Effect of ice storage on the functional properties of proteins from a few species of fresh water fish (Indian major carps) with special emphasis on gel forming ability

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Abstract In the present study the effect of ice storage on physico-chemical and functional properties of proteins from Indian major carps with special emphasis on gel forming ability have been assessed for a period of 22 days. The solubility profile of proteins in high ionic strength buffer and calcium adenosine triphosphatase (ATPase) enzyme activity reduced significantly ($p < 0.05$), while that of total volatile base nitrogen (TVB-N) increased significantly ($p < 0.05$) at the end of 22 days of ice storage. The major protein fraction showed association-dissociation-denaturation phenomenon during ice storage as revealed by gel filtration profile and viscosity measurements. The gel forming ability of three fish species both in fresh and during different periods of ice storage was assessed by measuring the gel strength of heat induced gel. Among the three species the gel strength of the gel obtained from *Catla catla* and *Cirrhinus mrigala* was higher (586 and 561 g.cm) than the gel obtained from *Labeo rohita* (395 g.cm) in fresh condition. The gel forming ability of three species was significantly affected ($p < 0.05$) during ice storage. The TVB-N values of fish meat as a function of ice storage was within the prescribed limit up to 17 days of the ice storage.

Keywords Indian major carps · Gel forming ability · Ice storage · Solubility

Introduction

The demand for fish is increasing world over due to its high nutritive value. However, it has become increasingly difficult to meet the demand due to short supply. Though, the world fish production has increased from 77.45 mt in 1983 to 142 mt in 2008, this increased supply is not sufficient to meet the demand. Nearly 64% of the total production is obtained by capture fishery mostly from marine sources and 36% from aquaculture (FAO 2010). The fish production in India is different from the world scenario, wherein, 4.61 mt have been obtained from inland sources (mainly aquaculture) and 2.99 mt from marine sources during the year 2008–09 (Pandian 2010). The freshwater fish production in India is dominated by Indian major carps which include three species viz. *Catla catla* (*C. catla*), *Labeo rohita* (*L. rohita*) and *Cirrhinus mrigala* (*C. mrigala*). With improved aquaculture practices the production of Indian major carps is likely to increase and post harvest management will be the key issue for effective utilization. At present the disposition pattern of Indian major carps is mainly in the fresh or iced form and the potential for the preparation of mince based products is high.

As fish is highly perishable commodity, better utilization calls for deeper understanding on the composition, spoilage process and stability to different processing conditions. The fish species is so diverse; the rate of perishability varies from one species to another. Assurance of both quality and safety of the food will be major challenge faced by humankind in recent time. The deterioration of quality of both wild and farmed fish species is mainly due to action of intrinsic enzymes and microbes (Hsieh and Kinsella 1989; Pigott and Tucker 1987). In order to cater the supply of fresh fish, short term preservation by using ice or any

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chilling medium is more practical and economical. It is well known that lowering the body temperature will help to extend the keeping quality of fish (Pigott and Tucker 1990). During chilling storage biochemical changes are known to take place in the proteins and lipid fractions. As a consequence of these events, deterioration in sensory quality, loss of nutritional value and changes in physico-chemical properties have been reported (Bennour et al. 1991; Nunes et al. 1992; Olafsdottir et al. 1997). Though the traditional methods of preservation like salting, drying, canning and smoking are applied, it is preservation by chilling and freezing which are widely accepted by consumers. It is important to understand the changes in the properties of proteins that occur in fish meat during chill storage which will have bearing on the final quality of product. The technological value of any fish meat is determined by its functionality which is mainly contributed by proteins. The properties such as gelation, emulsification, water binding and lipid binding are the manifestation of conformational status of the protein system (Kinsella and Melachouris 1976). Different processing methods including icing will lead to changes in the properties of proteins from fish leading to alteration in the functional properties (Sikorski 1994).

In order to improve the utilization pattern of fresh water fish consumption to processed products, there is a need to understand changes occurring in muscle components during ice storage. The demand for processed fishery products especially mince based products is high from the urban population. It is worthy to study the effect of ice storage of Indian major carps on the suitability for preparation of mince based products like fish sausage, kamaboko and fish cake. The primary requirement for preparation of these products is the gel forming ability of the mince. It is expected that in the years to come the demand for mince based products from Indian major carps will be on the rise. Hence, objectives of the present investigation are to assess changes in the physico-chemical and functional properties of three species of fish during ice storage and to evaluate the suitability of ice stored Indian major carps for preparing gel products by assessing gel forming ability.

