



A Comparative Study on Total Muscle Protein Content of Different Fish Species in Fresh and Smoked - Dried Condition and to Analyze their Banding Pattern through SDS-PAGE

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Abstract: Fish plays an important role in human nutrition. They are known to be a very healthy food item. They are excellent protein sources that also deliver various minerals and vitamins necessary for good health. Due to the high content of polyunsaturated fatty acid, fish flesh and fish oil are beneficial in reducing the serum cholesterol. In addition to that, the special type of fatty acid, omega-3 polyunsaturated fatty acid, is recognized as an important component to prevent a number of coronary heart diseases. Fish proteins contain all the essential amino acids in the required proportion and hence have a high nutritional value, which contribute to their high biological value. In fresh fish muscles, the water content is strongly bound to the proteins and cannot be easily removed even under higher pressure. For the present study, three different fish species, *Clarias batrachus* (E1), *Mystus tengara* (E2) and *Puntius ticto* (E3) were taken to study their protein content and analyze the electrophoretic protein banding pattern for both fresh and smoked dried conditions. Protein extract was made from the muscle tissue of the fishes taken. The protein content was measured by Lowry's method using BSA protein as standard. The optical density was measured at 660nm. The electrophoretic protein banding patterns were determined using SDS-PAGE gel electrophoresis. The results showed that the total protein concentration of samples for fresh condition E₁F, E₂F and E₃F was found to be 7.15±0.247 mg/ml, 7.20±0.224 mg/ml and 4.98±0.283 mg/ml respectively and for smoked dried condition E₁D, E₂D and E₃D was 8.48±0.273 mg/ml, 8.31±0.222 mg/ml and 5.81±0.335 mg/ml respectively. The SDS-PAGE electrophoresis showed significant increase in the protein bands of three types of smoked dried fish samples when compared to fresh ones. Therefore, the percentage of protein content in selected smoked dried fishes was found more.

Keywords: Electrophoresis, Fish, Fish flesh, Protein, Nutrition, smoked dried fish, SDS-PAGE

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1. INTRODUCTION

Fish make up almost half of the total number of vertebrates in the world. India is one of the mega biodiversity countries in the world.¹ The north east India has a unique topography and hence an attractive field for ichthyologic studies. This part of India is identified as a global hotspot of fresh water fish diversity.² Recently many new species have been documented from the status of north east India opening the scope for exploring the nutritional qualities of the fishes.³⁻⁵ Due to the presence of endemic fish species, the north east India is familiar as 'Global hotspot' for fish faunal diversity.⁶ The nearest wetlands are the only source of fish for the rural poor people.⁷ Fishery sector occupies an important place in the socio-economic development of the country. Fish play an important role in human nutrition. Good and adequate nutrition plays a very important role in the expression of mental, physical and intellectual qualities in man. Fish belongs to high protein and low lipid classes. Fish foods contain lower caloric content per unit of protein than lipid and they provide the animal protein for use in controlled diets.⁸ The principle component of fish muscles are proteins. In fresh fish muscles, the water content is strongly bound to the proteins and cannot be easily removed even under higher pressure. Fish proteins are generally enriched in two essential amino acids called lysine and methionine. The protein is present chiefly in the skeletal muscles- the fish flesh. Fishes can be classified as oily fish or fat fish (fat content more than 8%), average fat fish (fat content between 1-8%) and lean fish (fat content less than 1%). These fats are chiefly the triglycerides esters of fatty acids with small amounts of free fatty acids, some vitamins, sterols, hydrocarbons, phospholipids etc.⁹ Fish spoil naturally, and ancient people had used different kinds of techniques to preserve fish. The heat of the sun and air cause the fish to dry by reducing moisture content to 75% or less depending on its oil level. But sun drying may expose fish to attack by insects, vermin and contamination by sand and dirt with no control over drying time. Refrigeration by chilling or freezing and smoking are other ways of fish preservation. The smoking method does not only preserve, but also give fish, a desirable odour and flavor. The process involves cleaning, brimming, soaking, drying and smoking for about 30-45 minutes with 85°C using hardwoods and or plant leaves. The dried product can be stored for a longer period of time and consumed as and when desired. It provides an important source of protein. Apart from generating food production, it stimulates the growth of a number of subsidiaries for processing and production of various value added fish products so much, so that the sector has been recognized as a powerful employment and income generator. The present study was therefore undertaken to determine the total muscle protein content of three different fish species, i.e., *Carias batrachus*, *Mystus tengara* and *Puntius ticto* in different experimental conditions- fresh and smoked dried conditions and also to analyze the electrophoretic banding pattern of muscle protein from the above mentioned species through SDS-PAGE.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of samples

