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Biochemical and Amino Acid Profiles of Two Smoke-dried Fish Species From the Hill Streams of Manipur, India

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Abstract - Two smoke dried fish Schizothorax and Ompok species from the hill streams of Manipur were studied. Proximate composition, and amino acid values were determined. High protein content (70.08%) was in Schizothorax and lipid content was high(19.62) in Ompok species. Moisture range was between 9.34 and 10.45% and ash was between 5.34-3.23%. All the amino acids were present except arginine and cysteine. Lysine was in maximum level 12.25-9.93g/100gm) among the essential amino acids of the fishes examined. Among the non- essetial amino acids, maximum level (20.76-18.21g/100gm) was with glutamic acid followed by aspartic acids 11.37-10.24g/100gm). The present study indicates that the fish species were good sources of protein with different essential amino acids.

Keywords: fish, proximate composition, proteins and amino acids.

1. INTRODUCTION

Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals. Fish is a vital source of food provided by the aquatic sources of the earth. Its protein is easily digestible and favorably complements dietary protein provided by cereals and legumes that are typically consumed in many developing countries. Experts agree that, even in small quantities, fish can have a significant positive impact in improving the quality of dietary protein by complementing the essential amino acids that are often present in low quantities in vegetable-based diets. But recent research shows that fish is much more than just an source of animal protein. Fish oils in fatty fish are the richest essential fatty acids that is vital to normal brain development in unborn babies and infants. Without adequate amounts of these fatty acids, normal brain development does not take place.

The FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein (FAO, 2000). Proteins containing selenium can intercept free radicals and are thought to prevent cancer. Taurine, a metabolite product of protein is important for the development of the brain and retinal tissue. Moreover it plays a key role in the development of cell membranes and in the detoxification of the body. Vitamin D, which very few foods contain in worthwhile amounts, mainly occurs in fatty fish. Followed by niacin, vitamin B6 and vitamin B12. Fish contains all the important amino acids for human nutrition, including those known as “essential amino acids” which the human metabolism cannot synthesize himself. A unique contribution of high quality protein and vital nutrients makes fish an invaluable food. Research has proved that fish is a food for physical and mental health increasing performance ability for wealth and success.

Most of the hill stream fishes are traditionally smoke processed and sold in smoked forms in markets of Manipur. Its important reasons are lack of good transportation and cold storage facilities in this area of hills and mountains. Sometimes people have to walk on foot for many miles in the hilly terrains. So smoking is

the most popular method of fish processing in the hilly region from the early time. Smoking involves heat application to remove water and inhibit bacterial and enzymatic action on fish (Eyo, 2000). It imparts aroma, taste and colour on processed fish (Olley et al. 1988), today the smoking process is a traditional method of considerable economic importance worldwide (Huda et al., 2010). The processing and preservation of fish were of utmost importance to prevent economic losses since fish is highly susceptible to physical deterioration. Productions of smoked herrings, salmons and other fishes are reported from other parts of the worlds (Espe et al, 2001). (Kolodziejska et al., 2002, Usydus et al, 2009), (Yanar, 2007). (Amin and Tjipto, 2001).

The purpose of smoking is to preserve and increase shelf life of fishes. Methods of drying and smoking of fish vary between different countries and within the same country depending on the species of fishes used and the type of fish products desired. The process may use unsalted fish or various salt addition giving salt concentration in the final products ranging from less 2% to over 20%. The variation makes it difficult to arrive at general conclusion regarding the processing effects of smoking and drying on protein quality and the nutritional values of the final products (Obstvedt, 1988). Smoked and dried fishes are a traditional part of the diet of a large section of the world's population.

The purpose of the paper is to study the proximate composition and further is to study the amino acids composition of the smoked-dried two fishes after analyzing using HPLC.

2. MATERIALS AND METHODS

The smoked dry fishes Schizothorax sp., and Ompok sp., with lengths 29-34.5cm, 18.5-22.5cm, 27-28.5, 21-20cm,18-21.5 were purchased from the markets of hill districts of Manipur during the months of March to May. Each species of the fish sample was oven-dried in an electric oven at 600C until the sample had constant weight. Because of variations in composition of different regions, they were homogenized separately without including any bones and used for different analyses.

2.1 Chemical Reagents

Chemicals purchased from Merck and Sigma were of analytical grades. Water used was of special grade obtained from Millipore filter system (USA).

2.2 Proximate Composition analysis

The values of proximate composition were determined by the method of AOAC,(1975). Nitrogen was estimated colorometrcaly by spectrophotometer. Protein values was calculated by multiplying the nitrogen values of the fishes by 6.25. Lipid was determined following the methods of et al ,1957.

