FUNCTIONAL PROPERTIES AND PROXIMATE ANALYSIS OF FRESHWATER BIVALVE *LAMELLIDENS MARGINALIS* (LAMARCK, 1819) MUSCLE PROTEIN

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Abstract
The objective of this research was to study the functional properties viz solubility, Foaming capacity, foam stability, emulsifying activity index, emulsifying stability index, water binding capacity (WBC) Oil binding capacity (OBC) and proximate analysis of Lamellidens marginalis muscle proteins. The present study was carried out because of dearth in information on its nutrient and functional properties. it was observed that the solubility showed an increase at both the extreme pH viz at pH 264.32% and pH 10 10 70.88% and maximum pH was found at pH 4. L. marginalis showed lowest solubility of 34.64 % at pH 4 and a highest solubility of 70.88% at pH 10. Foam capacity was found about 18% and foam stability 6.33minutes. The emulsifying activity index and emulsifying stability was 30% and 41.42 minutes respectively. The water binding capacity (WBC) and oil binding capacity (OBC) being 75% and 65% respectively. The proximate analysis for 100g muscle protein was crude protein 34.1%, moisture 6.6%, ash 11.1% crude fibre 0.1%, carbohydrate 43.1% and fat 4.3%. . L. marginalis is a good alternative protein source for human consumption. The results are discussed in relation to muscle protein.

1. INTRODUCTION

Lamellidens marginalis, the freshwater bivalve is commonly used as food, as a source of medicine, for pearl culture, and shells are used to extract edible lime in India, Bangladesh and Nepal (Ramakrishna and Day, 2007; Anchu *et al.,* 2005). As a food source, this bivalve are widely consumed, however, its commercial exploitation is relatively less, this is because, practically there is no information is available on its functional and physical properties of muscle protein. According to some, this properties constitute an important factor for food processing and food formulation (Furlan *et al.,* 2011; Jayasena *et al.,* 2010).
Zayas (1997) first studied the role of animal protein and their potential use in food industries. Since then various workers studied animal protein (Martin, 2010; Martin and Pagare, 2013). In the present study, the freshwater bivalve *L. marginalis* is taken to analyze the functional and proximate analysis of muscle.

2. MATERIALS AND METHODS

2.1. Materials

The freshwater bivalve *Lamellidens marginalis* (Lamark, 1819) were procured from Godavari river, near Kaigaon toka Aurangabad, Maharashtra, India. Immediately on reaching the laboratory, the bivalves were thoroughly washed in cold water to remove slime, dirt etc. They were acclimated to laboratory conditions for two weeks prior to experimentation in plastic troughs. The bivalve were maintained in normal D:L cycle at $25\pm1^{o}C$. The bivalve of average weight $43.4\pm0.4g$ and length of $8.6\pm0.1mm$ were used for the experiments.

2.2. Protein solubility

The solubility was determined accordingly to the method described by Vani and Zayas (1995). 45ml aliquots of *L. marginalis* muscle homogenate (muscle: water; 1:6; w:v) were adjusted to pH 2,4,6,8,10 and 12 by adding 2N HCl or 2N NaOH. At each pH level, 1.5ml homogenate was transferred into 2ml micro-tubes and centrifuged at $10000\times g$ at $4^{o}C$ for 20 minutes. The insoluble pellets were discarded. Soluble protein in the supernatant was assayed

in 0.5ml samples by the Biuret method (Torten *et al.,* 1964) using bovine serum albumin as the standard.

2.3. Foaming properties

Foaming properties of the *L. marginalis* muscle protein, such as, foaming capacity and foam stability were determined according to the method described by Sathe and Salunkhe (1981) with slight modifications. 200ml of 20mg/ml protein solution was taken in 250ml cylinder and pH adjusted to pH 8. This was followed by centrifugation at 10000 rpm for 1 minute to incorporate air at 25°C. The total volume after whipping was read immediately and used to calculate the foaming capacity; based on the following equation (Sathe and Salunkhe, 1981).

$$Foaming\ capacity = \frac{A - B}{B} \times 100\%$$

A-volume after whipping; B-volume before whipping

$$Foaming\ stability = \frac{A - B}{B} \times 100\%$$

A-volume after standing; B-Volume before whipping.

