

Original Research Article

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Chilled Storage Induced Changes in Functional Properties of Fresh Water Eel (*Mastacembelus armatus*)

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ABSTRACT

The changes in functional properties viz. solubility, total sulfhydryl groups, Ca²⁺ ATPase activity, emulsion activity index (EAI), emulsion capacity (EC), viscosity, foam volume stability (FVS), foam volume capacity, gelation, water holding capacity (WHC) of muscle proteins from fresh water fish eel (*Mastacembelus armatus*) during ice storage was evaluated for a period of 16 days with the analysis of each parameter monitored for every alternate day. The solubility of muscle proteins i.e. MFP decreased from 59.16 to 51.89 mg/g during chill storage. The gel strength of MFP decreased slowly from 250 to 247g.cm during first 4 days and then markedly decreased from 240 to 100 g.cm from 6th day onwards up to 16th day of storage. The total sulfhydryl groups of MFP fraction of eel muscle proteins decreased steadily during ice storage. The Ca²⁺ ATPase activity of actomyosin gradually decreased during storage. The emulsion activity index (EAI) of MFP and SPP fraction at concentration of 2.5 and 5.0 mg/ml also decreased continuously from 2.8 to 2.0 and 3.1 to 1.3 m²/g and 2.5 to 1.4 and 2.2 to 1.0 m²/g respectively. The emulsion stability (ES) of MFP and SPP fraction at concentration of 2.5 and 5.0 mg/ml decreased gradually from 55 to 28 and 60 to 40 and 32 to 15 and 40 to 20 min respectively during chill storage. The foaming expansion and foaming volume stability (FVS) at concentration of 5.0 mg/ml showed decreasing trend during chill storage. The viscosity of MFP and SPP at concentration of 5.0 mg/ml decreased gradually during chill storage. The water holding capacity (WHC) of muscle also reduced during chill storage. The result of fish with respect to functional properties showed noticeable changes during ice storage.

Keywords

Eel fish, Functional properties, Ice storage

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Introduction

The spiny eel, *Mastacembelus armatus* is one of the most common species of fresh water eel found in Asia. Demand for the fish almost always exceeds supply, particularly in India and its neighbouring countries such as Pakistan, Sri-Lanka and Bangladesh, although the fish has also been reported in Myanmar,

Thailand, Malaysia and Southern China. In northern and eastern India, the fish is very popular when sold alive; it occurs in a variety of freshwater habitats in the plains as well as in hills of India (Talwar and Jhingran, 1991).

Proteins are endowed with a number of physico-chemical and functional characteristics, which make them suitable for

varied food applications such as thickeners, emulsifiers etc. The functional properties of eel muscle were evaluated in order to examine its potential in the preparation of emulsion or sausage type foods. Fish proteins are unique in nature and exhibit high degree of functional properties. Functional properties can affect the processing and behaviour of proteins in the food system as judged by the final quality of the product (Kinsella, 1981). Many proteins with different functional and biological activities share common structural elements (Richardson, 1981). Post harvest changes in fish muscle affect the quality of protein and hence its functional properties (Partiban *et al.*, 2005).

The changes in functional properties of fish proteins during ice storage have been reported in hake (Crupkin *et al.*, 1981), threadfin bream (Yongswawatdigul and Park, 2002), tilapia (Parthiban, *et al.*, 2005, 2015), Atlantic salmon (Wang *et al.*, 2005), rohu (Mohan *et al.*, 2006) and *L. calbasu* (Yathavamoorthy *et al.*, 2012). In view of limited literature available on changes in functional properties in eel fish proteins during ice storage, the present study investigates the changes in physico-chemical and functional properties of eel during chill storage.

Materials and Methods

Fresh eel (*Mastacembelus armatus*) harvested from Krishna River in Sangli district and brought in ice condition to Ratnagiri fish landing center were purchased. The fish had total length of 30 to 55 cm and weight ranged between 225±13.22 g.

