

Estimation of Soluble Protein Content of Fish *Puntius sophore* (Hamilton) in Fresh, Refrigerated and Sundried condition

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Abstract

To estimate the soluble protein of fish *Puntius sophore* (Hamilton) which were collected from Sixmile fish market of the city of Guwahati. Samples of *P. sophore* in different conditions were taken i.e. Fresh, Refrigerated and Sundried conditions. The average body weight of *P. sophore* in Fresh, Refrigerated and Sundried conditions were 6.5 ± 0.52 g, 7.2 ± 0.67 g and 7.6 ± 0.95 g respectively. These results indicate that the average protein content of *P. sophore* in fresh condition was 1.67 ± 0.03 mg/g, in refrigerated conditions was 1.56 ± 0.03 mg/g and in dried condition was 3.6 ± 0.23 mg/g. Variation of protein content in different conditions were observed. It showed that the protein content between the fresh and refrigerated condition remain unchanged. But, significant alteration of maximum protein under sundried condition has been evident. Also, an inverse relationship could be drawn between the moisture and protein content in this investigation. Therefore, it could well be recommended that sun drying method is effective for the storage fish in terms of protein content. It could be planned a conservation plan of small indigenous fishes like *P. sophore* on the basis of their food value.

Keywords: Different conditions, Moisture, Protein, *Puntius sophore*

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I. INTRODUCTION

Puntius sophore(Hamilton in 1822) commonly known as Stigma barb is a tropical fresh water and brackish fish belonging to the *Puntius* genus in the family Cyprinidae. It is widely distributed throughout the Indian sub-continent including India, Bangladesh, Bhutan, Nepal, and Pakistan [19, 24, 32]. This fish is being reported from Afghanistan [23], China [32] and Myanmar [21]. It is native inland water inhabits like river, streams, ponds, beels, flood plains, boars, hoars in plains and sub-montane regions dominantly [19,25,31], who has been used in variety of traditional fishing gears [14]. Although, *P. sophore* is being declined rapidly due to heavy fishing and in recent studies from the Indian waters, it is categorized as lower risk near threatened in the Western Ghat [3]. This fish is a major source of animal protein and micronutrients in the diet of rural small-scale farmers [28]. It is an important small indigenous fish species and very much famous food fish item [25] and can also be used as an aquarium fish [8].

Fish is being a good source of protein, which then needed for the development of human body and availability of these vital sources of nutrients, depends to a large extent on the methods of storage [10, 6] such as drying and freezing. Fish are known to be a very healthy food item. They are an excellent protein source that is minerals and vitamins which is necessary for good health [4]. Fish is an important component of human diet. Fish serves as one of the most sources of protein to about one billion people in developing countries [17].

The main constituents of the fish body include water, lipid, ash and protein having the carbohydrates and non-protein compounds have been important constituents which are present in a small amount [7, 16, 30,]. Mostly, the fish consists of about 70-80% of water, 20-30% of protein and 2-12% of lipid [16]. Fishes are valuable sources of high grade protein and other organic products. Fishes are the primary sources of protein and are being valued in the diet because they provide a good quantity of protein, particularly sulfur containing amino acids [15].

Protein requirement of fish is higher in earlier stage but little attention has been paid in the different area of the country. Fish is a highly perishable commodity recording losses in quantity before consumption. So the method for storing fisheries product is a vital factor in the consumption of fish. We cannot expect the value of nutrition that we get in the fresh fishes due to the preservation technique, which can be done by icing, freezing, salting, roasting, drying, sun drying etc. [30].

Puntius sophore is one of the nutritionally superior small indigenous fish which have been reported earlier, the complete nutrient profile is not available; more over nutritional composition varies with such variables as the zoogeography, size, season, etc. [18]. *Puntius sophore* is the common indigenous fish is being considered for their nutritional value, affordable price and highly contributed to the local fishery for the present study on this species was selected.

II. MATERIALS AND METHODS

2.1 Sample Collection

The sample of fresh *Puntius sophore* was collected from Six Mile fish market of the city of Guwahati, Assam, India. The samples were collected freshly and transported to the laboratory of Zoology department of University of Science & Technology, Meghalaya for subsequent experiments. Collected *Puntius sophore* samples in a fresh condition having the mean weight was 6.50±0.52 g and the mean length was 8.08±0.09 cm. The collected samples of *Puntius sophore* kept in refrigerator having the mean weight was 7.2±0.67g and the mean length was 8.1±0.33cm and the samples kept for sun drying having the mean weight was 7.6±0.95g and the mean length was 6.6±0.57 cm.

2.2 Sample Preparation

To estimate the soluble protein in fresh, refrigerated and sundried conditions total five samples are kept in each condition.