2.4. Emulsifying properties

Emulsifying properties of *L. marginalis* muscle proteins such as, emulsifying activity index (EAI) and emulsion stability index (ESI), were determined according to the method described by Pierce and Kinsella (1978). 10ml of soya oil were added to 30ml of protein solution and the mixture homogenized and centrifuged at 10000 rpm at room temperature for 1 minutes. 50µl of the emulsion was pipetted out from the bottom of the mixture at 0 and 10 minutes after homogenization and diluted to 5ml with 0.1% (W/V) dodecyl sulfate sodium salt (SDS). The absorbance of the diluted solution was measured at 500nm, using a UV spectrophotometer. The absorbance (A_o and A_{10}) were used to calculate the EAI and ESI

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times 100 \times A}{c \times 0.25 \times 10000}$$

Where, A=Absorbance at 500nm; c=protein concentration (g/ml).

$$ESI\ (minutes)\frac{Ao \times 10}{Ao - A10}$$

2.5. Water binding and Oil binding capacity

The water binding capacity (WBC) and Oil binding capacity (OBC) for *L. marginalis* muscle protein was determined by the method of Shahidi *et al.,* (1995). 1g sample was suspended in 10ml distilled water, vortexed for 2minutes followed by centrifugation at 4500×g for 30minutes. The free water was decanted and the water binding by the sample was expressed as grams of water absorbed per 100g *L. marginalis* muscle protein. Similarly the Oil binding capacity (OBC) was determined by dispersing in 10g of refined soy oil and repeated the above operation. The OBC was expressed as grams of fat absorbed per 100g of *L. marginalis* muscle protein.

2.6. Proximate analysis

Moisture, fat, ash, crude fibre and protein were determined by according to the methods of AOAC (2005). Carbohydrate by difference and calorific value were determined by the method (Gopalan *et.al.,* 1996).

2.7. Statistical analysis

The data was subjected to statistical analysis, using the MS Office. The data was subjected to ANOVA test.

3. RESULTS AND DISCUSSIONS
3.1. Protein Solubility

Protein solubility profile of bivalve *L. marginalis* showed a solubility of 70.88±0.37% at pH 10 and 63.29±0.46 % at pH 2 A least solubility of 31.64±0.865 at pH 4. The solubility profile for *L. marginalis* is shown in Figure 1.
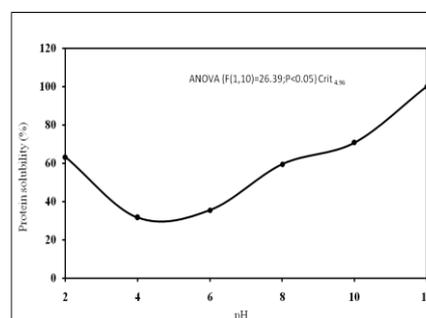

Fig. 1. Percentage solubility curve of *L. marginalis* muscle protein

Figure 1 showed a U-shaped curve for *L. marginalis* muscle protein, showing higher solubility at acidic and alkaline pH. Similar, results were reported by various workers (Palafox *et al.,* 2009; Mohan et al., 2007; Wachirattanapong et al., 2009). Kristinsson, (2003) reasoned that the increased solubility at extreme pH may be due to the co -extraction of the restraining proteins at neutral pH. Hultin et al., (2005) suggested that solubilization of muscle protein appear to be almost instantaneously at extreme acidic or alkaline pH.

The *L marginalis* protein showed least solubility at pH 4 and thereafter, an increase was observed at alkaline and acidic pH, however the solubility showed a non-significant decrease {F (4, 5) = 0.153, p<0.05}. Similar results were reported by Wachirattanapong *et al.,* (2009) with least solubility at pH 5.5 for hybrid catfish, Kristinsson and Ingadottir, (2006) found least solubility at pH range of 5 to 5.5. Mohan *et al.,* (2007) too reported a low solubility for mullet (*M. cephalus*) at pH 6.3. The solubility variation observed in the present study may be attributed to both net charge on weakly acidic and basic side chain groups of peptides, which increases as pH moves away from pI and surface hydrophobic interactions as suggested by Thiansilakul *et al.,* (2007) and Nalinanon *et al.,* (2011).