2.3 Amino acid Composition

The samples for the amino acid analysis were prepared according to the method of Ishida et al, 1981). Protein is hydrolyzed into their constituent amino acids by 6N hydrochloric acid. The amino acids are separated by an ion-exchange column in a HPLC and the eluted amino acids are identified based on the fluorescence they produce with o-ophthalaldehyde in the presence of 2-mercaptoethanol. The mobile phases are low pH (buffer 'A') and high pH (bffer 'B') respectively.

2.4 Preparation of sample

100 mg of sample was taken in a test tube and 10 ml of 6N HCl was added to it. The tube was filled with nitrogen gas, heat scaled Whatman No.42 filter paper quantitatively after breaking open the tube and the

filtrate. Was flash evaporated thrice using distilled water to remove HCl and was made to definite volume in 0.05 M HCl

2.5 Amino acid analysis

The digested samples was filtered using 0.45 ⊠m membrane filter and 20 ⊠l of this was injected through the sample loop of Shimadju

HPLC-LC, fitted with a packed column (ISC-07/51504-Na) of length 19 cm and diameter 5 mm. Oven temperature was adjusted for 600C. The column was kept in an oven and the sample was carried to the column length a guard column. The amino acids were detected by spectroflourometer after post column derivatization with ophthaldehyde. Stepwise elution of amino acids starting from acid then neutral and alkaline took place. The chromatograph was recorded by a data processor.

2.6 Estimation of Tryptophan

It was done following the method of Sastry and Tummuru, 1985.

Tryptophan was hydrolysed in alkali. The 5-hydroxy furfural resulting from sucrose under acidic condition forms pale green-coloured condensation product with thioglycolic acid, which reacts with tryptophan giving a pink coloured complex. The colour intensity is measured at 500 nm.

0.2g of sample was taken in a test tube to which 10 ml of 5% NaOH solution was added and heated to 1300C (or filled with nitrogen gas), heat sealed and kept at 1100C in an oven for 24h. Tube has broken and the content was neutralized with 6NHCL. Mixture was made to 100 ml and filtered, and pH was checked to be neutral .0.1 ml of 2.5% sucrose and 0.1 ml of 0.6% thioglycolic acid was added successively in to a series of test tubes containing 4 ml of 50% H2SO4 and mixed with vortex misture. Test tubes were kept in water bath for 5 min at 45-500C. One of the tubes was kept as a blank and 0.8ml 0.1N HCL was added to it. In the others varying concentrations of sample or standard as well as 0.1N HCl were added to make up to 5 ml. Absorbance of the sample and standards were measured by taking reading at 500 nm.

Calculation: The nitrogen content of the sample is estimated by kjeldahl method.

3. RESULTS

In the smoke dried fishes (Table 1), the protein ranges from 31.14 to 72.58% and were significantly higher (72.58%) in Schizothorax sp. The lipid contents varied from 1.43 to 5.13% and the highest was recorded in Ompok sp. (5.13%) and the lowest (1.43%) in Schizothorax sp. In smoke-dried fishes lipid range from 18.73 to 6.36% and were significantly higher (18.93%) in Ompok sp. Ash contents was varied from 4.32 to 5.33% in smoked fishes.

Table -1: Proximate composition of the smoke-dried fishes.

Species	Moisture%	Protein%	Lipid%	Ash%
Schizothorax sp.	12.35±0.06a	72.58±2.43c	6.36±0.08a	5.23±0.01b
Ompok sp.	10.14±0.07b	31.20±1.70a	18.73±0.29d	5.20±0.01a

Values are mean of six replicates.

Means (±SD) within a row followed by the different letters are significantly different (P≤0.05).

3.1 Amino acid profile

Amino acid composition of the smoke-dried hill stream fishes are shown in Table 2. A total number of 16 amino acids were present in the fishes examined, as shown in Table 2. The most dominant amino acid was glutamic acid in the smoke-dried fishes. In the smoke-dried fishes the level of glutamic acid was 20.76 g/100g in Schizothorax sp. and 18.21 g/100g in Ompok sp. The second most dominant amino acid was aspartic acid. Aspartic acid also was higher (11.42 g/100g) in Schizothorax sp. and 10.24 g/100g in Ompok sp. Other dominant amino acids were leucine, lysine, alanine, valine. Alanine was 8.17 g/100g in smoke-dried Schizothorax sp. and 7.46 g/100g in Ompok sp.

Among the essential amino acids lysine was most dominant in the fishes examined. In smoke-dried fishes, lysine was higher (12.25 g/100g) in Schizothorax sp.,and 9.93 g/100g in Ompok sp. Leucine was 8.30 g/100g In smoke-dried fishes Schizothorax sp. and 7.50 g/100g in Ompok sp. Cysteine and arginine were not detected in the analysis by HPLC.

Table -2: Amino acid composition of the smoked fishes (g/100g protein).

