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FUNCTIONAL PROPERTIES OF FISH *MASTACEMBELUS ARMATUS* (LACEPEDE, 1800) MUSCLE PROTEIN

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ABSTRACT
The functional properties *viz*. solubility, emulsion activity, emulsion stability, foam stability and activity of muscle protein of *M aramatus*, a species of freshwater spiny eel was studied. The solubility profile showed a 'U' shaped curve at different pH. The percentage solubility was higher at extreme pH. Emulsion Activity Index and Emulsion stability index was 8.96 and 41.11 minutes respectively. Foaming capacity and stability was 13.67% and 25 minutes respectively. The muscle protein showed a water binding capacity (WBC) of 76% compared with Oil binding capacity (OBC) 61%. The study conclude that fish, *M. armatus* muscle protein have good functional properties. These functional properties effect the sensory characters of food and plays a significant role in physical behavior of foods or food ingredients during their preparations, processing and storage.

INTRODUCTION

Mastacembelus armatus, is a species of freshwater spiny eel having great economic value especially in India (Talwar and Jhingram, 2001). Mastacembelidae or spiny eels are elongate, eel like medium sized to large acanthomorph teleosts that also occur in tropical Africa and Middle East Asia (Berra, 2000). This fish received an urgent attention, due to the declining wild population. The decline in population is attributed to the loss of habitat, poisoning of alien species, diseases, pollution, siltation, poisioning, dynamite and other destructive fishing (Camp, 1998). The biological studies on fecundity, reproductive potential, reproductive behavior and evaluation of commercial potential of this fish is only recently studied by various workers (Targonska *et al.,* 2012; Uthayakumar *et al.,* 2013).

The functional properties of fish are important, because they affect the sensory characteristics of food and play a major role in the physical behaviour of foods or food ingredients during their preparation, processing and storage. Functional properties of various fish protein were studied viz. *Cyprinus carpio* and *Epinephelus tauvina* (Rao, 2014), *barracuda* (Ramachandran *et al.,* 2007), *Clupea harengus* (Liceaga *et al.,* 1999), *Decapterus maruadsi* (Thiansilakul *et al.,* 2007; Hu *et al.,* 2014), jumbo squid *Dosidicus gigas* (Rocha-Estrada *et al.,* 2010), *Labeo rohita* by (Mohan *et al.,* 2006) crab *Barytelphussa cunicularis* (Pagare and Martin, 2013; Martin, 2010) and in Maize (Sharma *et al.,* 2015).

M. armatus is found commonly in river Godavari, which passes through this region. Godavari river is the major river of these region and considered as life line for water and aquatic bioresources for people living near the bank of this river. This fish has as high market value and is widely consumed for its nutritive and organo-leptic properties by local population (Talwar and Jhingram, 2001). The literature survey shows that there are no studies carried out on its functional properties of muscle protein. Thus, in this context the studies were carried out to ascertain the functional properties of its muscle protein.

MATERIALS AND METHODS

The freshwater fish *M. armatus* were procured from Godavari River, Kaigaon toka, near Aurangabad and were maintained in glass aquarium in laboratory conditions for two weeks prior to experimentation. The fish were maintained in 20L of water at temperature 25 ± 1^{o}C, dissolved oxygen between 6.54-9.83mg/L. The water was changed daily. Fish of average weight 18 ± 0.15g and length of 20.33 ± 0.57cm were only considered for functional properties studies.

Solubility

The solubility of protein was determined accordingly to the method described by (Vani and Zayas, 1995) with modifications. The fish were dissected and 1g of muscle was taken in 100mL of chilled distilled water, homogenized, and centrifuged at 10,000xg for 10minutes at 4^{o}C. From this 10ml aliquot was transferred to each test tube and pH was adjusted to 2,4,5,6,7,8,10, and 12 using either 0.1N NaOH or 0.1N HCl. The protein content was estimated by following Lowry's

method (Lowry *et al.,* 1951). The exact protein content in the supernatant was calculated from the standard curve of Bovine serum albumin. The solubility profile is expressed as percentage protein solubility. The pH solubility at pH 12 was taken as 100%. Percentage Protein solubility was assessed according to the following formula;

$$\text{Percent protein solubility} = \frac{\text{Amount of Soluble Protein}}{\text{Total amount of protein}} \text{ X } 100$$