Fishes were de-skinned and filleted. The fillets were minced in a kitchen mixer/grinder and boneless meat was used under a temperature of 2-4°C throughout the experiment. Extraction of muscle protein fraction was estimated according to the method of King

and Poulter (1985). Protein determination of MFP and SPP extracts were estimated according to Gornall *et al.*, (1948) by Biuret method. Extraction of natural actomyosin was prepared according to the method described by Benjakul *et al.*, (1997).

The ATPase assay of actomyosin was estimated according to the method of MacDonald and Lanier (1994). Inorganic phosphates were estimated by the method of Fiske and Subbarow (1925). The total SH groups of myofibrillar protein fraction were estimated according to Sedlak and Lindsay (1968).

The ability of the proteins, SPP and MFP to form emulsion was estimated as emulsion activity index (EAI) according to the method of Pearce and Kinsella (1978) and as per modification of Cameron *et al.*, (1991). About 20 ml sample was prepared for estimation of viscosity of salt soluble and water-soluble protein at different concentrations (2.5 and 5.0 mg/ml). It was determined with a (Model DV II + Pro, Brookfield) viscometer at shear rate 100 rpm as described by Mohan *et al.*, (2006). Foamability of the protein was determined by the method of Wild and Clark, (1996). The water-washed fish mince was used to get the concentrate of MFP. Heat-induced gels were prepared from MFP concentrate by grinding with 3% Sodium chloride for 2 min at 4°C (Lan *et al.*, 1995). Water holding capacity (WHC) of mince was carried out by the method of Kocher and Foegeding (1993) with slight modification. The data of experiment was analyzed by Man-Whitney test and t-test (Zar, 2005).

Results and Discussion

Solubility of protein

The initial solubility of sarcoplasmic and myofibrillar fractions were observed to be

44.93 and 59.16 mg/g respectively for the fresh eel stored in ice (Fig. 1). The SPP showed decreasing trend throughout the storage ($p < 0.05$). The decrease in solubility of SPP could be attributed to its instability due to the aggregation behavior under the conditions of storage and subsequent leaching out in the melt ice (Mohan *et al.*, 2006; Parthiban *et al.*, 2015).

The solubility of MFP of eel meat showed gradual decreasing trend ($p < 0.05$). The solubility of proteins during ice storage decreased by 3% in two days and it remained almost constant up to 8 days, then further it decreased steadily up to 16th day. By the end of 16 days of storage, 13% of MFP became insoluble. According to Partiban *et al.*, (2005), the solubility of proteins during ice storage decreased by 8% in two days in tilapia stored in ice and it remained almost constant up to 7 days. By the end of 14 days of storage, 24% of total salt soluble proteins became insoluble. A similar decrease in salt soluble proteins of croaker and pink perch during ice storage was observed by Srikar (1979). Mohan *et al.*, (2006) noted decreasing trend in protein solubility of rohu during iced storage. Parthiban *et al.*, (2015) observed that the total soluble proteins of tilapia decreased during storage in ice.

Ca²⁺ ATPase activity of actomyosin of eel

ATPase activity has often been monitored as an indicator of the extent of myosin unfolding (denaturation) and aggregation (Lanier, 1986). Ca²⁺ATPase are localized in the myosin head region and play a significant role in the muscle contraction. Any alteration in the structural integrity of myosin affects the activity of enzyme and is widely used as the measure of myosin integrity (Nagai *et al.*, 1990 and Benjakul *et al.*, 1997). The Ca²⁺ATPase activity of actomyosin of eel in the present study showed decreasing trend during storage

of fish. The decrease in the activity was marginal up to initial four days of storage in ice as was evidenced from the gel strength value observed for the sample on the 4th day. Thereafter, it decreased significantly ($p < 0.05$) from initial value of 0.50 $\mu\text{mole Pi/mg protein/min}$ on the 1st day of storage to 0.20 $\mu\text{mole Pi/mg protein/min}$ on 16th day of storage (Fig. 2).