Three different fish species, both in fresh and smoked-dried conditions were collected from the market (*Carias batrachus*, *Mystus tengara* and *Puntius ticto*). All the collected samples were healthy and free from fungal and bacterial skin infection.

Three fresh samples and three smoked dried samples were processed to prepare the protein extracts of all the samples on the same day along with freshly killed samples.

2.2 Preparation of protein extract from muscle

The fishes were cut along the dorsal most part of their body, separating the skin from muscle, so that the muscles became well exposed. The bones were carefully removed, then to each 1g of sample (kept in separate test tubes), 0.1N saline was added using a micropipette. Since proteins are thermo-sensitive molecules, all the test tubes containing the muscle extracts were kept in a beaker containing ice so as to maintain their respective experimental conditions. The muscles were properly homogenized by using tissue homogenizer and centrifuged at 3000 rpm for 8-10 minutes. Supernatants were taken out, remaining sample was discarded. Supernatant kept in eppendorf tubes using micropipette and stored at -20°C for further analysis of the samples.

2.3 Estimation of protein concentration

The protein concentration of the samples were estimated by using Lowry's Method by using standard protein (Bovine serum albumin).¹⁰ This method is based on the reaction of copper ions produced by the oxidation of peptide bonds with Folin-Phenol reagent (a mixture of phosphotungstic acid and phosphomolybdic acid in the Folin-Phenol reagent). This method is more sensible than ultraviolet (280nm) absorbance reading, it is less disturbed by turbidity. The optical density of each sample was measured using a spectrophotometer at a wavelength of 660 nm and recorded the data in tabular column.

2.4 SDS- PAGE Gel Electrophoresis

Electrophoresis can be used to separate and visualize proteins. In sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE), proteins are separated based on size.¹¹ When protein samples are applied to such gels it is usually necessary to know the protein content or the protein concentration of the sample. This makes it possible to apply a volume of samples of the gel so that samples have a comparable amount of total protein. An intact SDS-PAGE electrophoresis system should include a tank, lid with power cables, electrode assembly, and cell buffer dam, casting stands, casting frames, combs (containing wells) and glass plates. The gel plates were assembled according to the manufacturer's instructions. The mixture for 12% resolving gel was poured inside glass mould and immediately overlaid with n-butanol. The casting frame was kept undisturbed for 30 minutes. n-butanol was discarded and the gel top washed with distilled water. The mixture for 5% stacking gel was then pipetted over the resolving gel up to top of the plates. The comb was washed with the remaining solution and immediately inserted into the stacking gel. The casting frame was kept undisturbed for 30 minutes. Protein concentration of each sample was estimated after performing Lowry's method. Using the data of protein concentration, the volume of the required loading protein sample was calculated in such a way that each loading sample contained 40µg of protein. The loading protein samples were mixed with a sample buffer in properly labeled Eppendorf tubes (were kept on ice) using micropipette. The comb from the mould was removed carefully and the wells were properly washed with distilled

water. Electrophoretic tank was filled with an electrophoresis buffer and the unit containing the prepared gel plates was placed at the right place. The loading samples were loaded in the wells using micropipette and the top of the chamber was covered. Then the apparatus was attached to the power supply unit and applied 70V for the stacking gel. Once the samples crossed the stacking gel, the voltage was adjusted to 150V. Power supply was turned off once the samples reached the bottom of the resolving gel. After that, the gel plates were carefully removed from the electrophoretic apparatus. The gel plates were removed and was placed in a fixative solution and left for 30 minutes. After the fixation, the gel was placed in a petri dish containing staining solution for about 4 hours at room temperature. The petri dish was placed on an electronic rocker. Finally after the staining

process, the gel was placed in destaining solution and left overnight.

