Proximate composition and Amino acid analysis in epidermal mucus of freshwater spiny eel *Mastacembelus armatus*

R. SIVASAKTHI[1], S. UMARAJESWARI[1*]AND M. UMAMAGESHWARI [2]

PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College Meelaneelithanallur- 627953, Tenkasi Dist, Tamil Nadu
Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, Tamil Nadu, India

ABSTRACT

Epidermal membranes cover the skin of fish and secrets protective mucus which serves as a first line of defense against pathogens. The purpose of this work was to determine the proximate composition and amino acid profile of *Mastacembelus armatus* epidermal mucus. Proximate composition was determined using AOAC method. Using an Automatic amino acid analyser, the amino acid composition was determined. The result obtained from the analysis revealed that proximate composition of fish epidermal mucus was moisture 53.41%, carbohydrates 29.3%, total ash 3.29%, total lipid 13.1% and total protein 32.49%. Present study amino acid analysis of the mucus reveals that is high content of glutamic acid (1.221%), lysine (0.747%), aspartic acid (0.741%), arginine (0.737%), leucine (0.741%), moderate amount of serine (0.531%), histidine (0.503%), alanine (0.481%), threonine (0.415%), valine (0.427%), isoleucine (0.361%), and phenylalanine (0.361%) were observed. Amino acid such as tyrosine (0.279%), proline (0.275%), methionine (0.057%) was found in lesser quantity. The proximate composition and amino acid profile from the epidermal mucus of *Mastacembelus armatus* contains most of the essential components required to play a possible role on antimicrobial activity and it is also used as good food for a human being.

KEYWORDS: Fish mucus, proximate composition, amino acid

1. INTRODUCTION

Mastacembelus armatus is commonly known as tire-track spiny eel/ zig-zag eel. It is a common fish species of Indian subcontinent. It belongs to the family Mastacembelidae under the order

Synbranchiformes. It is one of the most popular table fishes with delicious flesh quality having a special flavour characteristic texture and high protein, oil and vitamin C content [1-3]; and thus has a good market demand [3,4]. Mucus, on the other hand, is the final protective barrier between fish and the environment. In most conditions, it is regarded as useless and discarded during fish processing [5]. The coating of mucus that makes fish so slippery is produced by epidermal mucous cells and is believed to serve primarily for protection [6,7].

During the past few years, fish have been proven having good sources of monounsaturated, polyunsaturated fatty acids and amino acid constituents. Together with vitamins and minerals compositions, the mucus is providing potential sources in alleviating health diseases and disorders such as arthritis and inflammatory disorder [8]. Curiously, several studies have proved that preparations from fish skin secretions can enhance the rate of wound healing and antimicrobial activity in animals and the healing of diabetic foot ulcers in humans [9,10].

However, in the recent reports it was established that the freshwater fish contain relatively large amounts of EPA (Eicosapentaenoic acid) and DHA (Decosahexaenoic acid) [11]. Ghosh and Dua, (1997) [8] stated that Indian fresh water fish have high concentration of amino acids. Amino acid is sub-components of a complex protein and very important in the mechanical pathway in defense mechanism of organisms. Concha et al. [12] has detected the presence of apolipoprotein A-I (apoA-I) in the skin and epidermal mucus of carp (C. carpio) which is the principal protein constituent of HDL (high density lipoprotein) that has resulted in a high abundance of protein plasma acts as innate defense mechanism in teleost fish [13].

Fish mucus is secreted by epidermal goblet cells and comprises of mucins and other substances such as inorganic salts, immunoglobulin, proteins and lipids suspended in water giving it characteristic lubricating properties [14]. *Mastacembelusarmatus*is a medically important fish and its epithelial surface secretes large amount mucus compared with another teleost. The mucus is secreted by the epidermal goblet cells in the epidermis which composed from inorganic salts, immunoglobulins, lipids and gel forming macromolecules such as mucins, and other glycoproteins suspended in water,[15] which giving the mucus lubricating properties [16]. The mucus layer is continuously replaced which protect the eel from stable colonization by bacteria, parasites and fungi [17]. The functional properties of the mucus depend on its ability to form a gel on the epithelial surface [18]. which produce antimicrobial molecules serve as the first line of a host's defence against microbial invasion [18,19]. The main objective of

this was performed to identify the proximate composition and amino acid components that might be the potential role as defense mechanism in *Mastacembelus armatus.*

2. MATERIALS METHODS

2.1 Sample collection

The healthy live freshwater fishes *(Mastacembelus armatus*) approximately 6 months old weight about 500 gms of each were caught from Thamirabarani River in Tharuvai Village, Tirunelveli Dt, Tamil Nādu. Mucus collection was done with certain modifications following the method of Subramanian et al (2008) [20] Before collection of mucus, Fish were kept of water in specimen tray for 1 hour. After one hour mucus secreted on the epidermal surface of the body of fish was collected as samples. A sterile spatula was used to scrap mucus from the dorsal body surface of the fish. Mucus was not collected in the ventral side to avoid intestinal and sperm contaminations. Then the mucus obtained were pooled and centrifuged at 1,500× g for 10 min, 4 ° C. The supernatant obtained was stored at -80 ° C. The mucus then put in the freezer drier (± 36 h) until dried completely.