The net increase of positive charge in muscle protein at low pH may result from neutralization of negative charge at high alkaline pH. This results in de-protonisation of basic groups such as, the imidazole, guanidyl and lysyl side chains of histidine, arginine and lysine and from deprotonation of phenolic side chains as in tyrosine, this was reported by various workers (Cortes-Ruiz *et al.,* 2001; Undeland *et al.,* 2002). Hultin *et al.,*(2005) reported that solubilization of muscle protein is instantaneous at extreme acidic or alkaline pH. pH above or below isoelectric pH (pI), the proteins become negatively or positively charged depending on the pH, resulting in electrostatic repulsion between molecules and hydration of charged residues, contributing to the solubility of proteins, strong electrostatic repulsions among protein molecules and the increased protein solvent interaction at pH could have contributed to increase solubility (Kristinsson, 2006).

3.2. Foaming

Foaming capacity and stability of *L. marginalis* muscle protein is shown in (Figure 2). Foaming capacity was 18.% and foaming stability was observed at 15minutes.
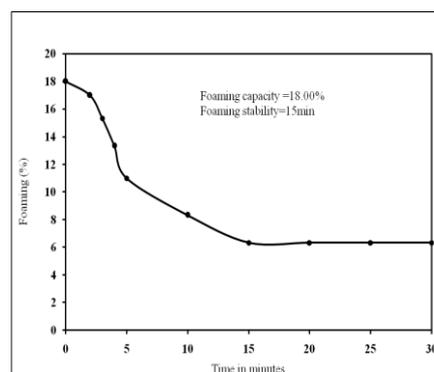

Fig. 2. Foaming capacity and stability of *L. marginalis* muscle protein

In *L marginalis,* 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 them to form and stabilize foams, also protein rapidly absorb at newly formed air/liquid interphase during bubbling. It is further reported by Lone (2015) and Rao (2014) that foam ability is an important functional property of protein, which form a flexible cohesive film to entrap air bubbles.

The phenomenon of foam formation at the air water interface is governed by three factors viz. transportation, penetration and reorganization of protein molecules (Nalinanon *et al.,* 2011). According to Ogunwolu *et al.,* (2009) good foaming activity is facilitated by the movement of protein at the interface. The others factors such as, type of protein, degree of denaturation, pH, temperature and whipping methods (Saetae and Suntornsuk, 2011). Based on studies, it was concluded that, low foaming capacity may be due to protein, present in bivalve 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 (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 18% 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 increase in foam stability formation due to formation of stiffer foams and is enhanced by flexile protein domains, viscosity of aqueous phase, protein concentration the high molecular weight peptides and surface hydrophobicity of unfolded protein (Klompong *et al.,* 2007; Van der *et al.,* 2002; Lawal, 2004). The globular proteins forms a highly elastic adsorbed layer (Wildeand Clark, 1996) with increased viscosity, thus contributes to the foam stability. In the present study, the high molecular weight protein present in bivalve 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).

3.3. Emulsion

EAI and ESI of *L. marginalis* was analyzed and results are presented in (Figure 3). EAI was 29. 68%, 28.65%, 27.63%, 26.61%, 25.58%, 24.56%, 22.51%, 20.47%, 18.42%, 14.32%, 11.25%, 9.21%, 8.18%, 7.57%, 7.57% m^2/g for 0 to 50 minutes and ESI 41.42 minutes respectively.

L. marginalis showed decrease in Emulsifying activity index (EAI) with increase in time and Emulsifying stability index was observed at 41.42 minutes. The EAI is a function of oil volume fraction, protein concentration and of the type of equipment used to produce the emulsion in fish emulsion stability is due to optimum hydrophobicity of the protein (Lone *et al*., 2015). Emulsifying properties are related

to the hydrophobic-hydrophilic balance of proteins. Mc Clements, (2005) reported that at high electrostatic repulsion between oil droplets leads to greater stability. Similar mechanism may be happening in the present study with bivalve muscle protein. The instability may occur under pH condition close to the protein's isoelectric point (or high ionic strength) droplets where flocculation/aggregation dominates leading to coalescence and instability (Mc Clements, 2005). Depending on the protein's size, structure and conformation of protein segment radiate from the interface comprised of mainly hydrophilic amino acids, to create stearic stabilization, physically restricting droplets from coming together (Damodaran, 1996; Tcholakova et al., 2006a). The presence of protein within the continuous phase also acts to increase emulsion viscosity, reducing the mobility and diffusing of oil droplets within the emulsion (Jafari et al., 2012).
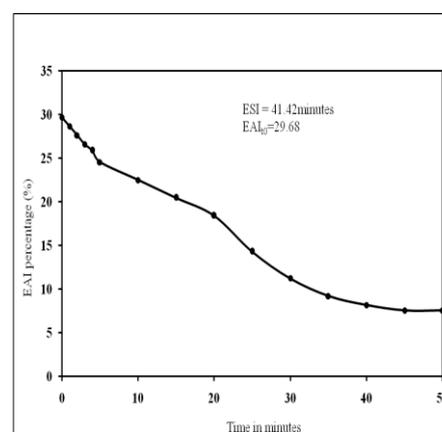