Emulsion activity index (EAI) and emulsion stability index (ESI)

Emulsifying properties were determined according to the method described by Pearce and Kinsella, (1978) with minor modifications as described below. An emulsion was prepared by homogenizing 1ml groundnut oil and 3 ml of 0.1% fish muscle solution in 0.1M sodium phosphate buffer pH 7.4 in a homogenizer (REMI) at a high speed setting for 1 minute. 100μl of emulsion was taken from the bottom of the test tube, after standing for 0,1,2,3,4,5,10,15,20,25,30,35,40,45 and 50 minutes and diluted with 5ml of 0.1% Sodium dodecyl sulfate. The absorbance of diluted emulsion was measured at 500nm. The relative emulsification was then measured at 500nm, immediately after emulsion formation at time (0). After emulsion formation the sample was used to calculate the emulsion activity index (EAI) and emulsion stability index (ESI) using the following formula:

$EAI = 2T\varphi C$

Where, T is the turbidity ($T = 2.303A_{500}/l$; A_{500} is absorbance at 500nm; l is path length); F is oil volume fraction (0.25) and C is protein concentration.

$$\text{EAI } (m^2/g) = \frac{2 \text{ X } 2.303 \text{ X } Abs_{500nm}}{0.25 \text{ X Protein Weight (g)}}$$

$$\text{ESI (min)} = \frac{A_0 \text{ X } \Delta t}{\Delta A}$$

Where, $\Delta A = A_0 - A_{10}$ and Δt = 10min.

Foaming properties

Fish sample containing 0.5g was whipped with 50ml distilled water (pH adjusted to 8) at high speed using a grinder mixer (Kenstar) for 2 minutes. The whipped mixture is immediately transferred into 250ml graduated cylinder. The foaming capacity was assessed on the basis of percent foam volume increase calculated according to the following equation of (Sathe and Salunkhe,1981).

$$\% \text{ volume increase} = \frac{\text{total volume after whipping – Volume before whipping}}{\text{Volume before whipping}} \text{ X } 100$$

To determine foam stability, the total volume at time intervals of 0, 2.3.4.5, 10, 15, 20,25 and 30 minutes was noted.

The whipped sample was allowed to stand at 25ºC for 30 minutes and the volume of whipped sample was then recorded. Foam stability was calculated by following method as described by Sathe and Salunke (1981).

$$\text{Foaming stability (\%)} = \frac{\text{Volume after standing - Volume before Whipping}}{\text{Volume before whipping}} \text{ X } 100$$

Water and Oil Binding capacity

Water and oil absorption capacities were determined according to the method of Adebowale *et al.* (2005). The results calculated as gram of water or groundnut oil absorbed by per gram water and oil binding capacity.

Statistical analysis

The data were subjected to ANOVA test using MS Office.

RESULTS AND DISCUSSION

Protein solubility

Protein solubility profile of fish, *M. armatus* showed a highest percentage solubility 77.94 ± 0.325 at pH 10 and 53.29 at pH 2 respectively. The least percentage solubility 29.4 ± 1.035 was observed at pH 6. The solubility profile of fish protein solution to different pH is shown in Figure 1. The protein concentration of fish muscle was 0.307g/ml. The protein solubility showed a significant {$F(1, 10) = 24.65; P < 0.05$ $_{cri}4.96$}difference with pH.

Solubility profile of *M. armatus* muscle protein showed a typical U-shaped curve. Similar profile were reported in *Pacific whiting* (Chen *et al.,* 2009; Wang *et al.,* 2015), rockfish (Yongsawatdigul and Park, 2004) *Channel catfish* (Kristinsson *et al.,* 2005) and *Atlantic croaker* (Kristinsson and Liang, 2006). The other factor which influences solubility in food systems are temperature, ionic strength, freezing, heating, drying and shearing (Vaclavik and Christian, 2003; Bolontrade *et al.,* 2013). The changes in protein solubility results in conformational changes of protein (Sankar, 2009). Mohan, *et al.,* (2007) reported an increase in protein extraction with an increase in ionic strength and extreme pH condition viz. pH 2 and pH 12. Mohan *et al.* (2007) also reported a 5 times solubility in comparison with extract of *Mugil cephalus* at pH 6-7. At pH above or below the isoelectric point, the protein acquires net negative or positive charges making it more hydrophilic exhibiting protein solvent interaction than protein-protein interaction thus increasing protein solubility, similar, observations were also seen by Hamm, (1960). In present study we observed a observed a 2 times increase in solubility at pH 2 and 3 times in pH 12. Our studies are in confirmation with studies of Mohan *et al.* (2007).

