there is an aggregation process after 10 d of ice storage. One of the possible reasons that can be ascribed for the increase in G′ values is aggregation/ denaturation of proteins during ice storage. The viscoelastic nature of the gels prepared from the ribbonfish meat, ribbonfish-cornstarch mixture, and ribbonfish-tapioca starch mixture during different periods of ice storage was assessed with frequency sweep test. The slope of regression of G′ values of gels obtained during different periods of ice storage is given in Table 4. There was a significant difference between samples as well as ice storage period ($P < 0.05$). However, this needs further validation using large strain test.

The temperature at which sol-gel transition occurred in ribbonfish meat-starch mixture as a function of ice storage period is given in Figure 8. In all the samples, the 1st transition occurred between

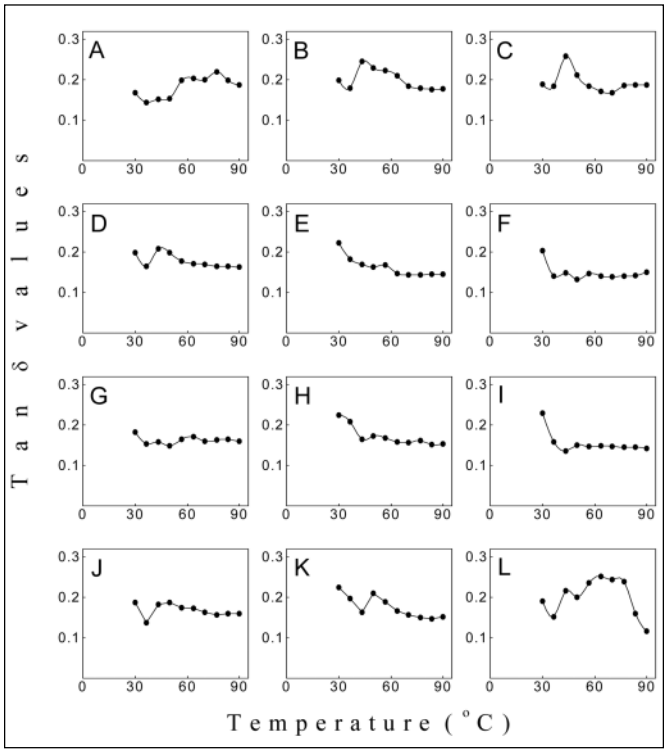

Figure 8—Tan δ values of ribbonfish meat with and without starch (corn/tapioca) at 9% w/w level as a function of ice storage days; Day 0: (a) meat alone; (b) meat + cornstarch; (c) meat + tapioca starch. Day 5: (d) meat alone; (e) meat + cornstarch; (f) meat + tapioca starch. Day 10: (g) meat alone; (h) meat + cornstarch; (i) meat + tapioca starch. Day 20: (j) meat alone; (k) meat + cornstarch; (l) meat + tapioca starch.

36.7 °C and 43.3 °C that is due to the myosin molecule, and the 2nd and 3rd transitions varied between 63 °C and 83 °C, indicating both were due to gelatinization of starch and further denaturation of myosin and actin molecules. The transition temperature occurring beyond 70 °C is due to gelatinization of starch. The DSC profile of 10% (w/v) cornstarch indicated gelatinization peak temperature of 66.90 °C, whereas that of tapioca starch was 60.41 °C. In a fish-starch mixture, the gelatinization of starch will be shifted to the higher side because of less available moisture, and there will be variation in transition temperature from sample to sample. However, the G′ values of the fish-starch mixture were consistently higher than the fish meat alone at any period of ice storage.

Conclusions

The effect of ice storage on the physicochemical and viscoelastic properties of ribbonfish (*Trichiurus spp*) meat has been evaluated. There was a significant ($P < 0.05$) reduction in protein solubility, emulsion capacity values, and Ca^{++} ATPase enzyme activity and an increase in reduced viscosity values during 15 d of ice storage indicating an aggregation/denaturation process. The VBN content reached an approximate value of 40 mg N/100 g meat at the end of 10 d of ice storage, which is normally taken as the limit of chemical spoilage. Ribbonfish had moderate gel-forming ability, which was enhanced by the addition of either 9% corn or tapioca starch. The dynamic viscoelastic behavior of ice stored ribbonfish meat indicated that higher G′ values were possibly due to the aggregation/denaturation process induced during storage. The viscoelastic property of ribbonfish meat and fish meat-starch mixture altered significantly ($P < 0.05$) during ice storage period. The cornstarch had better ability to enhance the viscoelastic property of ribbonfish meat.

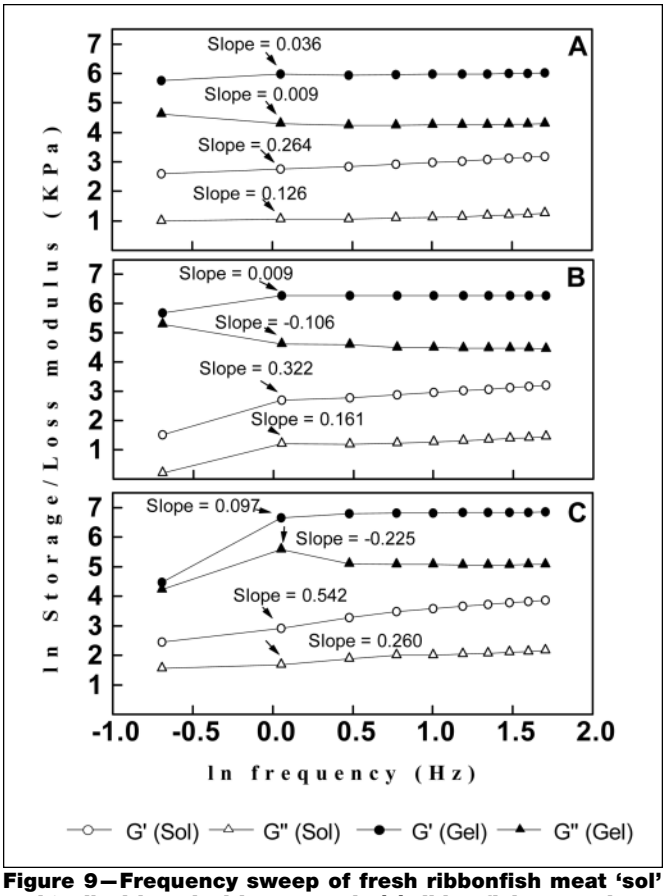

Figure 9—Frequency sweep of fresh ribbonfish meat 'sol' and 'gel' with and without starch; (a) ribbonfish meat alone, (b) ribbonfish meat + 9% cornstarch; (c) ribbonfish meat + 9% tapioca starch.

Table 4—Slope of regression of G′ values of the gels prepared from ribbonfish meat with and without corn/tapioca starch during different periods of ice storage[a,b]

Ice storage days	Ribbonfish meat alone[1]	Ribbonfish meat+9% cornstarch[2]	Ribbonfish meat+9% tapioca starch[3]
0	0.036[a]	0.009[a]	0.097[a]
10	0.052[b]	0.011[b]	0.130[b]
20	0.049[c]	0.018[c]	0.091[c]

[a]The G′ values obtained were as a function of frequency sweep. From the plot of ln G′ and ln frequency, the slope of regression of G′ values was calculated.
[b]Letters after values denote significant difference ($P < 0.05$) between storage period.
Superscripts 1, 2, and 3 denotes significant difference ($P < 0.05$) between samples.

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