

Original Research Article

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## Physico-chemical and Functional Properties of Fresh Eel (*Mastacembelus armatus*) Muscle Proteins

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### ABSTRACT

In the present study, physico-chemical and functional properties such as solubility, Ca<sup>2+</sup>ATPase activity, total sulfhydryl group, emulsion activity index, emulsion stability, foam volume stability (FVS), foam volume capacity, gelation, water holding capacity (WHC) of muscle proteins from fresh eel (*Mastacembelus armatus*) fish were studied. Solubility value of sarcoplasmic (SPP) and myofibrillar protein (MFP) was 44.93 and 59.16 mg/g respectively. It is higher in MFP than SPP fraction. Ca<sup>2+</sup>ATPase activity of actomyosin was 0.50 μmole Pi/mg protein/min, total sulfhydryl group of MFP fraction was 48.27 μmole SH/g protein. The emulsion activity index and emulsion stability was higher in MFP than SPP fraction at 2.5 and 5.0 mg/ml concentration. The values for viscosity of MFP and SPP were more at concentration of 5.0 mg/ml than 2.5 mg/ml. The viscosity was more in MFP fraction than SPP fraction. Foam expansion and foam volume stability of MFP and SPP fraction at concentration of 2.5 mg/ml was lesser than 5 mg/ml. The gel forming ability of fresh eel protein was 250 g.cm and water holding capacity of eel mince was 2.53 g / g muscle. The present study conclude that eel muscle protein have good functional properties. Functional properties of muscle proteins revealed that the eel fish can be one of the promising candidate species for utilization in preparation of functional foods like emulsion-type and/or sausage products.

#### Keywords

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### Introduction

The freshwater spiny eel (*Mastacembelus armatus*) has great economic value especially in inland areas of India (Talwar and Jhingran, 1991). Among the commercially exploited fresh water fishery resources Indian major carps, minor carps, Chinese carps and eels constitute an important commercial fishery in riverine and reservoir fisheries. Eels represent an important commercially exploited fish in

the local fish markets in the inland areas. 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. Functional properties of proteins play a significant role in food applications and are very much influenced by their structure in food systems (Ramachandran *et al.*, 2007). The molecular basis of functionality is related to their

structure and ability to interact with other food ingredients (Zayas, 1997). Actin and myosin constitute the basic functional component of the myofibrillar proteins (MFPs). There are reports that fish undergo dramatic changes in the fiber type composition (myosin expression and organization of fiber type) and in the isoforms of myofibrillar molecule during post hatching growth (Watabe and Ikeda, 2006). Understanding physico-chemical characteristics is of utmost importance as it is directly related to the final quality of products like surimi, sausage and battered products. The functional properties of meat depend mainly on myofibrillar proteins (Goll *et al.*, 1977) and are related to the composition and structure of proteins and their interaction with other substances present in the food (Colmenero and Borderias, 1983). The functional properties of myofibrillar proteins are important in determining the quality of the product (Roura and Crupkin, 1995). Functional properties of various fresh water fish protein were studied viz. tilapia (Parthiban *et al.*, 2005, 2015), rohu (Mohan *et al.*, 2006).

## **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  $225 \pm 13.22$  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  $2-4^{\circ}\text{C}$  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). Foam ability 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  $4^{\circ}\text{C}$  (Lan *et al.*, 1995). Water holding capacity (WHC) of mince was carried out by the method of Kocher and Foegeding (1993) with slight modification.

## **Results and Discussion**

### **Solubility of proteins**

The functional properties of proteins are often affected by protein solubility and those most affected are thickening, foam expansion, emulsifying and gel strength. As an indication whether or not denaturation has taken place in myofibrillar protein, a method commonly used is to measure the quantity of myofibrillar protein extracted from the muscle by salt solution with 0.45–0.6 ionic strength (Suzuki,

1981). Extractability is related to the solubility of the protein and characteristics of the muscle structure. The solubility characteristics of proteins are related to the amino acid composition at protein surface and its interaction with the solvent (Bigelow, 1967). In the present study, solubility of sarcoplasmic and myofibrillar fractions of fresh eel were observed to be 44.93 and 59.16 mg/g respectively.

