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Amino acid and Fatty acid Profiles of the Marine Gastropod *Turbo brunneus* (L. 1758) along the Gulf of Mannar Region of Thoothukudi

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***Abstract*:** The majority of marine mollusks of economic importance are distributed in the coastal zone of the Gulf of Mannar. In the present study amino acids and fatty acid profiles of *Turbo brunneus* have been carried out. Twenty amino acids were estimated in both male and female *Turbo brunneus*. Among them, the essential amino acids (EAA) 20.30% and non essential amino acids (NEAA) 7.90 were found to present in males and 24.30 % of EAA and 14.10% NEAA in females. Fatty acids profiles revealed the presence of SFA 4.91% and 6.65% in males and females respectively. MUFA recorded in males and females were 1.12% and 4.02% respectively. The PUFA estimated in the males and females were 4.17% and 5.64% respectively. The result of the present study shows that the tissue of marine gastropod *Turbo brunneus* is a valuable food for human beings as it contains well balanced amino acid and fatty acid contents.

Key words: *Molluscs. Amino acid. Fatty acid. Turbo brunneus. Gastropo*

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I. INTRODUCTION

Amino acids are the essential components of all living cells. They are the building blocks of proteins. Animals and plants contain about 200 amino acids. For the purpose of understanding the significance of the metabolism of individual amino acids, they may be classified under two groups namely, essential and non-essential amino acids. Essential amino acids are indispensable and are required for nutrition, promotion of normal growth and maintenance of nitrogen balance. They are not synthesised in the body and are required to be supplied in adequate amounts through diet. Other amino acids are non-essential and do not present unusual features. But a few of them are physiologically important and take part in the general metabolic reactions. This section will deal with the metabolism of individual amino acids (Ambika Shanmugam, 1992).

Fatty acids are an organic compound consisting of a hydrocarbon chain and a terminal carboxyl group. The fatty acids of sea food differ from vegetable fatty acids in length. In the presence of Omega-3 fatty acids, the action of prostaglandins on epinephrine is diminished and thus constriction or narrowing of blood vessels is prevented.

Babu *et al.*, (2010) studied the proximate composition, amino acid and fatty acids profiles in a mesogastropod *Bursa spinosa*, Palpandi *et al.*, (2010) studied the proximate composition and fatty acid profile of different tissues of the marine gastropod *Cymbium melo* and Ihsan Ekin and Mehmet Bashan (2010) reported the fatty acid composition of selected tissues of *Unio elongatulus* from Tigris River, Turkey. The malnutrition problem in our country can be overcome by effective utilization of nutrient rich molluscan sea food. Malnutrition is considered as a serious problem, which is faced by the developing countries. In India 20-30% of the population does not get adequate nutrition (Babu *et al.*, 2010).

The molluscs are excellent sources of sea food. In order to fulfil the demand for malnutrition, the present study was carried out to analyse the aminoacid and fatty acid profiles of *Turbo brunneus.*

II. MATERIAL AND METHODS

Monthly samples of about 50 to 60 specimens of *Turbo brunneus* were collected from the coastal waters of Thoothukudi over a period of 12 months. The animals were brought to the laboratory, cleaned and were removed from the shells, separated into sexes and were dried in hot air oven at 70^0c. Estimation of amino acids and fatty acids were carried out in dry tissues of the animals and the values were expressed in percentage of dry weight.

A. Estimation of amino acids

To the 500mg of sample, 5ml of 10% TCA was added which was kept in a water bath for half an hour. The contents were cooled and centrifuged at 5000 rpm for about 5 minutes. The supernatant liquid from the residue obtained was removed. To this residue 5ml of 6M HCl was added and kept in a water bath until the contents were evaporated to 1ml for hydrolysis. The solution was stored in refrigerated condition till the time of derivatization.