Fig. 3. Emulsifying activity and stability index of *L.marginalis* muscle protein

3.4. Water binding capacity (WBC) and Oil binding capacity (OBC)

The water and oil binding capacity of *L. marginalis* is shown in (Figure 4). In *L. marginalis* the water binding capacity and oil binding capacity was found to be 77% and 65% respectively.

The study shows that water is retained in muscle protein. This is feasible due to the three dimensional network of filaments in myofibrils provides an open space for water to be immobilized. Puolanne and Peltonen (2013)

reported that majority of water is retained in the space between thick and thin filaments. High content of acidic and basic amino acids impairs a high electrical charge to these proteins and determines a 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).
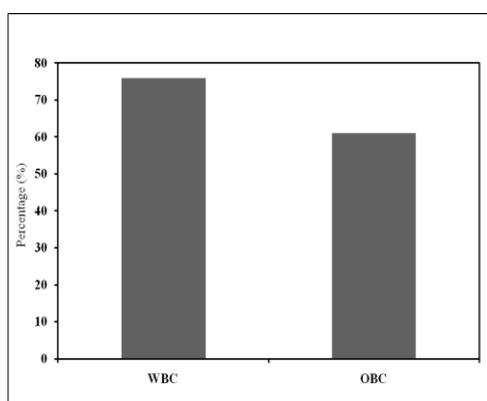

Fig. 4. Water binding and Oil binding capacity of L.marginalis muscle protein

The amount of oil bounds is markedly affected by the method used, the protein content, the charge area, the surface area, hydrophobicity and liquidity of the oil. 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).

3.5. Proximate analysis

Proximate composition of the *L. marginalis* is shown as percentage in Figure 5. In *L. marginalis* the total protein content was 34.1%, Ash 11.1%, Moisture 6.6%, Crude fiber 0.1%, Fat 4.2% and the carbohydrate by difference (%) 4.9. The calorific value being 436Kcal/100g.
The Figure 5 depicts the results of the mean percentage proximate composition (i.e. moisture, fat, Ash content, crude fiber, protein, carbohydrate). *L. marginalis* showed high crude protein content, less fat and carbohydrate being 43.1%.
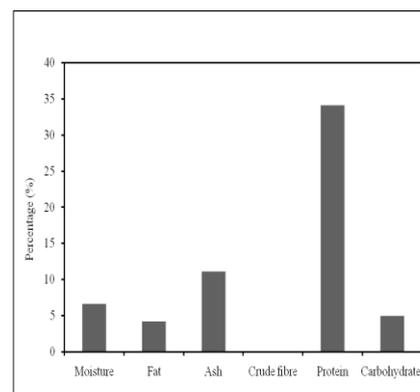

Fig. 5. Proximate analysis of L.marginalis

The fat is less, however, it is essential, since, it helps in absorption of fat soluble vitamins. Baby *et al.,* (2010) reported the proximate composition of six commercially important mollusc viz. *Pila globusa*, *Bellamya bengalensis*, *Melania tuberculata*, *Lamellidens marginalis*, *Anisus convexiuscalus* and *Helix sp*. They reported highest amount of protein in *Anisus convexiuscalus* (12.927±0.57) followed by *Melania tuberculata* (12.357±0.34%). The species *Melania tuberculata* is the highest fat containing species. *Anisus convexiuscalus* contains more than 4.6% of ash, which is followed by *Melania tuberculata* and *Bellamya bengalensis*. *Melania tuberculata* contain more than 7.5% carbohydrate in dry flesh. *Pila globusa* has 2.902±0.03% carbohydrate. About three fourth of the flesh of mollusc is water by weight, ranging from 74.6% to 85.9%.

4. CONCLUSIONS

The *L.marginalis* muscle contained high pH solubility at extreme pH. The functional properties values fulfills the requirements for better physic functional characteristics or functionality. The proximate analysis of bivalve muscle showed good nutritional values.

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