Materials and methods

Material The three species of Indian major carps viz. *C. catla*, *L. rohita* and *C. mrigala* were used in the present study. The fishes were harvested from culture ponds in Shimoga, Karnataka and transported to the laboratory in iced condition in poly- urethane boxes. The average length and weight of all the three species of Indian major carps were between 33 and 43 cm and 519–869 g. The fish was iced in the ratio of 1:1 (fish: ice). The fish and ice were

packed in an alternative layer in the box and transported to the laboratory. The time taken to reach the laboratory from harvest centre was about 5–6 h. The three species were segregated and transferred to thermocole (expanded poly styrene) boxes and iced in the ratio of 1:1. The boxes were kept in chill room (4°–6 °C) for storage studies. The ice was replenished once in 24 h after draining the melt water. The total period of ice storage was 22 days.

Preparation of sample The samples were drawn for analysis at 0, 3, 6, 9, 12, 15, 18 and 22 day of ice storage. The meat samples were analyzed for physico-chemical and functional properties. The term 0 day refers to day of sample drawn after 10 h of harvest. The fish was removed from thermocole box and dressed (removal of head, entrails and fins). The meat was separated manually devoid of fins and bones and the separated meat was macerated well using pestle and mortar in ice bath and used for analysis.

Proximate analysis The proximate analysis of fresh meat from all three species was carried out. The moisture content was determined by hot air oven method (AOAC 2010). The crude protein content of the meat was determined by estimating its total nitrogen content by Kjeldahl method (AOAC 2010). The nitrogen obtained was multiplied by a factor of 6.25 to get protein content. Total volatile base nitrogen (TVBN) content of meat was determined by Conway's micro-diffusion technique (Beatty and Gibbon 1937) and expressed as mg N/100 g meat. The non protein nitrogen (NPN) content of meat was analyzed according to the method as described by Velankar and Govindan (1958) and expressed as mg N/100 g meat.

Protein solubility in high ionic strength buffer The solubility of total proteins was determined using phosphate buffer, (50 mM, pH 7.5, containing 1 M sodium chloride) as solvent. This buffer containing 1.0 M sodium chloride here afterwards referred as extraction buffer (EB). The ratio of fish meat to extraction buffer was 1:10 (meat : buffer). The fish meat with buffer was homogenized in high speed using laboratory homogenizer (Ultra-Turrax homogenizer) at 9000 rpm for 2 min. The slurry was centrifuged at 9000×g at 4 °C for 15 min using high speed refrigerated centrifuge (IEC B22, USA). The clear supernatant was taken for protein determination by Kjeldahl method. The protein solubility was expressed as percentage of total protein of meat.

Calcium ATPase enzyme activity Calcium ATPase enzyme activity was measured by using the method of Naguchi and Matsumoto (1970). About 1 g of fresh and ice stored samples of meat was macerated in 10 ml, 0.2 M Glycine - NaOH buffer, pH 9.2 and slurry was filtered through

Whatman no. 1 filter paper and the filtrate was used as enzyme solution. The reaction mixture comprised of 0.06 ml of ATP (0.05 M) solution, 0.4 ml calcium chloride (0.1 M), 2 ml Glycine-NaOH buffer (0.2 M, pH 9.2). 0.4 ml of enzyme was added to reaction mixture and incubated for 5 min at 27 °C. The reaction was stopped by adding 2 ml of 15% TCA. Appropriate blanks were maintained. The mixture was filtered through Whatman no. 1 filter paper, and the inorganic phosphorus content was determined by method of Taussky and Shorr (1952). To the 3 ml filtrate, 2 ml freshly prepared ferrous sulphate –ammonium molybdate solution (10%) was added. The intensity of the color developed was measured at 660 nm (Spectro UV- VIS Double, Labomed. Inc., USA). The liberated inorganic phosphorus was calculated using standard curve obtained by using potassium di- hydrogen phosphate as a standard. The ATPase enzyme activity was expressed as µg Pi/min/mg protein.

Gel filtration profile Gel filtration profile of total proteins extracted from three species of fish using extraction buffer was carried out. The gel used was sepharose 6B packed in glass column of 1.6 cm×95 cm (dia.×height). The total bed volume was 200 ml. The eluent used was extraction buffer. The void volume (V_0) was determined using blue dextran and found to be 72 ml. Gel filtration was carried out at room temperature. The protein concentration loaded to the column varied between 10 and 18 mg/ml. The flow rate was adjusted to 30 ml/h and after eluting void volume of 72 ml, the fractions of 3 ml were collected manually in a series of test tubes. The protein concentration of fractions obtained was measured by taking the absorbance at 280 nm (Spectro UV- VIS Double, Labomed. Inc., USA). A plot of concentration of protein against elution volume was obtained to get filtration profile.