Derivatization

1ml of 0.5M sodium borate buffer (p^H 9.0) and 1ml of FMOC-CL solution (9-fluorenyl methoxy carbonyl chloride) (3MM in acetone) were mixed with micro litres of analyte solution. After 2 minutes, 2.5 ml of ADAM (1 – Adamantyl amine) (40mM in acetone –water 3:1) and again after 2 minutes 3.8 ml of a mixture of 0.5M sodium acetate buffer (p^H 4.0) and Acetonitrile (1:1) were added to 200 microlitres of the reaction mixture. The values were obtained in the following chromatographic conditions:

Columns : Lichro CART 250 – 4 superspher R P – 18, 4, microns
Pre column : Lichro CART 4 – 4 Lichrospher RP – 18, 4 microns
Pump: Merck Hitach L -7100
Pump mode: Gradient
Mobile phase (A) : 100mM sodium acetate p^H 4.6 – Tetra hydro Furan – DMF (90:5:5)
Mobile phase (B) : 100% Acetonitrile
Gradient programme : 7-15% B in 0-10 minutes
15-50% B in 10-35 minutes

50-100% B in 35-40 minutes
Equilibrate with 7% B for 10 minutes
Detection: Fluorescence detector (L-7485), Ex -263nm, Em-313 nm
Temperature : 40^0C
Injection Volume : 100 microlitres
Flow rate: 1 ml/ minute

B. Analysis of fatty acids

Preparation of samples was oven dried at 70^0C for 24 hrs until no more weight reduction was observed. After that samples were grounded finely with pestle and mortar.

To the 100-200 mg of finely ground tissue samples 1:1 ratio of chloroform: methanol (2ml) was added and kept for 30 seconds. The residual matter was removed by filtering through Whatman No.1 filter paper (125 mm). This was washed with 1 ml of chloroform: methanol (2:1 vol) to remove the inorganic substances from the combined extract by partition and treated with chloroform: methanol: water (8:4:3) where the lower phase evaporated to dryness. The dried matter was subjected in a sealed test tube with 3% Methanolic HCL at 80^0C for 18 hrs. To this 2ml of hexane was added to extract the Fatty acid methyl esters (FAME) obtained from methanol phase in Hexane. Top 1ml of the hexane phase was collected in a micro vial. The residual fraction was dissolved in 10/µl of ethyl acetate and injected 1/µl aliquot into a gas chromatograph (Model Agilant 6890, 1997) equipped with flame identification detector and column HP ULTRA -2 (25m, 0.2mm ID) (Kashiwagi *et al.*, 1997).

III. RESULTS

Results of seasonal and sexual variations observed in the amino acids and fatty acids profile of whole body tissues of *Turbo brunneus* are shown in the table 1-4.

A. Amino acids

Total aminoacid content in the body tissues of male *Turbo brunneus* was 31.20 %. Among them, the essential aminoacids (EAA) 23.30 % and non essential aminoacids (NEAA) 7.90% were found to be present. The results of present study revealed that threonine, 3.04%; Arginine, 2.32%; Tyrosine 1.03%; Histidine, 2.24%; Valine, 0.34%; Methionine, 0.48%; Isolucine, 3.03%; Phenylalanine 1.10%; Leucine, 2.24%; Lysine, 4.03% and Tryptophan, 3.44% are the major EAA recorded. Aspartic acid 1.13%; Glutamic acid 0.09%; Asparagine 1.13%; Serine 2.04%; Glutamine 0.01%; Glycine, 1.13%; Alanine, 0.03%; Cysteine 0.22% and Proline 1.14% are the NEAA recorded. Lysine showed the highest concentration (4.03%); Valine (0.34%) and Methionine (0.48%) contributed the lowest concentration of EAA. Of the NEAA Serine (2.04%) showed the maximum concentration and Glutamic acid (0.09%) showed the minimum concentration (Table 1).