The results of the present study revealed denaturation of myofibrillar proteins particularly myosin that underwent denaturation during ice storage. The loss in ATPase activity may also be attributed to the tertiary structural changes caused by ice storage and increase in the ionic strength of the system. Further, rearrangement of proteins via protein-protein interactions is also presumed to contribute to the loss in ATPase activity (Benjakul and Bauer, 2000).

Total sulfhydryl content of MFP of eel

In the present study, the content of total sulfhydryl groups of MFP fraction of eel decreased steadily from initial value of 48.27 $\mu\text{mole SH/g protein}$ on the 1st day to 45.95 $\mu\text{mole SH/g protein}$ on 16th day of chill storage (Fig. 3). The decrease in sulfhydryl content was coincidental with the decrease in Ca²⁺-ATPase activity. It was presumed that conformational changes of myosin, especially in the head region occurred rapidly. These changes probably resulted in the exposure of reactive sulfhydryl groups, which were prone to oxidation of disulfide interchange (Benjakul *et al.*, 2003). Wang *et al.*, (2005) observed that total SH groups increased during first two days, followed by gradual decrease in farmed Atlantic salmon (*Salmo salar*) during storage at 4°C. Mohan *et al.*, (2006) reported decrease in -SH groups of rohu during 11 days of ice storage. Parthiban *et al.*, (2015) reported that SH groups content was 23 $\mu\text{ moles/min/mg actomyosin}$ which increased on 4th day of iced

storage and the increase was more than five - times on day 14 indicating protein denaturation.

The decrease in sulfhydryl groups with a concomitant oxidation resulted in the formation of disulfide bonds through oxidation of sulfhydryl groups or disulfide inter changes (Hayagawa and Nakai, 1985). Changes in SH groups are an indication of either the formation of disulfide bond or fresh exposure of reactive SH groups to the bulk solvent from the interior of the protein molecule (Murthy *et al.*, 2012).

Emulsion activity index (EAI) and Emulsion stability (ES) of SPP and MFP of eel during chill storage

The emulsifying properties of proteins are evaluated by several methods, such as size distribution of oil droplets formed, emulsifying activity, emulsion capacity and emulsion stability (Kinsella and Melachouris, 1976). In the present study, the ES was calculated on the basis of time taken for attaining half the initial OD at 500 (Pearce and Kinsella, 1978). Myosin and actomyosin molecules by virtue of their structure and conformation due to both hydrophobic and hydrophilic residues, act as excellent emulsifier (Galluzzo and Regenstein, 1978).

In the present study, the EAI of MFP of eel at concentration of 2.5 mg/ml decreased gradually from 2.8 m²/g on 1st day to 2.0 m²/g on 16th day of storage. EAI of MFP of eel at concentration of 5.0 mg/ml decreased gradually from 3.1 m²/g on 1st day of storage to 1.3 m²/g on 16th day of chilled storage (Table 1) and the EAI of SPP of eel at concentration of 2.5 mg/ml was observed to be 2.5 m²/g on the 1st day which decreased to 1.4 m²/g on 16th day. EAI of SPP was recorded at concentration of 5.0 mg/ml as 2.2 m²/g on the 1st day which also decreased to 1.0 m²/g on

16th day of chilled storage (Table 1). EAI was reported to have a clear correlation with surface hydrophobicity, solubility and viscosity of the protein (Mohan *et al.*, 2008). The EAI decreased in the present study which may be attributed to the decrease in viscosity and solubility of MFP during storage.

The emulsion stability of MFP of eel at concentration of 2.5 mg/ml decreased steadily from initial value of 55 min on 1st day of storage to 28 min on 16th day of storage. Emulsion stability of MFP at concentration of 5.0 mg/ml changed from initial value of 60 min on 1st day of storage to 40 min on 16th day of chill storage (Table 2). The emulsion stability of SPP of eel at concentration of 2.5 mg/ml decreased gradually from initial value of 38 min on 1st day of storage to 17 min on 16th day of storage. Emulsion stability of SPP at concentration of 5.0 mg/ml changed from initial value of 40 min on 1st day of storage to 20 min on 16th day of chilled storage (Table 2).