Apparent reduced viscosity The apparent reduced viscosity of total proteins from three Indian major carps during different periods of ice storage was determined. The total proteins were extracted using extraction buffer as described. The apparent reduced viscosity was determined using Ostwald viscometer with a capillary diameter of 0.5 mm. The viscosity was measured at 25 °C±0.5 °C. The protein solution of 12 ml was loaded to viscometer and the solution was equilibrated at 25 °C in a water bath where the temperature was maintained. The protein solution was taken up by suction well above the marking of the capillary of viscometer and the time taken for protein solution to travel between two points was recorded with the help of stop watch ((Rocar, Switzerland with 0.2 s accuracy). The experiment was repeated three times and average flow time (in sec) was taken for calculation. The relative viscosity was determined by noting the time taken for protein

solution and the solvent (EB) separately by using the formulae given below

$$\eta_{rel} = \frac{t_1}{t_0}$$

Where,

t_1 time taken for protein solution in Sec

t_0 time taken for solvent (EB) in Sec

The relative viscosity at different protein concentration was determined and reduced viscosity (η_{red}) was calculated using equation given by Yang (1961).

$$\eta_{red} = \frac{\frac{t_1}{t_0} - 1}{C(mg/ml)}$$

A plot of η_{red} viscosity vs protein concentration was obtained and η_{red} viscosity at single protein concentration (3 mg/ml) was derived from the plot.

Gel forming ability

Preparation of gel About 500 g of separated meat was macerated using pestle and mortar in chilled condition (4°–5 °C) with 2.5% sodium chloride. The time of maceration was 10–12 min. About 100 g of viscous meat paste was stuffed into krehalon casing of 50 mm×250 mm (dia×length) using hand stuffer. The casings were sealed with aluminum wire using ringer machine. Prior to stuffing one end of casing was sealed and after stuffing the other end of casing was sealed. The stuffed casings were heat processed in a constant temperature set water bath on 90 °C±2 °C for 45 min and cooled in chilled water for 15 min. The prepared gels were kept in refrigerator overnight and used for the measurement of gel strength.

Measurement of gel strength The gel strength was measured both by instrument and folding test. The instrumental measurement was carried out using texture analyzer (TA. XT plus Stable Micro System, Surrey, England). The prepared gels were brought to room temperature and cut into 25 mm×30 mm (dia×length) and placed on the platform. A 5 mm spherical probe was used for measurement. A trigger force of 10 g and distance of 20 mm was programmed in the instrument. During the measurement spherical probe pierces the gel to a distance of 20 mm and peak load exerted by the instrument was recorded. The gel strength was calculated by multiplying the peak load (g)×programmed distance (mm). Gel strength of the sample was expressed in g.cm. The average of three replicates was reported as gel strength of sample.

Folding test Folding test was conducted to assess the quality of gel prepared according to the method described by Okada (1963). Gel size of 3 cm dia and 3 mm thick was folded once into semicircle or twice into quadrant. Folding test of gel was classified as follows - no cracks when folded into quadrant (AA), crack along the edges when folded into quadrant (A), no crack when folded into a semicircle (B), cracks along the edges when folded into semicircle (C), breaks into two pieces when folded into semicircle (D).

Emulsion capacity Emulsion capacity of meat samples was determined according to the method of Swift et al. (1961). 25 g of meat was homogenized with 100 ml of chilled extraction buffer at 9000 rpm for 2 min using Ultra Turrex homogenizer (T25 Janke Kunkel, Germany). Slurry was kept in refrigerator for 15 min. The protein content of slurry was determined by kjeldahl method. To the 12.5 g of slurry, 37.5 ml of chilled extraction buffer and 50 ml of refined sunflower oil were added and homogenized at 9000 rpm for 10 s using Ultra Turrex homogenizer with 525 N-G18 dispersing tool. Homogenization was continued at high speed (23000 rpm) with addition of oil at the rate of 0.5–0.6 ml/s until visual phase inversion was recorded. Emulsion capacity was calculated after considering initial volume of oil added and expressed as ml of oil per mg of protein. Average of three replicates was reported as emulsion capacity values.