The total aminoacid content recorded was 38.40% in the female *Turbo brunnues.* Among them 24.30% contributed EAA and NEAA contributed 14.10%. The results of the present study revealed that Threonine, 1.12%; Arginine, 3.01%; Tyrosine, 3.11%; Histidine, 1.12%; Valine, 1.24%; Methionine, 3.32%; Isoleucine, 1.14%; phenylalanine, 3.04%; Leucine, 1.12%; Lysine, 3.04% and Tryptophan, 3.01% are the major EAA recorded. Aspartic acid 1.31%, Glutamic acid, 2.03% Asparagine, 1.13%; Serine, 1.03%; Glutamine, 2.15%; Glycine, 2.04%; Alanine, 2.21%; Cysteine, 1.10% and Proline, 1.12% are the NEAA recorded. Of the EAA Arginine (3.01%); Tyrosine, (3.11%); Methionine, (3.32%), Phenylalanine (3.04%), Lysine (3.04%) and Tryptophan (3.01%) contributed more than 3.0%. Histidine (1.12%) Leucine (1.12%) and Threonine (1.112%) were the EAA showed the lowest concentration. Glutamic acid (2.03), Glutamine (2.15), Glycine (2.04%), Alanine (2.21%) were the higher concentration of NEAA recorded in female *Turbo brunneus.* Serine (1.03%) showed the lowest concentration among the NEAA (Table 2).

B. Fatty acids

In the present study, six individual fatty acids were identified in the male *Turbo brunneus*. Among them the saturated fatty acids were found to be dominant (4.91%) and most of which were C16:0 (2.12%) and C17:0 (1.81%). The poly unsaturated fatty acids (PUFA) were next to the most common fatty acids (4.17%) with the higher levels of C18:2 (3.04%). The mono unsaturated fatty acid (MUFA) occupying the third position contributed 1.12% of total fatty acids are represented by C18:1. At the same time Omega 6 fatty acid is accounted for 3.04% of the total PUFA (Table 3).

In the female individuals of *Turbo brunneus* also the saturated fatty acids were dominant (6.65%) and most of which were C16:0 (3.31%) and C18:0 (3.31%). The poly unsaturated fatty acids (PUFA) were also the next to the most common fatty acids (5.64%) with the higher levels of C18:3 (3.41%). The mono unsaturated fatty acid (MUFA) occupying the third position contributed 4.02% of total fatty acids are represented by C18:1. At the same time Omega 6 fatty acid is accounted for 2.23% of the total PUFA (Table 4).

IV. DISCUSSION

Totally 20 amino acids were estimated as percentage composition of the total protein in both male and female *T. brunneus* respectively. Among them, the essential amino acids (EAA) 24.30% and non – essential amino acids (NEAA) 7.90% were found to present in male *T. brunneus* where as in female *T. brunneus* 24.30% was contributed by EAA and 14.10% by NEAA. To support the present study, similar to this findings were made by Babu et. al., (2010) in *Bursa spinosa.*

Ajaya Baskar (2002) observed that amino acid content in the molluscs *Perna viridis, Crossostrea ma drasensis* and *Meretrix casta.* Totally 18 amino acids were recorded. The total amino acids in the *P. viridis* was 95.76% , among them, essential amino acid 47.28% in C. *madrasensis* total amino acid 98.4%, among them essential amino acid 54.52% and in the *M. casta* the amount of aminoacid observed was 65.17%, among them, essential amino acid contributed 38.17%. Babu *et al.*, (2010) reported 50.1% of EAA and 46.79% of NEAA in *Bursa spinosa.*

The present study revealed that totally 20 amino acids were estimated both in male and female *Turbo brunneus.* The total amino acid concentrations were exhibited high levels of lysine in males and methionine, followed by lysine phenyalanine, Tyrosine and Tryptophan

in females, based on the quantum of availability of EAA in the tissues of *Turbo brunneus.* This clearly indicated the potential source for EAA and for human consumption. Therefore, it is strongly recommended that *Turbo brunneus* it fit for human consumption.