3. STATISTICAL ANALYSIS

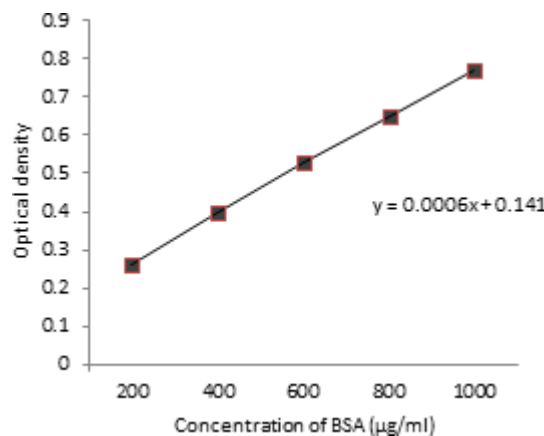
The data obtained were analyzed using Microsoft Excel 2010. The statistical data were presented as mean ± standard deviation (SD). Student’s (paired) “t” test was used for analysis of comparison. Probability value (P) of less than 0.05 was considered statistically significant.

4. RESULTS

The measured optical density values and concentration of the standard BSA are given in the following table (Table 1):

Concentration of BSA (µg/ml)	Optical density
200	0.26
400	0.40
600	0.53
800	0.65
1000	0.77

The above mentioned concentrations and optical density were plotted on x-axis and y-axis respectively to get the calibration curve and hence the $y = mx + c$ relation (Graph 1).



Graph 1: Relation between BSA concentration and optical density

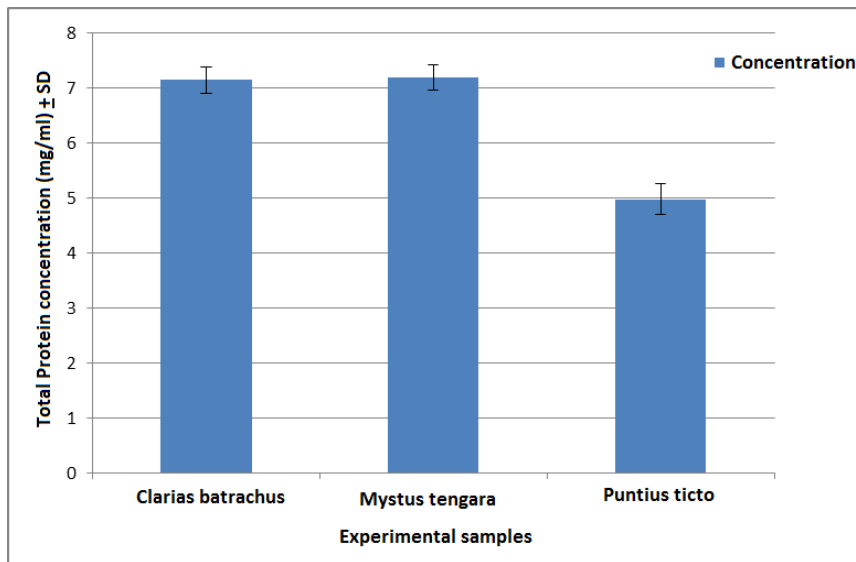
From the above graph (Graph 1), the relation between BSA protein concentration and respective optical density values were obtained by the relation, $y = 0.0006x + 0.141$.

Experimental samples	Optical density ± SD	X value obtained from equation $y=0.0006x+0.141$	Concentration (µg/ml)	Concentration (mg/ml) ± SD
<i>Clarias batrachus</i> (E ₁ F)	0.57 ± 0.014	715	7150	7.15 ± 0.247
<i>Mystus tengara</i> (E ₂ F)	0.58 ± 0.013	720	7200	7.20 ± 0.224
<i>Puntius ticto</i> (E ₃ F)	0.44 ± 0.016	498	4980	4.98 ± 0.283