Samples	Schizothorax sp.	Ompok sp.
Aspartic acid	11.37	10.24
Threonine	5.81	6.38
Serine	5.43	4.80
Glutamic acid	20.76	18.21
Proline	3.72	5.72
Glycine	3.01	3.11
Alanine	8.17	7.46
Cysteine	-	-
Valine	5.60	6.57
Methionine	3.22	3.99
Isoleucine	5.51	5.62
Leucine	8.30	7.50
Tyrosine	3.20	4.38
Phenyl alanine	5.76	6.46
Histidine	4.73	4.56
Lysine	12.25	9.93
Arginine	-	-
Tryptophan	1.54	1.65

4. DISCUSSION

Discussion of the results of analysis based on the various experiments of the smoke-dried fishes, Schizothorax sp, and Ompok sp. are adopted here under separate headings to give explanation and justification to the obtained values of the fishes.

The important key to health and active longevity may be sufficient and appropriate exercise and healthy eating to ensure adequate intakes of protein and most other key nutrients to maintain muscle and bone strength and mobility. The demand for animal protein will no doubt continue to grow in the emerging economies, because meat is a preferred food in most societies (Millward, 2008).

The nutritional value of fish meat comprises the contents of moisture, protein, lipids, vitamins and minerals plus the caloric value of the fish (Evangelos, 1989; Chandrashekar and Deosthale. 1993; Steffens, 2006). Moisture contents are different in smoked fish (Table 2). Water is essential to all living cells. The fluid acts as the medium of transport of nutrients metabolites etc. in absence of water the system will not function. There is a inverse relationship between water and fat contents (Nair and Mathew, 2000).Water contents are different in the fresh and smoked fishes. Water is lost from the tissue during smoking and drying process. Percentage of moisture of smoked dried fish depends on the level of dryness.

In smoked fishes, the value was highest (72.58%) in Schizothorax sp.. The lowest protein was 31.20% in smoked samples of Ompok sp. The value of protein percentage of the smoke-dried fishes is in agreement with the values of the fishes reported by Lilabati and Vishwanath (1990). So, the fishes of the present study are a good source of protein. Protein content of fish varies widely depending on factors such as natural feeding habits and availability of food, fasting during spawning, migration etc (Nair and Mathew, 2000). The lipid values of 4.34% - 10.73% in some smoked fishes were reported by Lilabati and Vishwanath (1990).

The relatively high to moderate percentage of protein could be attributed to the fact that fishes are good sources of pure protein, but the differences observed in the present values might also be attributed to fishes' consumption or absorption capability and conversion potentials of nutrients from their diet or local environment into such biochemical attribute needed by the organism's body (Burgress, 1975; Adewaye and Omotosho, 1997).

4.1 Amino Acid Profiles

Amino acid profiles of the fishes (Tables 2) shows different values for different fish species. Evaluation of protein quality aims to determine the capacity of food protein sources and diets to satisfy the metabolic demand for amino acids and nitrogen. The fish species of the present study as sources of protein show essential and non-essential amino acids in the composition of the protein. There is variation of amino acids contents in the different species. The amino acid composition varies among fresh and smoked fishes too. Presence of high amount of glutamic acid in the fishes contributes to the sweet taste of the fishes. High level presence of histidine in L. pangusia will contribute to better taste. Presences of total aromatic and sulphur amino acids vary among the different species signifying their different characters. Amino acids cysteine and arginine were detected in this fishes as effects of smoke and drying process. Dominant presence of total essential amino acid over total non-essential amino acids in the fishes indicates the strength of quality. Presence of all the essential amino acids in the fish protein makes them complete protein. In the study of Indonesian smoked cat fishes by Huda et al. (2010), the most dominant amino acids was glutamic acids (8.11-9.18 g/100g protein) in Macrones nemerus and Orypterus microneme; lysine contents was 4.97 to 5.80 g/100g in the two fishes. In the study of Indian major carps, Rohu, Catla, and Mrigal by Sankar and Ramachandran (2002), the dominant acid was glutamic acids (15.63 to 20.06 g/100g

protein) followed by aspartic acid (10.26 to 20.25g/100g protein); and important amino acid lysine was 3.01 to 8.20 g/100g. These are found similar in our present study.

5. CONCLUSION

The analysis of the proximate composition shows that the fishes contain protein, lipid, minerals,(ash) carbohydrate, some amount of water which is indicative of nutritional values .Schizothorax sp. contains high amount of protein while Ompok sp. contains high amount of lipid which is sources of essential fatty acids. The further analysis of the protein shows the smoke-dried fishes contain all the essential amino acids which indicates high quality proteins (complete protein). Cysteine and arginine are absent as result of smoking and drying effects. However good care is important in the processing for maintaining the quality of the fishes.

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