The decrease in EAI could be related to the formation of disulfide bond between the protein molecules by the oxidation of SH groups exposed during protein unfolding during the initial periods of storage. Tendency of the proteins to aggregate resulted in decreased emulsion stability. The low temperature induced denaturation of proteins in mince of Indian oil sardine (*Sardinella longiceps*) resulted in the reduction of emulsion capacity of extract (Mathew and Prakash, 2007).

Parthiban *et al.*, (2005) observed that the EAI increased by 2-3 times during the storage of fish in ice up to 7 day and after that EAI decreased and continued till the end of storage period. Similar results were observed by Mohan *et al.*, (2006) where the emulsion stability of the protein gradually decreased during storage of rohu fish in ice.

Table.1 Changes in emulsion activity index of MFP and SPP of eel

Storage days	EAI (m ² /g) of MFP		EAI (m ² /g) of SPP	
	Protein concentration			
	2.5 mg/ml	5.0 mg/ml	2.5 mg/ml	5.0 mg/ml
0	2.8±0.05	3.1±0.14	2.5±0.15	2.2±0.18
2	2.7±0.08	3.0±0.12	2.4±0.11	2.0±0.08
4	2.5±0.03	2.8±0.08	2.3±0.09	1.9±0.05
6	2.4±0.11	2.7±0.05	2.1±0.12	1.8±0.11
8	2.3±0.08	2.6±0.16	1.8±0.05	1.7±0.16
10	2.2±0.15	1.9±0.12	1.7±0.18	1.6±0.20
12	2.1±0.03	1.5±0.07	1.5±0.06	1.4±0.14
14	2.0±0.06	1.4±0.11	1.3±0.15	1.2±0.17
16	2.0±0.04	1.3±0.15	1.4±0.03	1.0±0.07

Table.2 Changes in emulsion stability of MFP and SPP of eel

Storage days	ES (min) of MFP		ES (min) of SPP	
	Protein concentration			
	2.5 mg/ml	5.0 mg/ml	2.5 mg/ml	5.0 mg/ml
0	55±0.57	60±0.33	32±0.88	40±0.57
2	52±0.57	58±0.88	30±0.33	38±0.88
4	50±0.33	55±0.57	27±0.57	36±0.57
6	48±0.57	52±0.57	24±0.88	32±0.88
8	46±0.33	50±0.57	22±0.57	30±0.88
10	40±0.57	48±0.57	22±0.57	27±0.57
12	35±0.57	45±0.57	20±0.88	24±0.57
14	30±0.57	43±0.33	17±0.57	22±0.57
16	28±0.88	40±0.88	15±0.57	20±0.57

Table.3 Changes in viscosity of MFP and SPP of eel

Storage days	Viscosity (cP) of MFP		Viscosity (cP) of SPP	
	Protein concentration		Protein concentration	
	2.5 mg/ml	5.0 mg/ml	2.5 mg/ml	5.0 mg/ml
0	2.3 ± 0.05	2.5±0.05	1.9 ±0.12	2.3 ±0.05
2	2.2 ±0.08	2.4±0.08	1.8 ±0.08	2.2 ±0.03
4	2.2 ± 0.06	2.3±0.07	1.7 ±0.03	2.1 ±0.06
6	2.1 ±0.08	2.3±0.12	1.6 ±0.05	1.9 ±0.08
8	2.0 ±0.05	2.2±0.05	1.5 ±0.03	1.8 ±0.02
10	1.9 ±0.09	2.1±0.06	1.3±0.07	1.7 ±0.03
12	1.8 ± 0.12	2.1±0.02	1.2±0.06	1.5 ±0.12
14	1.7 ±0.11	2.2±0.03	1.2 ±0.02	1.4 ±0.11
16	1.6 ±0.03	2.0±0.08	1.1 ±0.07	1.3 ± 0.03