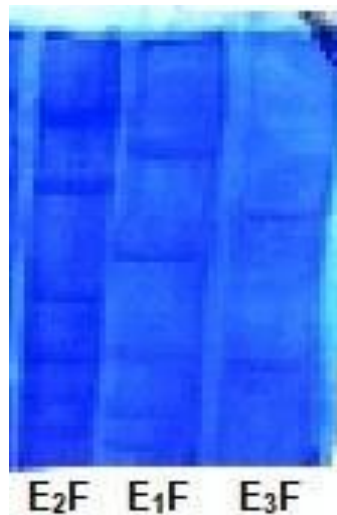
Values are mean ± SD; (n=10), P<0.05

The protein extracts prepared from the experimental fresh sample shows variation in terms of their total protein concentration. The total protein concentration of sample E₁F, E₂F and E₃F was found to be 7.15±0.247 mg/ml, 7.20±0.224 mg/ml and 4.98±0.283 mg/ml respectively. Thus, from the

study it was found that the muscle protein concentration was highest in *Mystus tengara* (E₂F), *Clarias batrachus* (E₁F) followed by *Puntius ticto* (E₃F) out of the three experimental fishes (Table 2 and Graph 2).



Graph 2: Graphical representation of total protein concentration (mg/ml) in three different fresh experimental samples



(E₁F= *Clarias batrachus*, E₂F=*Mystus tengara*, E₃F=*Puntius ticto*)

Fig 1. SDS-PAGE gel showing muscle protein bands of three different fish species (fresh condition)

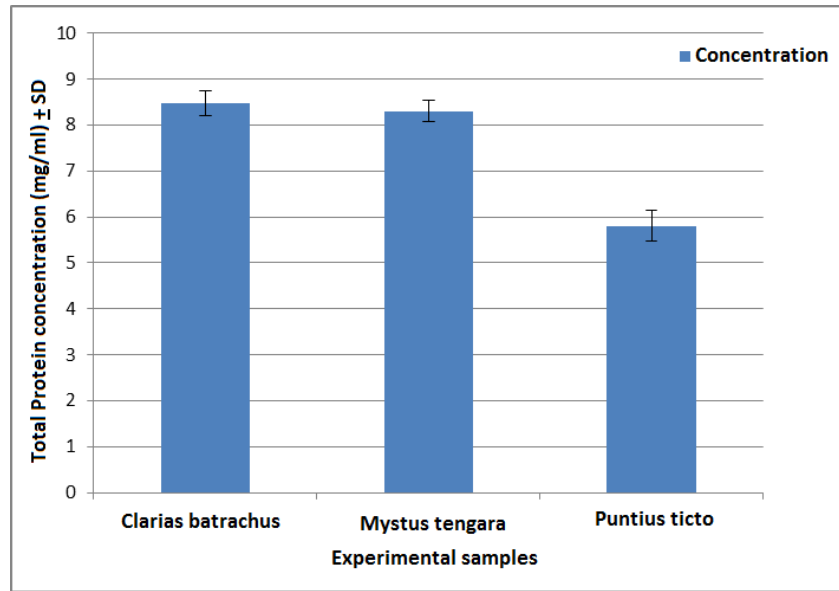
The SDS-PAGE electrophoresis is done in the case of three different freshly killed fish species viz. *Clarias batrachus* (E₁F), *Mystus tengara* (E₂F), and *Puntius ticto* (E₃F) shows the following electrophoretic banding patterns (Fig. 1).

Table 3. Optical density and protein concentration of the three experimental smoked dried samples				
Experimental samples	Optical density ± SD	X value obtained from equation $y=0.0006x+0.141$	Concentration (µg/ml)	Concentration (mg/ml) ± SD
<i>Clarias batrachus</i> (E ₁ D)	0.65 ± 0.016	848	8480	8.48 ± 0.273
<i>Mystus tengara</i> (E ₂ D)	0.64 ± 0.013	831	8310	8.31 ± 0.222
<i>Puntius ticto</i> (E ₃ D)	0.49 ± 0.02	581	5810	5.81 ± 0.335

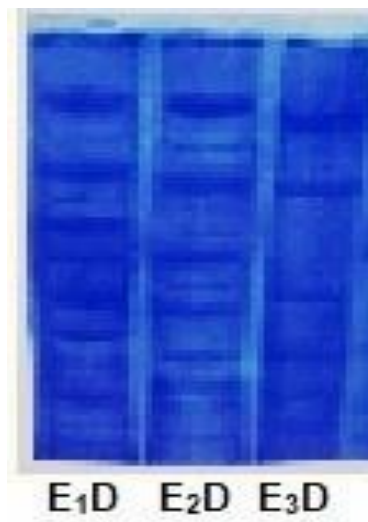
Values are mean ± SD; (n=10), P<0.05

The protein extracts prepared from the experimental smoked dried fish sample shows variation in terms of their total protein concentration. The total protein concentration of sample E₁D, E₂D and E₃D was found to be 8.48±0.273 mg/ml, 8.31±0.222 mg/ml and 5.81±0.335 mg/ml respectively.