Statistical analysis The data obtained was analyzed by one way ANOVA (Snedecor and Cochran 1962). The analysis of variance was carried out for number of days of ice storage with TVBN, protein solubility and gel strength. All the experiments were carried out in three replicates. Data was also analyzed to evaluate the Karl Pearson correlation coefficient between different parameters and ice storage period by the method as described by Yamane (1964).

Result and discussion

Proximate composition, physico-chemical and functional characteristics of fresh meat of the *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*: The proximate composition of fresh meat from three species is given in Table 1. The moisture content was slightly higher than reported values of cultured carps from Indian waters (Ganesh et al. 2006; Joseph et al. 1988; Ramachandran et al. 2009). The protein content of the fish varied from 17.94 to 19.14. The fat content in all the three species was less than 2% and can be considered as lean varieties. It has been well established that fat content varies with sex, age and season (Gopakumar 1993). The ash content of meat of the three species varied from 1.5 – 2%. Since the ash content was determined in the meat it is expected to have lower value than from whole fish including bone.

The total volatile base nitrogen content (TVB-N) of three species stored in ice showed an increasing trend during ice storage (Fig. 1 a). In case of *C. mrigala* a decrease in TVB-N content was recorded at the end of 12 days of ice storage and with further increase in ice storage period, the values increased. The increase in TVB-N as a function of ice storage was found to be significant ($p < 0.05$) at the end of ice storage. The increase in TVBN content mainly arises from degradation of NPN constituents by intrinsic enzymes or by bacterial activity during ice storage (Barassi et al. 1987). The TVBN content for finfish in the range of 35–40 mg N/100 g of meat is taken as the limit of chemical spoilage (Connell 1995). The increase in TVBN values during ice storage is species specific and TVBN values for the fresh water fishes like Thai pangasius and silver carp have shown 5–20 times increase for a period of 25–35 days (Hossain et al. 2005; Fan et al. 2008). The increase in TVBN values for *L. rohita* for a period of 25 days of ice

Table 1 Proximate composition, physico-chemical and functional characteristics of fresh meat of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*

Proximate composition	Catla catla	Labeo rohita	Cirrhinus mrigala
Moisture (g/100 g meat)	76.2±1.68	78.0±1.15	78.0±0.36
Protein (g/100 g meat)	18.3±1.14	19.1±0.36	17.9±0.56
Crude fat (g/100 g meat)	2.0±0.11	0.99±0.02	1.6±0.05
Ash (g/100 g meat)	1.5±0.05	1.6±0.51	2.0±0.06
Volatile base nitrogen (mg/100 g meat)	9.8±1.77	7.9±1.37	11.2±0.00
Non Protein Nitrogen (mg/100 g meat)	282.0±1.73	311.4±8.4	305.5±23.71
pH	6.9	6.8	6.8
Solubility (as% of total nitrogen)	76.0±1.35	71.4±3.14	75.2±0.62
Ca ²⁺ ATPase enzyme activity (µg Pi/min./mg protein)	0.64	0.72	1.04
Viscosity (dl/mg of protein)	0.058	0.055	0.050
Gel strength (g.cm)	586.5±38.22	394.9±51.97	561.6±19.22
Emulsion capacity (ml oil/mg protein)	0.64	0.36	0.31

Values are means±SD, n=3

Fig. 1 Changes in **a** total volatile base nitrogen, **b** protein solubility, **c** calcium ATPase activity, **d** apparent reduced viscosity, **e** gel strength and **f** emulsion capacity of Indian major carps during the period of Ice storage. Mean values of three replicates ($n=3$) was used for plotting

storage was not significant as reported by Joseph et al. (1988). In the present study the TVBN values were less than 40 mg N/100 g of meat at the end of 17 days of ice storage. If one compares the TVBN production of fresh water fish and marine water fish, the increase in marine fish is higher than fresh water fish (Dileep et al. 2005; Binsi et al. 2007). This is possibly due to higher non protein nitrogen content in marine fishes. There was a significant ($p<0.05$) correlation was found (r value more than 0.9) between increase in the TVBN value and days of ice storage for all the three species.