Partiban *et al.*, (2005) observed the 66% solubility of MFP and 34% of SPP of total soluble protein of fresh tilapia fish, Mohan *et al.*, (2006) reported the solubilities of SPP and MFP of rohu fish were 47.6 and 76.5 mg/g respectively. Ramachandran *et al.*, (2009) observed the solubilities of SPP of *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* were 32.15, 53.29, 46.65 and 66.67 mg/g tissue respectively whereas, the MFP solubilities of *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* were 152.34, 116.85, 80.91 and 152.54 mg/g tissue respectively. Parthiban *et al.*, (2015) observed the solubilities of SPP and MFP of tilapia fish were 21.26 and 35.23 g/100g respectively.

### **Ca<sup>2+</sup> ATPase activity of actomyosin of eel**

The myosin globular has ATPase activity (which releases the energy for muscle contraction) and binds to actin in the absence of ATP (post-mortem). Therefore, Ca<sup>2+</sup>ATPase activity can be used as an indicator of the integrity of myosin molecules (Benjakul *et al.*, 1997) as the globular heads of myosin are responsible for Ca<sup>2+</sup>ATPase activity (Benjakul *et al.*, 2003; Ramachandran *et al.*, 2007). In the present study, the Ca<sup>2+</sup>ATPase activity of actomyosin of fresh eel was 0.50  $\mu$ mole Pi/mg protein/min. Ramachandran *et al.*, (2009) reported the Ca<sup>2+</sup>ATPase activity of MFP of *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* were 0.41, 0.59, 0.41 and 0.28 respectively. The Ca<sup>2+</sup>ATPase activity of actomyosin fraction

was 0.81  $\mu$ mole Pi/mg protein/min in the fresh tilapia (Parthiban *et al.*, 2015).

### **Total sulfhydryl content of MFP of eel**

Sulfhydryl groups are considered to be the most reactive functional groups in proteins and any destruction of cysteine or cystine during storage of fish is indicated by disappearance of SH groups. The SH groups represent the reactivity of the proteins and the content of surface reactive SH groups increases with the unfolding of protein during exposure to extreme conditions (Sankar and Ramachandran, 2005). In the present study, the content of total sulfhydryl groups of MFP fraction of fresh eel was 48.27  $\mu$ mole SH/g protein. The Mohan *et al.*, (2006) reported SH groups content of 88  $\mu$ moles/min/mg in AM from fresh rohu fish. Ramachandran *et al.*, (2009) the concentration of reactive sulphhydryl groups ranged from 23.5  $\mu$  moles SH/g protein to 44.7  $\mu$  moles SH/g protein among fishes and the highest values were recorded in MFP from silver carp and the lowest in common carp. Parthiban *et al.*, (2015) observed the total SH content of actomyosin of fresh tilapia fish was 23 $\mu$ moles/min/mg AM.

### **Emulsion activity index (EAI) and Emulsion stability (ES) of SPP and MFP of eel**

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). The physical and sensory properties of protein-stabilized emulsion depend on the size of the droplets formed and the total interfacial area created. The ability of proteins to bind fat in comminuted meat is of great importance. Proteins being amphoteric molecules, are surface-active agents and thus concentrate on fat-water interface. Emulsion

stability refers to the ability of a protein to form an emulsion that remains unchanged for a particular duration, under specific conditions. Protein-stabilized emulsions are often stable for days.

In the present study, the EAI of MFP and SPP of eel at concentration of 2.5 mg/ml were 2.8 and 2.5 m<sup>2</sup>/g respectively. Whereas, the EAI of MFP and SPP of fresh eel recorded at concentration of 5.0 mg/ml were 3.1 and 2.2 m<sup>2</sup>/g respectively. Partiban *et al.*, (2005) observed the EAI value was 124 m<sup>2</sup>/g for extracted total soluble protein from fresh tilapia fish. Mohan *et al.*, (2006) reported the EAI of SPP at concentration of 2.5 mg/ml was 11.86 m<sup>2</sup>/g whereas, the EAI content of MFP at concentration of 2.5 and 5.0 mg/ml were 1.09 and 6.25 m<sup>2</sup>/g respectively. Ramachandran *et al.*, (2009) observed the EAI of MFP values of fresh water fishes *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* with different habitat were 2.86, 5.22, 7.24 and 3.10 m<sup>2</sup>/g respectively.