Thus, from the study it was found that the muscle protein concentration was highest in *Clarias batrachus* (E₁D), *Mystus tengara* (E₂D) and lowest in *Puntius ticto* (E₃D) out of the three experimental smoked dried fishes (Table 3 and Graph 3).



Graph 3. Graphical representation of total protein concentration (mg/ml) in three different experimental smoked dried samples



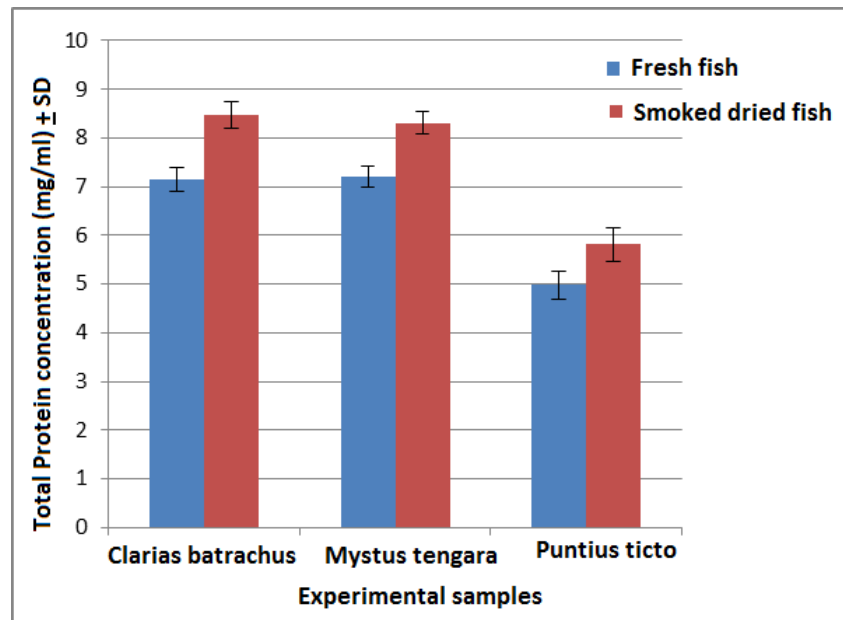
(E₁D= *Clarias batrachus*, E₂D=*Mystus tengara*, E₃D=*Puntius ticto*)

Fig 2. SDS-PAGE gel showing muscle protein bands of three different fish species (smoked-dried condition)

The SDS-PAGE electrophoresis is done in the case of three different smoked-dried fish species viz. *Clarias batrachus* (E₁D), *Mystus tengara* (E₂D), and *Puntius ticto* (E₃D) show the following electrophoretic banding patterns (Fig. 2). In Assam among the fresh water fish species, *Clarias batrachus*, *Mystus tengara* and *Puntius ticto* are very delicious, have high market price, nutritious and popular to consumers. Therefore, these

fishes are very important due to commercial purpose. Among the 3 species studied, protein content obtained varied from 4.98 mg/ml to 7.15 mg/ml in fresh fishes and 5.81 mg/ml to 8.48 mg/ml in smoked dried fishes which corroborates with the findings of Anon (1962).¹² Azam *et al.* (2003) also found similar results in 14 species of dried fishes.¹³

Genus studied	Local name	Protein concentration in Fresh Fish (mg/ml) ± SD	Protein concentration in Smoked dried fish (mg/ml) ± SD
<i>Clarias batrachus</i>	Magur	7.15 ± 0.247	8.48 ± 0.273
<i>Mystus tengara</i>	Tengara	7.20 ± 0.224	8.31 ± 0.222
<i>Puntius ticto</i>	Puthi	4.98 ± 0.283	5.81 ± 0.335