Protein solubility in high ionic strength buffer The protein solubility showed an increasing trend in *Catla catla* and *L. rohita* up to 12 and 9 days of ice storage respectively and thereafter the values decreased (Fig.1 b). In the case of *C. mrigala* there was a marginal increase in soluble protein up to 6 days of ice storage thereafter the value decreased. The

reduction in solubility during ice storage is attributed to the behavior of myofibrillar proteins as affected by ice storage. The process of association–dissociation–denaturation is the main contributing factor for reduction in solubility. Changes in protein solubility are a direct evidence of conformational changes of protein molecule. A moderate increase in the protein solubility during initial period of ice storage is attributed to weakening of fibrous linkages in muscle structure (Zayas 1997). The solubilization of myofibrillar proteins is the major factor affecting the functional properties of fish protein both during ice and frozen storage (Regenstein and Regenstein 1984; Borderias et al. 1985). The aggregation/denaturation will be more severe during frozen storage (Shenouda 1980). In the present study the analysis of variance and correlation coefficient values indicated significant reduction in protein solubility during ice storage. The changes in solubility of total proteins could have occurred due to alterations in

conformational status which can be monitored by assay of Ca^{2+} ATPase enzyme activity, gel filtration and viscosity measurement.

Ca²⁺ ATPase enzyme activity The initial Ca^{2+} ATPase enzyme activity of *C. mrigala* was higher in comparison to *C. catla* and *L. rohita* (Fig. 1. c). The slope of the curve clearly indicates that there was sharp reduction in activity in *C. mrigala* at the end of 6 days of ice storage (Fig. 1 c). The Ca^{2+} ATPase enzyme activity of natural actomyosin of cod at various periods of ice storage revealed that the activity could be detected even after 30 days of ice storage (Colmenero et al. 1988). The changes in the solubility profile of myofibrillar protein will lead to decreased activity of enzyme (Kamal et al. 1991). In the present study the enzyme activity could not be detected in *L. rohita* after 18 days of ice storage. If one compares the solubility data it is evident that there is a significant decrease in solubility which may be one of the important factors contributing to reduction in ATPase enzyme activity. Factors that can cause the denaturation of myofibrillar proteins can affect ATPase enzyme activity (Mac Donald and Lanier 1994). The possibility of action of proteolytic enzymes in the muscle can also reduce the enzyme activity (Quali and Valin 1981). Measurement of ATPase enzyme activity and calculating the inactivation rate constant for measuring the extent of protein denaturation has been arrived at by many workers (Fukuda et al. 1984; Tsai et al. 1989).

Gel filtration profile The gel filtration profile of the total protein extracted from *C. catla*, *L. rohita* and *C. mrigala* revealed three fractions. The major fraction eluting at an elution volume between 80 and 86 ml is more likely to be actomyosin complex. The gel filtration profile as function of ice storage revealed the phenomenon of association – dissociation occurring in all the three species studied. The volume at which the major fraction eluted in *C. catla* at the end of 3, 6, 9, 12, 17 and 22 days of ice storage was 83, 86, 77, 80 and 80 ml respectively (Fig. 2 a-b). This shift in elution volume is an indication of association (if elution volume is reduced) and dissociation (if elution volume is increased). A similar behavior was also observed in case of *L. rohita* (Fig. 2 b-d) and *C. mrigala* (Fig. 2 e-f) by taking into account the elution volume of the major peak. The concentration of the major peak at the end of 22 days of ice storage was significantly reduced in the profile obtained from *C. catla* and *L. rohita*. The profile obtained from *C. mrigala* (Fig. 2 e-f) indicated marginal reduction in high molecular weight components. Gel filtration profile did not reveal increase in low molecular weight components indicating that reduction in concentration of high molecular weight components is due to lower solubility in the solvent

used. It has been reported that association–dissociation–aggregation alters elution profile (Binsi et al. 2007). This association–dissociation–aggregation is one of the reasons for reduction in solubility and Ca^{2+} ATPase enzyme activity.

Apparent reduced viscosity The reduced viscosity profile at a single protein concentration (3 mg/ml), a derivative value vs ice storage period is given in Fig. 1d. The η_{red} viscosity values as a function of ice storage showed a fluctuating trend in all the three species studied. However at the end of 22 days of ice storage, η_{red} viscosity values of proteins from *C. mrigala* showed a higher value from initial value. The change in η_{red} is an indication of changes in conformational status of major protein fractions. The measurement of viscosity of protein solution has often been used to determine the protein denaturation. The association–dissociation of actomyosin molecule will lead to decrease in particle axis ratio leading to change in shape of molecule. The measurement of viscosity is considered as more reliable protein quality from fish than protein solubility or emulsion capacity (Colmenero et al. 1988). In the present study the gel filtration profile of proteins from the three species showed association–dissociation phenomenon which is reflected in viscosity measurement. Changes in the viscosity in the muscle protein during ice storage are due to exposure of hydrophobic groups to the bulk solvent (Roura and Crupkin 1995). The increase in η_{red} viscosity values at a concentration of 3 mg/ml in case of *C. mrigala* could be related to more of protein – protein interaction.