Table.4 Changes in foam expansion of MFP and SPP of eel

Storage days	Foam expansion (%) of MFP		Foam expansion (%) of SPP	
	Protein concentration		Protein concentration	
	2.5 mg/ml	5.0 mg/ml	2.5 mg/ml	5.0 mg/ml
0	3.1± 0.06	3.0±0.03	2.4±0.05	2.3±0.05
2	2.9±0.05	2.8±0.03	2.3±0.05	2.2±0.05
4	2.8±0.03	2.7±0.06	2.2±0.03	1.8±0.05
6	2.5±0.05	2.6±0.05	2.0±0.03	1.6±0.03
8	2.1±0.05	2.5±0.03	1.9±0.03	1.5±0.05
10	2.0±0.06	2.3±0.05	1.8±0.05	1.3±0.03
12	1.7±0.06	2.1±0.03	1.6±0.03	1.2±0.03
14	1.6±0.05	1.9±0.12	1.4±0.05	1.1±0.03
16	1.5±0.03	1.7±0.03	1.2±0.05	1.0±0.03

Table.5 Changes in foam volume stability of MFP and SPP of eel

Storage days	Foam Volume Stability (%) of MFP		Foam Volume Stability (%) of SPP	
	Protein concentration		Protein concentration	
	2.5 mg/ml	5.0 mg/ml	2.5 mg/ml	5.0 mg/ml
0	60.5± 0.38	65.3± 0.12	28.8± 0.17	35.6± 0.20
2	59.7± 0.72	65.4± 0.76	28.8± 0.23	35.2± 0.20
4	59.5± 0.27	65.2± 0.63	27.2± 0.58	34.6± 0.41
6	58.9± 0.34	64.8± 0.46	27.1± 0.28	34.3± 0.24
8	58.5± 0.82	64.5± 0.40	26.8± 0.43	33.8± 0.26
10	58.2± 0.66	64.3± 0.40	26.5± 0.65	33.5± 0.29
12	57.5± 0.53	63.2± 0.27	26.3± 0.37	32.2± 0.20
14	56.6± 0.78	62.8± 0.51	25.9± 0.20	32.0 ± 0.66
16	56.2± 0.75	62.2± 0.57	25.7± 0.40	31.8± 0.57