2.2 Proximate analysis

The chemical composition of the mucus samples was analyzed using the procedure of Association of Official Analytical Chemists [21]

2.2.1 Moisture Content

The amount of moisture in the organisms was determined according to AOAC [20]. Samples were dried in an air oven at 101°C for about 10 hours until constant weights were obtained, cooled in a desiccator and reweighed. Moisture content was taken to be the differential between fresh and dry weights

Determination of Moisture Content

$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where:

W_1 = Weight (g) of sample before drying

W_2 = Weight (g) of sample after drying

%DM = 100 - %Moisture

2.2.2 Ash Content

Dried samples obtained in the process of moisture content determination were heated in a muffle furnace at 550°C for several hours. The percentage of ash was calculated by subtracting the weight of ash from the initial weight.

Determination of Ash Content

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.2.3 Crude Protein Content and Carbohydrate

Crude protein was analysed by the Kjeldahl method [20]. The samples went through the three essential steps of digestion, distillation, and titration using a conversion factor of 6.25 to convert total nitrogen to crude protein.

Determination of Crude Protein

$$\%\text{Protein} = \frac{(A-B) \times N \times 14.007 \times 6.25}{W}$$

Where:

- A = Volume(ml) of 0.2 NHCL used sample titration
- B = Volume(ml) of 0.2 NHCL used in blank titration
- N = Normality of HCL
- W = Weight (g) of sample
- 14.007 = Atomic weight of nitrogen
- 6.25 = the protein-nitrogen conversation factor for fish and its by-product.

The percentage of protein in the samples was calculated thereafter. Subtracting the sum of fat content, protein content, ash content, and moisture from 100 gave the total carbohydrate content [6]. The carbohydrate content was calculated by using the following formula:

Carbohydrate content (%) = 100- (moisture+ ash+ protein+ lipid)

2.2.4 Fat Content

Crude fat was determined by weighing 5 g of each sample wrapped in a filter paper in a Soxhlet apparatus using petroleum ether. This was done each for 4 hours. The extracted materials left after the solvent had evaporated were weighed and the fat content was calculated.

Determination of fat content

$$\text{Fat\%} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.3 Determination of Amino Acid Composition of Epidermal mucus of *Mastacembelus armatus*

2.3.1 Determination of Amino Acid

The analysis of amino acid composition of epidermal mucus of *Mastacembelus armatus* was performed with minor modification, according to the methods described by Zakaria et al. [22]. The epidermal mucus of L. dimidiatus (0.1-0.2 g) was hydrolysed with 5 mL of 6 mol/L hydrochloric acid in a closed test tube, shaken for 15 min and then flushed with nitrogen for 1 min prior to being put in for 24 h at 110 o C. After cooling, 10 mL of the internal standard a-aminobutyric acid (AABA) was added to each sample prior to the addition of 20 mL redrying solution (methanol:water:triethylamine, 2:2:1, v/v/v) and 20 mL derivatization reagent (methanol: triethylamine:water:phenylisocynate, 7:1:1:1, v/v/v/v). The mixture was then poured into volumetric flasks and deionized water was added to a final volume of 100 mL. Approximately 5-15 mL of the upper layer was discarded; the rest of the upper layer was filtered through filter paper. The hydrolysed sample obtained after filtration was kept for up to four weeks at -20 ° C until use. Before injection onto an HPLC, the hydrolysed sample was

filtered using a syringe filter. Then, 10 µL filtered sample was put into a vial and the same volume of internal standard was added before the sample was dried under a vacuum for 30 min. The redrying solution (70 µL) was then added to the dried sample and the mixture was shaken vigorously for 15 min. The sample was dried again under vacuum for 30 min, followed by the addition of 20 µL derivatization reagent. The sample was kept at -20 ° C until analysis by HPLC.

2.3.2 High Performance Liquid Chromatography

Conditions

The amount of 5 µL of extract was subjected to High Performance Liquid Chromatography for further amino acid analysis. The dimensions of the AccQ-Tag Column were 3.9 mm × 150 mm [WAT052885] with Fluorescence Detector: Ex—250 nm, EM—395 nm. Mobile phase for the chromatography process are A: AccQ Tag Eluent A, B: 60% CAN. Column temperature was maintained at 36 °C. Peak integration was interpreted and calculated with the Software version 2.1 provided by the supplier. Amino acids were identified by comparison with the amino acid authentic standards.