Nalinanon *et al.* (2011) reported that pH effects the charges on the weakly acidic and basic side chain groups. Thiansilakul *et al.* (2007) reported that solubility variations is due to both net charge of peptides, which increases as pH moves away from pI and surface hydrophobic interaction. Kristinsson *et al.* (2005) too reported that an adequate solubility is essential to separate muscle proteins from insoluble material, such as, iso-electric precipitation of neutral solutions will lead to the highest recovery of protein. A similar mechanism may be taking place in the present study.

Foaming capacity and stability

Foaming capacity and stability of protein is shown in (Figure 2). Foaming capacity observed was observed 13.67% and

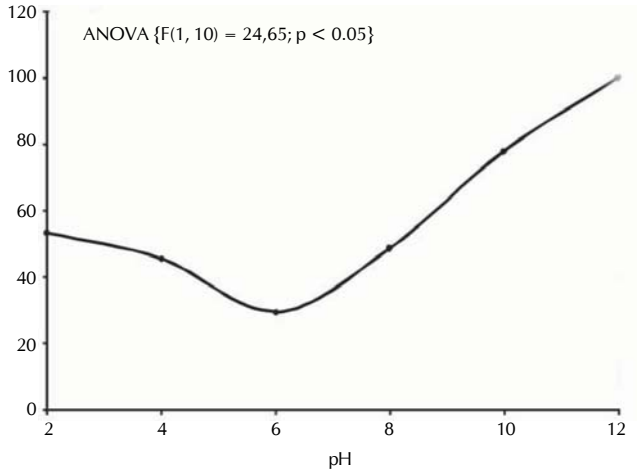

Figure 1: Percentage solubility curve of *M. armatus* muscle protein

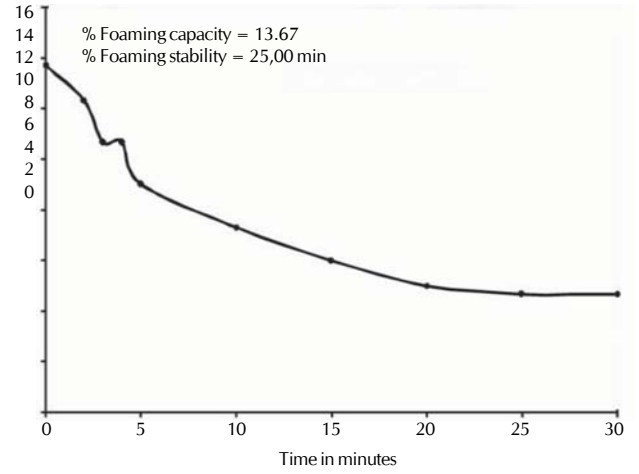

Figure 2: Foaming capacity and stability of *M. armatus* muscle protein

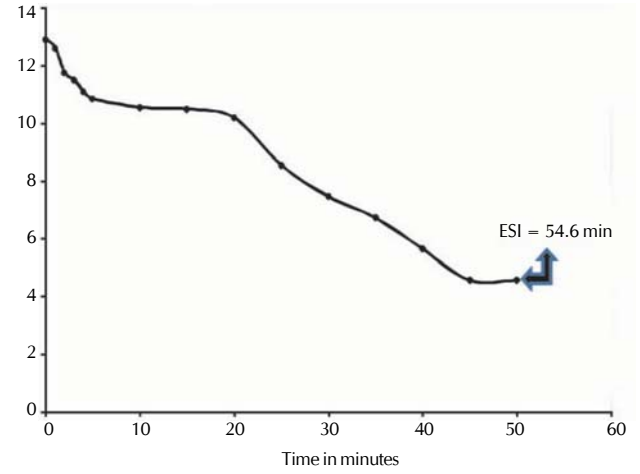

Figure 3. Percentage emulsifying activity index and emulsifying stability index (shown in arrow) in minutes of *M. armatus* muscle protein