Gel forming ability There was a significant reduction in gel strength values in all the three species at the end of 22 days of ice storage. The reduction in gel strength values of the gels prepared from *C. catla* meat was rapid at the end of 3 days of ice storage. The initial gel strength values of the gels from *C. catla* and *C. mrigala* were almost same while that of obtained from *L. rohita* was lower (394.91 g.cm). The process of gelation is cross linking of polypeptide chain to form well defined tri-dimensional network with entrapment of water in the muscle (Ziegler and Foegeding 1990). The strength of the gel depends on the extent of cross links that occur in the polypeptide chain. Proteins from fish differ in their ability to cross link to form network and found to be highly species specific. The present study revealed that the *C. catla* meat had higher gel forming ability in the fresh condition but in the ice stored samples the ability decreased significantly. The initial gel strength values for *L. rohita* were much lower than *C. catla* and *C. mrigala*. But as a function of ice storage period up to 9 days gel forming ability was good. The reduction in gel forming ability could be attributed to loss of protein solubility and Ca^{2+} activated ATPase enzyme activity. This is further

Fig. 2 Gel filtration profile of total proteins from meat of **a–b** *Catla catla*, **c–d** *Labeo rohita* and **e–f** *Cirrhinus mrigala* at 0 day and 22nd day of ice storage

reflected in gel filtration profile and viscosity measurement of protein from three fish species.

The instrumental analysis of gel strength of the gel was complimented by folding test. The results are given in

Table 2. There was a good correlation between gel strength values and folding test. The gel prepared from *C. catla* and *C. mrigala* on the 0 day (fresh sample) revealed 'AA' grade and the corresponding gel strength values were found to be

Table 2 Folding test of gels prepared from *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* meat during different periods of ice storage

Ice storage period (Days)	Folding test (Grade)		
	<i>Catla catla</i>	<i>Labeo rohita</i>	<i>Cirrhinus mrigala</i>
0	AA	A	AA
3	AA	A	AA
6	B	B	AA
9	B	B	A
12	C	C	B
15	-	C	-
17	C	-	C
18	-	C	-
22	D	D	D

586.54 and 561.67 g.cm respectively. The ice storage had a significant effect on the folding test of the gel and at the end of 22 days of storage 'D' grade was recorded in all the samples. Based on the results it is evident that catla had higher gel forming ability in fresh condition. But it is declined rapidly during ice storage. The gel forming ability of *C. mrigala* and *L. rohita* showed a decreasing trend as a function of ice storage.

Emulsion capacity (EC) The emulsion capacity of total proteins of three fish species stored in ice for different periods is given in Fig. 1f. The initial emulsion capacity of proteins from *C. catla* was higher than that from *C. mrigala* and *L. rohita*. The emulsion capacity of protein is an intrinsic property and varies with nature of protein molecule. The surface hydrophobic molecules play very important role in establishing the emulsion properties (Kinsella 1982). Emulsion imparts texture that contributes to desired mouth feel facilitate the inclusion of both fat soluble and water soluble ingredients (Dickinson and Stainsby 1982; Das and Kinsella 1989). The emulsion capacity of total proteins from *C. catla* showed a decreasing trend up to 6th day of ice storage and further storage in ice, the values decreased. A similar fluctuation was also recorded for *L. rohita* and *C. mrigala* (Fig. 1 f). It has been established that denaturation/aggregation reduces many functional properties including emulsion capacity. The association–dissociation may be the principle reason for fluctuating values of EC during ice storage. It has been reported that denaturation/aggregation of food proteins at time results in increased emulsification properties (Wang and Kinsella 1976; Aoki et al. 1980).

Conclusion

The three species of Indian major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* are suitable for the preparation of mince based gelled products as assessed by gel forming ability. The ice storage of three species of fish had a significant effect on major protein fractions in terms of association–dissociation/aggregation–denaturation as revealed by solubility in high ionic strength buffer, Ca²⁺ ATPase enzyme activity, and gel filtration profile and viscosity measurements. The ice stored samples were acceptable up to 17 days of ice storage. For the preparation of gelled products like fish sausage and kamaboko the fish stored in ice for a period of 9 days was acceptable.

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