3. RESULT

This present study demonstrated the presence of various amino acid and proximate composition of crude mucus extract

3.1 Proximate analysis

The result of the proximate analysis of *Mastacembelus armatusis* shown in the table 1 below. The result showed that the fish epidermal mucus has high percentage of moisture and crude protein content. Moisture (53.41%), Total Protein (32.49%), Carbohydrate (29.3%), Lipid (13.1%) and Ash (3.29%).

Table 1: - The proximate composition of *Mastacembelus armatus* epidermal mucus

Proximate Nutrients	**% Composition**
Moisture	53.41
Carbohydrate	29.3

Total Ash	3.29
Total Lipid	13.1
Total Protein	32.49

3.2 Amino acid analysis: -

The amino acid content of *Mastacembelus armatus* epidermal mucus is presented in table 2. The total content of amino acid in *Mastacembelus armatus* epidermal mucus was 7.171%. The skin mucus of *Mastacembelus armatus* were found to contain 15 aminoacids. Glutamic acid (1.221%), lysine (0.747%), aspartic acid (0.741%), arginine (0.737%), leucine (0.741%) is found at maximum percentage. Moderate amount of serine (0.531%), histidine (0.503%), alanine (0.481%), threonine (0.415%), valine (0.427%), isoleucine (0.361%) and phenylalanine (0.361%) were observed. Amino acid such as tyrosine (0.279%), proline (0.275%), methionine (0.057%) was found in lesser quantity.

Table 2: - The amino acid analysis of *Mastacembelus armatus* epidermal mucus

Free amino acid content	**% W/W freeze dried extract**
Aspartic acid (ASP)	0.714
Serine (Ser)	0.531
Glutamic acid	1.221
Histidine	0.503
Alanine	0.481
Proline	0.275
Threonine	0.415
Arginine	0.737
Tyrosine	0.279
Valine	0.427

Methionine	0.057
Lysine	0.747
Isoleucine	0.361
Leucine	0.741
Phenylalanine	0.361
Total AAs	7.717
Total EAAs	4.125
Total Non – EAAs	3.752
EAAs/Total AAs	0.575
EAAs/Non – EAAs	1.099

4. DISCUSSION

Fish mucus is multi-functional, playing a major role in communication, resistance to disease, respiration, ionic and osmotic regulation, feeding, reproduction and excretion. In the present study, estimation of bio- molecules confirmed the presence of proteins, carbohydrates and lipids. In general, the proximate composition of the fish mucus indicated the quality. Therefore, proximate composition of a fish helps to its nutritional value in terms of energy units. From this study epidermal mucus of fish has higher amount of moisture 53.41% and protein 32.49%. The 'slipperiness' of fish (mucus is synonymous with slime) is because of the high water (moisture) content and the presence of high-molecular-weight, gel-forming macromolecules [6]. In addition, a variety of the other materials has been identified where glycosaminoglycans, lysozyme, immunoglobulins, complement, carbonic anhydrase, a range of lectins and calmodulin have all been found in fish mucus [6]. Mucus from several species of fishes contained up to 20 times more lipid per unit area than human sebum and from previous study, its revealed free amino which may provide antioxidant agents and protection againts bacterial and fungal attack [23].

The major defensive proteins are involved in contributing to the mucus protective abilities against pathogens and particulate matter. These include the alpha and beta defensins, which are antimicrobial peptides [24,25] and lysozyme (muramidase), an enzyme that digests muramic acid mucopolysaccharide bacterial cell wall components. Structural proteins include protease inhibitors such as secretory leukocyte proteinase inhibitor and pancreatic secretory trypsin inhibitor. These proteins protect the mucus proteins from proteolysis, depolymerization

and loss of viscosity by enzymes of both host and pathogen origins [26,27]. This study proved in my work that the protein concentration was more in the *Mastacembelus armatus* epidermal mucus. Crude mucus extract of *Mastacembelus armatus* is constituted of protein (32.49%) as a major component followed by carbohydrate (29.3%) and lipids (13.1%). Manivasagan et al. (2009) [18] investigated that soluble gel of A. maculates was having 12.64 µg/g of protein content,0.08 µg/g of carbohydrate content and 0.005 µg/g of lipid content. which also supports our results. Wei et al. (2010) [28] also reported protein content in both crude and aqueous mucus extract of Channa straitus. Dhotre et al. (2013) [29] also characterized the biochemical composition of freshwater fishes viz. Channa punctatus, Channa gachua, C. carpio and A. dussmieri and found similar results. Similarly, protein has been reported as a major component of fish skin mucus of six freshwater fishes viz. Clarias gariepinus, Channa micropeletes, C. straitus, Oreochromis niloticus and Hemibagrus nemurus [30]. The presence of protein content was also investigated in the epidermal mucus of Gaint snakehead, striped snakehead, Tilapia mossambicus and bagrid catfish [31]. High amount of protein may be responsible for antibacterial activity shown by fish mucus [31].