In the present study, the ES of MFP and SPP of fresh eel at concentration of 2.5 mg/ml were 55 and 38 min respectively. The ES of MFP and SPP at concentration of 5.0 mg/ml was 60 and 40 min respectively. Partiban *et al.*, (2005) observed the ES value was 570 sec of fresh tilapia sample. Mohan *et al.*, (2006) reported the ES values for SPP at concentration of 2.5 mg/ml was 52 min whereas, the ES content of MFP at concentration of 2.5 and 5.0 mg/ml were 87 and 364 min respectively. Ramachandran *et al.*, (2009) recorded the ES of *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* were 53.33, 86.67, 53.33 and 72.60 min respectively.

### **Viscosity of SPP and MFP of eel proteins**

The consumer acceptability of several liquid and semisolid type foods (e.g., gravies, soups, beverages, etc.) depends on the viscosity or

consistency of the product. Viscosity is a functional property which is greatly exploited when proteins are added to liquid foods as thickeners, and it affects several other functional properties. 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 and 5.0 mg/ml was 2.3 and 2.5 cP respectively whereas, the viscosity of eel SPP at concentration of 2.5 and 5.0 mg/ml was 1.9 and 2.3 cP respectively. Partiban *et al.*, (2005) reported the viscosity of tilapia fish protein was 3.25 mm/sec. Mohan *et al.*, (2006) observed the viscosity of rohu SPP at concentration of 2.5 mg/ml was 1.36 cP and for eel MFP at concentration of 2.5 and 5.0 mg/ml was 4.45 and 16.20 cP respectively. Ramachandran *et al.*, (2009) recorded the viscosity of MFP of fresh water fish viz. *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* from different habitat. The values of viscosity was lowest in the MFP of *C. carpio* (3.29 cP) and highest in *H. molitrix* (13.39 cP).

### **Foam expansion (FE) and foam volume stability (FVS) of SPP and MFP of eel proteins**

Foams consist of an aqueous continuous phase and a gaseous (air) dispersed phase. The unique textural properties and mouthfeel of these products stem from the dispersed tiny air bubbles. In most of these products, proteins are the main surface active agents that help in the formation and stabilization of the dispersed gas (foam) phase.

In the present study, the foam expansion of MFP of eel at concentration of 2.5 and 5.0 mg/ml were 3.1 and 3.0% respectively. The foam expansion of SPP of eel at concentration of 2.5 and 5.0 mg/ml were 2.4 and 2.3% respectively. 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 and 5.0 mg/ml were 60.5 and 65.3% whereas, the FVS of SPP of eel at concentration of 2.5 and 5.0 mg/ml were 28.8 and 35.6% respectively. Mohan *et al.*, (2006) observed the FE of SPP of rohu at concentration of 2.5 mg/ml was 41.33% and the FE of MFP of rohu at concentration of 2.5 and 5.0 mg/ml were 105.33 and 134.66%. The FVS of SPP of rohu at concentration of 2.5 mg/ml was 27.33% whereas, the FVS of MFP of rohu at concentration of 2.5 and 5.0 mg/ml were 89.00 and 87.00% respectively. Ramachandran *et al.*, (2009) reported the FVS of fresh water fishes viz. *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* were 60, 70, 60 and 50% respectively; whereas, the FE of these fishes were 100, 70, 180 and 55% respectively. *H. molitrix* had better foam expansion it did not show much foam stability.

### **Gel strength of muscle proteins of MFP of eel fish**

Gel is made up of polymers cross-linked via either covalent or non-covalent bonds to form network i.e capable of entrapping water and other small molecular weight substances. 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 (Mehta *et al.*, 2011).

In the present study, the gel strength of fresh eel was 250 g.cm. The similar study done by other researcher, Partiban *et al.*, (2005) recorded the gel strength of fresh tilapia fish was 710 g.cm. Ramachandran *et al.*, (2009) also reported the gel strength of fresh water fish viz. *O. mossambicus*, *M. cephalus*, *H. molitrix* and *C. carpio* harvested from different habitat were 281.09, 282.72, 180.19 and 166.16 g.cm respectively.

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

In food applications, the WHC of the protein is more important than water binding capacity (WBC). It refers to the sum of bound water, hydrodynamic water and physically entrapped water. The physically entrapped water contributes more to the water holding capacity. Many functional properties of proteins depend on water-protein interaction as water molecules bind to several groups in proteins. 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 (WHC) of fresh eel mince was 2.53 g / g muscle. Similar study was done by Partiban *et al.*, (2005) observed the WHC of fresh tilapia was 2.8 g / g muscle.

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