Fatty acid profiles of other molluscs are usually dominated by SFA and the present study also revealed the same where maximum SFA (6.65%) was present in females and 4.9% in male *Turbo brunneus.*

The poly unsaturated fatty acids (PUFA) have been recognized as effective factors in human health and nutrition, especially for cardiovascular diseases (Bruckner, 1992). The fatty acid composition of oceanic gastropod *Xancus pyrum* have been analysed by combined GC-MS technique, and the result showed the presence of 8 saturated and 6 unsaturated fatty acids (Usmanghani, *et al.,* 1989). Babu *et al.,* (2010) have reported the presence of 6 SFA, 1 MUFA and 4 PUFA in *Bursa spinosa* species, where as in the present study 3 SFA, 1 MUFA and 2 PUFA were analysed from the body tissues of *Turbo brunneus.*

In the present study, Myrstic acid was lower (0.03%) in female and Stearic acid was lower (0.98%) in male individuals of *Turbo brunneus* and α-Linolenic acid was higher (3.41%) in females compared to SFA and MUFA. In males Linolenic acid (3.04%) was recorded in higher concentration than MUFA and SFA. The level of total fatty acids measured in the whole body of *Turbo brunneus* was not very high and there might be several explanations for this fact. Primarily lipids are not the main energy reserve in molluscs, which are characterized by low total fat contents (Bonnet *et al.,* 1974). Babu *et al.,* (2010) recorded the low fatty acid contents in the body tissues of *Bursa spinosa.* In the present study also very low fatty acids were recorded in the body tissues of *Turbo brunneus.*

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Table 1. Amino acid composition of *T. brunneus* studied - Male

Essential amino acids	Percentage of EAA
Threonine	3.04
Arginine	2.32
Tyrosine	1.03
Histidine	2.24
Valine	0.34
Methionine	0.48
Isoleucine	3.03
Phenylalanine	1.10
Leucine	2.24
Lysine	4.03
Tryptophan	3.44
Total	**23.30**
Non-essential amino acids	**Percentage of NEAA**
Aspartic acid	1.13
Glutamic acid	0.09
Asparagine	1.13
Serine	2.04
Glutamine	0.01
Glycine	1.13
Alanine	1.03
Cysteine	0.22

Proline	1.14
Total	**7.90**

Table 2. Amino acid composition of *T. brunneus* studied – Female

Essential amino acids	**Percentage of EAA**
Threonine	1.12
Arginine	3.01
Tyrosine	3.11
Histidine	1.12
Valine	1.29
Methionine	3.32
Isoleucine	1.14
Phenylalanine	3.04
Leucine	1.12
Lysine	3.04
Tryptophan	3.01
Total	**24.30**
Non essential amino acids	**Percentage of NEAA**
Aspartic acid	1.31
Glutamic acid	2.03
Asparagine	1.13
Serine	1.03
Glutamine	2.15
Glycine	2.04
Alanine	2.21
Cysteine	1.10
Proline	1.12
Total	**14.10**

Table 3. Fatty acid composition of *T. brunneus* studied – Male

Saturated fatty acids	**Position of the carbon atom**	**Concentration of SFA in percentage**
Palmitic acid	C16:0	2.12%
Stearic acid	C18:0	0.98%
Myristic acid	C17:0	1.81%
Total		**4.91%**
Monounsaturated fatty acids (MUFA)		
Oleic acid	C18:1	1.12%
Poly unsaturated fatty acids (PUFA)		
Linolenic acid	C18:2	3.04%
α-Linolenic acid	C18:3	1.13%
Total		**4.17%**

Table 4. Fatty acid composition of *T. brunneus* studied - Female

Saturated fatty acids	**Position of the carbon atom**	**Concentration of SFA in percentage**
Palmitic acid	C16:0	3.31%
Stearic acid	C18:0	3.31%
Myristic acid	C17:0	0.03%
Total		**6.65%**
Monounsaturated fatty acids (MUFA)		
Oleic acid	C18:1	4.02%
Poly unsaturated fatty acids (PUFA)		
Linolenic acid	C18:2	2.23%
α-Linolenic acid	C18:3	3.41%
Total		**5.64%**