Lipids may also contribute to the lubricating surface tension properties of the mucus layer. The rheological properties of mucus are also strongly affected by lipids [32,33]. Lipids also serve to prevent evaporation of the aqueous phase, for example in the tear mucus layer covering the eye [34,35]. My present investigation stated that the total lipid content is 13.1%.

The total content of amino acids (AAs)in *Mastacembelus armatus* epidermal mucus was 7.171%. *Mastacembelus armatus* fish mucus contains more essential amino acids than non-essential amino acids. Among 15 amino acids, nine amino acids including threonine, arginine, thyrosine, valine, methionine, lysine, isoleucine, leucine, phenyl alanine were Essential aminoacids and 6 amino acids like aspartic acid, serine, histidine, glutamic acid, alanine, proline were non-Essential aminoacids. It was discovered that, lysine, arginine, leucine was found to be the highest EAAs in epidermal mucus of *Mastacembelus armatus* representing 0.747%, 0.737%, 0,741%. Glutamic acid, aspartic acid was found to be highest non-EAAs representing 1.221%, and 0.741%. Glutamic acid, which is excretory amino acid was found in high concentration in *Mastacembelus armatus*, synthesis of glutamic acid occurs within all tissues, including adipose and brain but especially large amount produced by the muscle, lung, and skin 21Glutamic acid may also contribute to the defense mechanism in this fish towards parasitic infection, this is due to the function of this amino acid which act as an important role in the metabolism of cells in the immune system, lymphocytes and macrophages [36] .

Many organisms produce arginine and lysine rich polycationic peptides to protect themselves from pathogenic microbes [37]. It has been established that the peptides with lysine from higher animals and plants exhibit antimicrobial activity [38]. Balasubramanian et al (2013) [39] have also studied the mucus shows strong antifungal activity which might be due to highest content of lysine. According to Manivasagan *et al.* (2009) [18] who have reported higher quantity of lysine in the mucus of cat fish. Moderate quantity of phenyl alanine was reported in the three species of channa variety [40]). Higher quantity of the few amino acids such as lysine, phenyl alanine, glycine, proline, and leucine were reported in the freshwater fish *Mugil cephalus* [41]. Injured skin of cat fish *Parasilurus asotus* stimulated the high production or secretion of lysine in to the mucus layer [42]. It showed a strong antimicrobial activity towards Gram-positive bacteria, Gram-negative bacteria and fungi without any haemolytic activity. We observed that the mucus of the *Mastacembelus armatus* possess higher content of Essential amino acids including lysine and arginine, leucine. These amino acids with larger quantity present in the mucus of *Mastacembelus armatuus* may have important role on antimicrobial activity. Nevertheless, further analysis needs to be isolating and identification of new antimicrobial components.

5. CONCLUSION

Based on the results, it's concluded fish epidermal mucus contains structural proteins, carbohydrates, lipid, moisture, ash and amino acids that have the potentials. The *Mastacembelus armatus* crude epidermal mucus contains 15 amino acids in which glutamic acid, lysine, arginine, aspartic acid was found at maximum percentage. The proximate content of mucus the moisture, protein was found more. All the important amino acids and proximate composition present in *Mastacembelus armatuss* epidermal mucus are suggested to play an important responsibility for antimicrobial activity, these results indicate that mucus was prospective applications in fish and human therapeutics. Further study is necessary for the clinical applications and it their mode of action.

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AUTHORS

First Author - R. SIVASAKTHI, Research Scholar, PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Sankarankovil, Tenkasi Dist, Tamil Nadu, India. Email id sivasakthi2912@yahoo.com

Second Author - S. UMA RAJESWARI, Assistant Professor, PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Sankarankovil, Tenkasi Dist, Tamil Nadu, India. Email.id umarajeswari20@gmail.com

Third Author - M. UMAMAGESHWARI, Research Scholar, PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Sankarankovil, Tenkasi Dist, Tamil Nadu, India. Email.id umamageshwari190@gmail.com.

Correspondence Author- S. UMA RAJESWARI, Email.id umarajeswari20@gmail.com,

Contact no: 7373503579

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