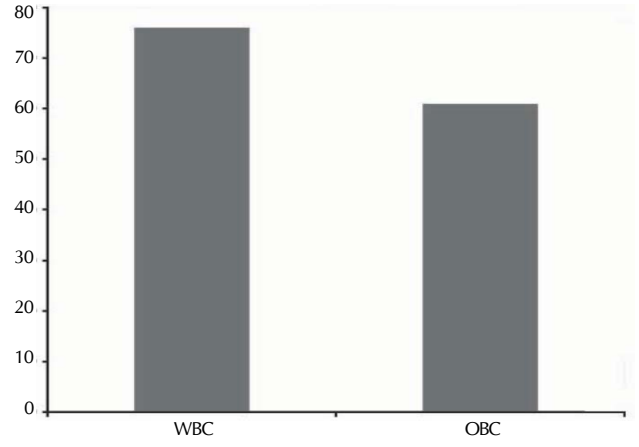

Figure 4: Water binding and Oil binding capacity of *M. armatus* muscle protein

foaming stability was at 25minutes. The foaming capacity showed a decrease with increase in time. Thiansilakul *et al.* (2007) reported that foaming properties is an important physico-chemical characteristics of protein that allows to form and stabilize foams. According to Lone (2015) and Rao (2014) foamability is an important functional property of protein, which form a flexible cohesive film to entrap air bubbles. Thiansilakul *et al.* (2007) observed that protein rapidly adsorb at newly formed air liquid interface during bubbling. Similarly, Nalinanon *et al.* (2011) reported foam formation to be governed by three factors, viz. transportation, penetration and reorganization of protein molecules at the air-water interface. According to Ogunwolu *et al.* (2009) good foaming activity is due to the movement of protein at the interface. This phenomenon is largely influenced by type of protein, degree of denaturation, pH, temperature and whipping methods used (Saetae and Suntornsuk, 2011). Based on studies, it was concluded that, low foaming capacity may be due to protein, present in this fish, which adsorb slowly and resist unfolding at the interface. Low foaming capacities could be due to inadequate electrostatic repulsions, lesser solubility and excessive protein-protein interactions as suggested by Butt and Batool (2010).Various workers have reported that weight and pH also influence increase in foam formation (Akin-Osanaiye *et al.*, 2009; Martin 2010; Ekpo and Ugbenyen 2011). The low foaming activity observed at 13.67% may be due to inadequate electrostatic repulsion, and lesser solubility as suggested by various workers (Akin-Osanaiye *et al.*, 2009; Martin 2010; Ekpo and Ugbenyen, 2011).

The foam stability was observed at 25 minutes. It was postulated by Lawal (2004) that an increase in foam stability, may be due to formation of stiffer foams, and is facilitated by flexile protein domains, viscosity of aqueous phase and protein concentration. Klompong *et al.* (2007) and Van der et al., (2003) reported, the role of high molecular weight peptides and surface hydrophobicity of unfolded protein is responsible for stability. In the present study, the high molecular weight protein present in fish muscle may be responsible for maintaining foam stability. The plausible mechanism for foam stability, observed may depend on proper balance of flexibility,

rigidity of protein at air-water interface and the ability to form the cohesive film with high resistance to shear deformation as suggested by Ramachandran *et al*. (2007).

Foh *et al*. (2011) reported that the low solubility and more hydrophobicity is responsible for lower stability. Foaming properties are governed by transportation, penetration and rearrangement of molecules at the air water interface. Hydrophobic regions of molecules determine the adsorption at the air water interface. The extent of protein-protein interaction within the matrix affects the nature of film formation at the interface and determines the foam stability (Mutilangi *et al.,* 1996). In the present investigation, *M. armatus* fish showed foam stability at 25 minutes. Foam stability contributes to smoothness, lightness, flavor dispersion and palatability.