Fig.1 Changes in concentration of MFP and SPP of eel

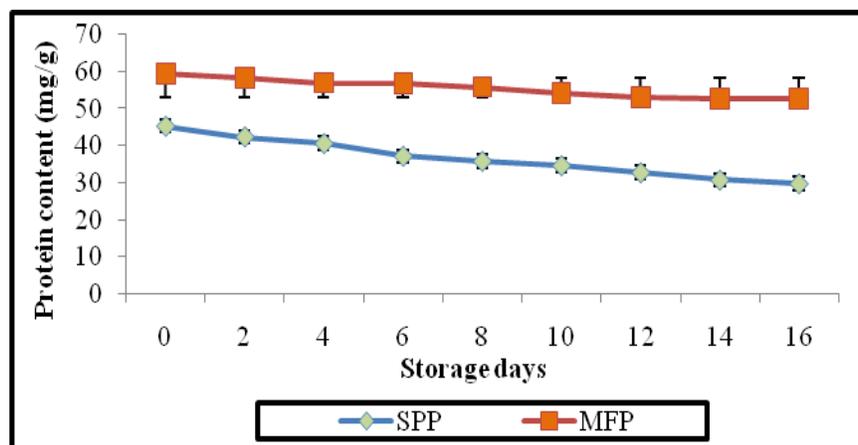


Fig.2 Changes in Ca^{2+} ATPase activity of actomyosin of eel

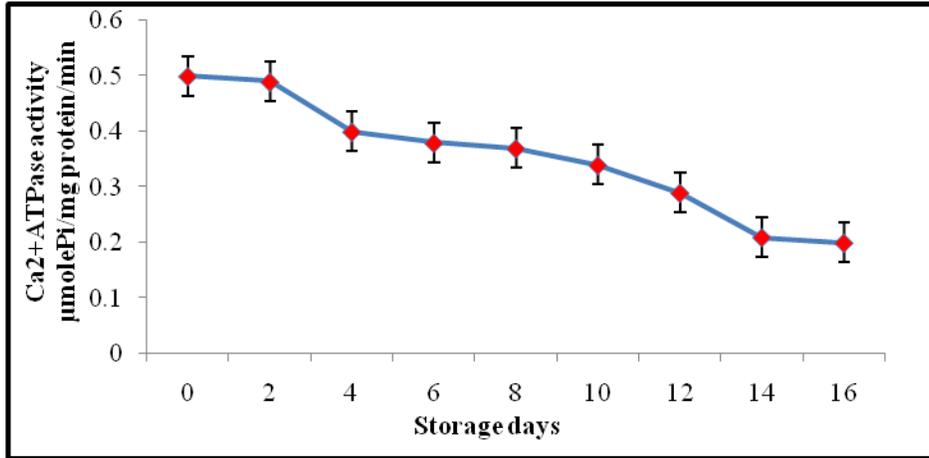


Fig.3 Changes in total sulfhydryl groups of MFP of eel

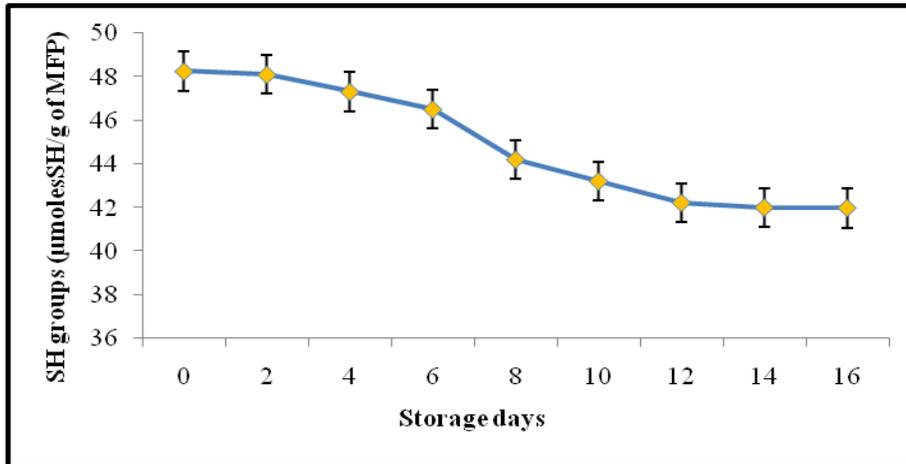


Fig.4 Changes in gel strength of eel mince

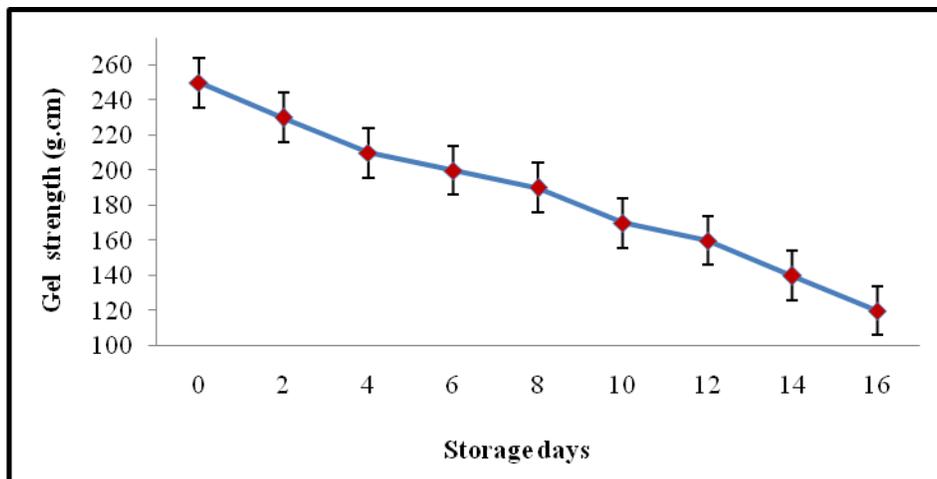
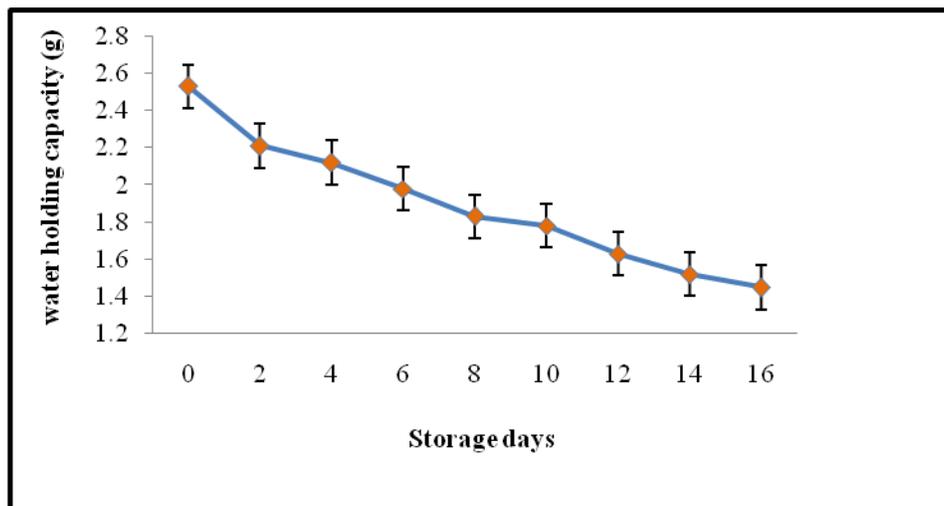


Fig.5 Changes in water holding capacity of eel mince



Viscosity of SPP and MFP of eel proteins

The viscosity of a solution relates to its resistance to flow under an applied force (or shear stress). Myosin present in muscle proteins is the major contributor to the viscosity of aqueous muscle extracts. In the present study, the viscosity of eel MFP at concentration of 2.5 mg/ml decreased gradually from initial value of 2.3 cP on the 1st day to 1.6 cP on 16th day of storage. Viscosity of eel MFP at the concentration of 5.0 mg/ml decreased steadily from initial value of 2.5 cP on 1st day to 2.0 cP on 16th day of storage (Table 3) and the viscosity of eel SPP estimated at concentration of 2.5 mg/ml decreased gradually from initial value of 1.9 cP on the 1st day to 1.1 cP on 16th day of storage. But, at SPP concentration of 5.0 mg/ml, it decreased from initial value of 2.3 cP on 1st day to 1.3 cP on 16th day of storage (Table 3). The results showed that with the increase in the concentration of protein there was increase the viscosity of protein.

Chalmers *et al.*, (1992) reported the highest viscosity after 6 days of storage and gradual decrease thereafter up to 9th day of storage of cod. Mehta *et al.*, (2011) observed the changes in viscosity of muscle protein of

freshwater fishes (Indian major carps) decreases during 22 days of ice storage. Mohan *et al.*, (2006) observed gradual change in viscosity of MFP for both concentrations i.e. 2.5 mg/ml and 5 mg/ml during iced storage of rohu (*Labeo rohita*).

Foam expansion and foam volume stability of SPP and MFP during chill storage

In the present study, the foam expansion of MFP of eel at concentration of 2.5 mg/ml decreased progressively from initial value of 3.1% on the 1st day to 1.5% on 16th day of storage. Its value at the concentration of 5.0 mg/ml decreased markedly from 3.0% on 1st day to 1.7% on 16th day of storage (Table 4) and the foam expansion of SPP of eel at concentration of 2.5 mg/ml decreased from initial value of 2.4% on 1st day to 1.2% on 16th day of storage. Its value at the concentration of 5.0 mg/ml decreased significantly from 2.3% on 1st day to 1.0% at 16th day of storage (Table 4).