Graph 4. Graph showing total protein concentration (mg/ml) in three different fresh and smoked dried experimental samples

The result observed in protein content was high in smoked dried fishes as compared to fresh fishes (Table 4 and Graph 4). The increase of protein in dried fishes may be due to dehydration which causes aggregation of proteins as reported by Ninawe and Rathnakumar (2008).¹⁴ The increased protein content in all the dried fishes observed is high due to the loss of moisture during smoking. This was based on the report of Kumolu *et al.* (2010) which assisted that spoilage of fish resulting from action of enzymes and bacteria can be slowed down during smoking.¹⁵ Thus, smoke drying can be used as an effective method of fish processing which can be useful in the efficient management of fish resources without losing their protein content.

5. DISCUSSION

In the field of fish nutrition, the preliminary evaluation of muscle protein can be used by SDS-PAGE electrophoresis. The SDS-PAGE electrophoresis is used to visualize and to quantify the level of protein through banding patterns. In Assam among the fresh water fish species, *Clarias batrachus*, *Mystus tengara* and *Puntius ticto* are very delicious, have high market price, nutritious and popular to consumers. Therefore, these fishes are very important due to commercial purpose. Among the 3 species studied, protein content obtained varied from 4.98 mg/ml to 7.15 mg/ml in fresh fishes and 5.81 mg/ml to 8.48 mg/ml in smoked dried fishes which corroborates with the findings of Anon (1962).¹² Azam *et al.* (2003) also found similar results in 14 species of dried fishes.¹³ As per the results obtained from SDS-PAGE photograph on the selected fish species, i.e., *Clarias batrachus*, *Mystus tengara* and *Puntius ticto*, there is a significant difference in the bands of both fresh and smoked dried fish samples. Significant increase is seen in protein bands in three types of smoked dried fishes when compared to the fresh ones. The increase of protein in dried fishes may be due to dehydration which causes aggregation of proteins as reported by Ninawe and Rathnakumar (2008).¹⁴ The increased protein content in all the dried fishes observed is high due to the loss of moisture during smoking. This was based on the report of Kumolu *et al.* (2010) which assisted that spoilage of fish resulting from action of enzymes and

bacteria can be slowed down during smoking.¹⁵ Thus, smoke drying can be used as an effective method of fish processing which can be useful in the efficient management of fish resources without losing their protein content. From this, it is clear that higher the number of bands, higher is the protein content of fish which implies higher nutritive or food value of fish species. Therefore, the dried species of *Clarius batrachus* contains the highest amount of muscle protein. *Mystus tengara* stands next to *Clarius batrachus* in its total muscle protein concentration, while *Puntius ticto* is found to be lesser as compared to the above two species. However, the fresh sample presents a slight drop in the protein content in a similar way, presenting a higher amount of muscle protein in *Mystus tengara* than *Clarius batrachus* and finally *Puntius ticto*.

6. CONCLUSION

Fish and fishery products play a great role in the nutritional picture because they are rich sources of nutrients and provide a good balance of proteins, vitamins and minerals and a relatively low caloric content. The study was undertaken to analyze the various nutritional parameters of three selected fresh and smoked dried fishes. The percentage of protein content in selected smoked dried fishes was found more. Fish belonging to the group of catfishes/live fishes are found to contain more protein as compared to other groups. However, the results of the present investigation also state that the percentage of protein is quite satisfactory in the fish species, i.e., *Clarius batrachus*, *Mystus tengara* and *Puntius ticto*. From these results, it can be concluded that smoked dried fish provides satisfactory nutrition in the form of protein. Therefore, increasing knowledge of dried fish as a health food will hopefully open up markets, leading to increase in the development of traditional markets, which will help in building up for target marketing.

7. ACKNOWLEDGEMENTS

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8. AUTHORS CONTRIBUTION STATEMENT

Dr. Devajit Basumatari derived the concept with proper planning and execution and carried out the research study. Ms. Dipika Doloi evaluated the results and contributed to the writing and revising of the manuscript.

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9. CONFLICT OF INTEREST

Conflict of interest declared none.