Emulsifying activity index and stability

EAI and ESI of *M. armatus* are shown in Figure 3. EAI was 8.96%, 8.60%, 8.12%, 7.75%, 7.27%, 7.15%, 6.78%, 5.93%, 5.81%, 5.09%, 4.48%, 3.99%, 3.99%, 2.54% and 2.54% m^2/g respectively. ESI was 41.11minutes. Lone, et al (2015), suggested that the EAI is a function of oil volume fraction, protein concentration and depends on the type of equipment used to produce the emulsion. Thus, the decrease in emulsifying activity index (EAI) with increase in time was observed, in *M. armatus,* this is because fish protein stabilizes at interface, due to optimum hydrophobicity of the protein, which stabilizes emulsions. The stabilization of emulsions is achieved by the interaction of the protein matrix with fat within the sample and the physical entrapment of fat globules *via* protein-protein interactions, which leads to the formation of an interfacial protein layer that surrounds and stabilizes the fat globules (Barbut, 1995). Mc Clements (2005) reported that at high electrostatic repulsion between oil droplets often leads to greater stability. Similar mechanism may be happening in the present study with fish muscle protein. The instability may occur under pH condition close to the protein's iso-electric point (or high ionic strength) droplets where flocculation/ aggregation dominates leading to coalescence and instability (McClements, 2005). Depending on the protein's size, structure and conformation of protein segment that radiate from interface comprises of mainly hydrophilic amino acids, which create steric stabilization, thus, physically restricting droplets from coming together as suggest by various workers (Damodaran, 1996; Tcholakova *et al.,* 2006a). The presence of protein within the continuous phase also acts to increase emulsion viscosity, thus reducing the mobility and diffusing oil droplets within the emulsion (Jafari *et al.,* 2008).

Philips (1997) reported that low value of EAI for hydrolysates is attributed to the inability of peptides to lower interfacial tension at the interface of oil and water. The hydrophobic and hydrophilic amino acid residues act as surfactant and promote the stability of oil in water emulsion system. It is reported that peptides should have a minimum of 20 residues to exhibit surface-active properties (Lee *et al.,* 1981). The low EAI to *M. armatus* may be attributed to low amino-acid residues which may be contributing to lower EAI. The difference observed in *M. armatus* fishes can be explained on the residue basis.

Water and oil binding capacity

The water and oil binding capacity is shown in Figure 4. The water and oil binding capacity was 76% and 61% respectively.

The present study the fish muscle showed retention of water in muscle protein. This may be because of the three dimensional network of filaments in myofibrils, which provides an open space between thick and thn filaments as suggested by Puolanne and Peltonen (2013). Puolanne and Peltonen (2013) reported that majority of water is retained in the space between thick and thin filaments, also a high content of acidic and basic amino acids imparts a high electrical charge to these proteins, thus, resulting in high water binding capacity. The extent of protein molecule hydration is basically the sum of the hydration of the amino acid side chain. Water binding capacity of myosin is related to large amounts of polar amino acids with a large content of aspartic and glutamic acid residues (Puolanne and Peltonen, 2013).

Water binding capacity of myosin is associated to large amounts of polar amino acids with a large content of aspartic and glutamic acid residues. The higher water binding capacity in muscles of *M. armatus* can be explained by large number of polar amino acids in muscle. Proteins have both hydrophilic and hydrophobic properties, therefore can interact with water and oil in foods (Butt and Batool, 2010). The functional properties of proteins in food system broadly depend on the water-protein interaction. WBC is affected by pH and ionic strength (*i.e.* salt). WBC reflects the extent of denaturation of the protein.

The oil binding capacity of *M. armatus* muscle protein was lower than the Water binding capacity. This suggested that *M. armatus* muscle protein may constitute more hydrophilic amino acids. Lawal and Adebowale (2004), reported their hydrophobic proteins, that have superior binding of lipids. The OBC of proteins is an important functional property as it improves the mouth feel and retains flavor in a food (Lone *et al.,* 2015). The OBC depends on the amount of non-polar amino acids in the side chain and the structure of the proteins (Butt and Batool, 2010). The major component affecting oil absorption capacity in protein is the composition of hydrophilic and hydrophobic parts. The mechanism of fat absorbtion is attributed mainly to the physical entrapment of oil and the binding of fat to the polar chain of protein (Shrivastava, 2013).

Thus, from the present studies it is concluded that *M. armatus* muscle protein showed a good solubility at extreme pH with least solubility at pH 6. The other properties such as emulsion and foaming properties too showed a good value thereby making muscle protein a potential source of useful ingredients for food formulations.

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