Foam stability refers to the ability of proteins to stabilize foam against gravitational and mechanical stresses whereas, the foam volume stability of MFP of eel at concentration of 2.5 mg/ml ranged from

60.5% on 1st day to 56.2 % on 16th day of storage. Foam volume stability of eel of MFP at concentration of 5.0 mg/ml ranged from initial value from 65.3% on 1st day to 62.2% on 16th day of storage (Table 5) and the foam volume stability of SPP of eel at concentration of 2.5 mg/ml decreased continuously from 28.8 % on 1st day to 25.7 % on 16th day of storage. Foam volume stability of eel of SPP at concentration of 5.0 mg/ml decreased progressively from 35.6% on 1st day to 31.8% on 16th day of storage (Table 5). The most direct measure of Foam volume stability is the reduction in foam interfacial area as a function of time. Foamability of MFP (5 mg/ml) showed a concentration dependent increase but it decreased gradually during the storage period. The strength of the foam derived from MFP was higher than the SPP which might be due to the differences in the molecular weight of the proteins as the foam refers to maximum weight a column of foam can withstand before its collapses. Parthiban *et al.*, (2005) observed a gradual decline in foamability during storage of tilapia fish under chilled condition. Mohan *et al.*, (2006) noted that foamability of SPP was relatively stable during six days of storage and showed a gradual decline subsequently. Foam volume stability of SPP was found to be increasing during storage whereas, foamability of MFP at both concentrations of 2.5 mg/ml and 5.0 mg/ml showed decreasing trend during storage period and FVS of MFP at both concentrations of 2.5 mg/ml and 5.0 mg/ml remained stable throughout the storage period.

Gel strength of muscle proteins of MFP of eel fish

Protein gelation refers to transformation of a protein from the *sol* state to a *gel like* usually high temperature, enzymes like transglutaminases or divalent cations under

appropriate conditions facilitate this transformation. In the present study, gel strength of MFP of eel mince decreased with increase in storage time of fish in ice. It decreased slowly from first 4 days and then markedly decreased from initial 250 g.cm on 1st day to 120 g.cm on 16th day of storage (Fig. 4). Gel forming ability of fish proteins is an important functional property (Murthy *et al.*, 2012). Benjakul *et al.*, (2003) observed that the degradation of myosin results in an inferior gel network formation, causing a lower elasticity with poor water holding capacity in the gel matrix.

Yongswatdigul and Park (2002) noted the steady decrease in breaking force and deformation of washed mince of threadfin bream with in storage time. Parthiban *et al.*, (2005) observed gradual decrease in gel strength with increase in length of storage of tilapia fish in ice. Mehta *et al.*, (2011) noted that there was a significant decrease in gel strength values in all the three species of Indian major carp at the end of 22 days of ice storage.

Water holding capacity of muscle proteins of eel fish (WHC)

Myofibrils are the largest water-holding filament lattices and most of the water in the meat is held within the myofibrils in the narrow channels between the filaments (Sankar, 2009). In the present study, the water holding capacity of eel mince during chill storage decreased continuously from initial value of 2.53 g / g muscle on 1st day to 1.45 g/ g muscle on 16th day of storage (Fig. 5).

Ramachandran *et al.*, (2007) observed a value of 2.49 g/g of WHC in group I (500 g) and 1.61g / g (1500 g) of barracuda fish. The water, which is immobilized in the protein structure, is released as a result of protein denaturation consequent of protein unfolding

(Partiban, 2005). During frozen storage water holding capacity of fish protein decreased due to denaturation and is supported by the increased thaw drip in frozen stored fish (Chang and Regenstein, 1997; Wang and Xiong, 1998; Benjakul *et al.*, 2005).

Similar trend was observed by Partiban *et al.*, (2005) with WHC of muscle protein of tilapia marginally increasing up to 4th day of storage and then decreasing up to 10th day, there was no further decrease. Further, Wang *et al.*, (2005) observed that the water holding capacity increased initially and then decreased progressively thereafter until 7th day and then increased again on 9th day of storage.

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