2.3 Extraction of Protein

- Two grams of tissue is taken from
- the each sample.
- Homogenize the tissue with 8ml of chilled distilled water.
- Take 2ml of homogenate into centrifuge tube and centrifuge it at 5000 rpm for 5-7 minutes.
- The supernatant was discarded, add 2.5 ml of cold 30% TCA into the centrifuge tube and centrifuged it at 5000 rpm for 5-7 minutes.
- The supernatant was discarded, add 2.5 ml of cold 10% TCA into the centrifuge tube and centrifuged it at 5000 rpm for 5-7 minutes. Repeat this step again.
- Discard the supernatant, add 2.5ml cold 5% TCA to the precipitate, centrifuged it at 5000 rpm for 5-7 minutes.
- Now add 2.5 ml ethyl alcohol to the precipitate and centrifuge it at 5000 rpm for 5-7 minutes.
- Discard the supernatant, add 2.5 ml chloroform and centrifuge it at 5000 rpm for 5-7 minutes.
- Now add 3 ml diethyl ether to the centrifuge tube and centrifuged it at 5000 rpm for 5-7 minutes.
- The supernatant was discarded and the precipitate was allowed to the air dry to powder form at room temperature.
- To the powder precipitate add 3ml of 0.1N NaOH, stir and incubate at 37° C over night and dilute it in 50ml in a volumetric flask.

2.4. Estimation of protein

The protein content was determined by Lowry's method (Lowry et al., 1951).

- 0.1% standard BSA solution (0.1 g BSA in 100 ml of distilled water) is prepared.
- Seven test tubes were taken and marked them as S₁, S₂, S₃, S₄, S₅, B and U.
- From standard BSA solution 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml solution was taken out and added to the S₁, S₂, S₃, S₄ and S₅ test tubes respectively and make each test tube having 1 ml volume by adding 0.2ml, 0.4ml, 0.6ml, and 0.8ml of distilled water respectively.
- 1ml of distilled water was taken in test tube B which would work as blank.
- In test tube U, 0.4 ml of extracted protein sample was taken and 0.6ml of distilled water was added to make the volume 1ml.
- 4.5ml of Reagent-I (48 ml of Solution A having 2% of Na₂CO₃ in 0.1N of NaOH + 1 ml of Solution B having 1% of Sodium Potassium Tartrate in distilled water + 1 ml of Solution C having 0.5% CuSO₄.5H₂O) was added to each of the test tube and incubated for 10 minutes at room temperature.
- 0.5ml of Reagent-II (Folin-Ciocalteu's Phenol Reagent and Distilled water added in equal proportion i.e. 1:1 ratio) was added to each of the test tube and incubated for 30 minutes at room temperature.
- Optical density of each test tube was taken at 630 nm and plot the standard curve.

- The amount of protein content of the sample was estimated using the following formula-

$$\text{Protein Content} = \text{Optical density of unknown Sample} / \text{Optical Density of known Sample} \times 100$$

2.5. Estimation of moisture content

The moisture content for refrigerated and sundried fish samples are estimated using the formula below-

$$\text{Moisture Content} = \text{Initial weight of the sample} - \text{Final weight of sample}$$

III. RESULT AND DISCUSSION

The protein content of the fish *Puntius sophore* as in fresh, preserved and dry matter condition are being presented in Table 1, 2 and 3 respectively.

Table 1:Protein content of *Puntius sophore* at Fresh condition

Sample No.	Protein content (mg/g body weight) ± S.D
1	1.69 ± 0.03
2	1.67 ± 0.03
3	1.63 ± 0.03
4	1.69 ± 0.03
5	1.65 ± 0.03
Mean ± S.D	1.67 ± 0.03

Table 2:Protein content of *Puntius sophore* at Freezing condition

Sample No.	Moisture Content (g) ± S.D	Protein content (mg/g body weight) ± S.D
1	1.29 ± 0.29	1.56 ± 0.03
2	0.8 ± 0.29	1.54 ± 0.03
3	0.50 ± 0.29	1.56 ± 0.03
4	1.2 ± 0.29	1.6 ± 0.03
5	0.9 ± 0.29	1.54 ± 0.03
Mean ± S.D	0.92 ± 0.29	1.56 ± 0.03

Table 3:Protein content of *Puntius sophore* at Sundried condition

Sample No.	Moisture Content (g) ± S.D	Protein content (mg/g body weight) ± S.D
1	2.8 ± 0.46	3.4 ± 0.23
2	2.0 ± 0.46	3.8 ± 0.23
3	1.8 ± 0.46	3.5 ± 0.23
4	2.8 ± 0.46	3.4 ± 0.23
5	2.3 ± 0.46	3.9 ± 0.23
Mean ± S.D	2.34 ± 0.46	3.6 ± 0.23

The average protein content of the fish *Puntius sophore* was found in fresh, refrigerated and sundried condition are 1.67 ± 0.03 , 1.56 ± 0.03 and 3.6 ± 0.23 respectively. It was observed that the proportions of the components of muscle tissues varied in different condition. The variation of the protein fraction may be due to the moisture content in the *Puntius sophore*. The highest protein content was found in sundried condition as because the moisture content was lesser. The amount of protein content found in the fresh and preserved condition is very less in comparison to sundried condition. The protein content was more in fresh than the preserved as the moisture content is high.

In preserved condition the time interval was about 3 days. It was found that after interval of 3 days of freezing, protein content was reduced from $1.67 \pm 0.03 \text{ mg/g}$ to $1.56 \pm 0.03 \text{ mg/g}$. There are other findings in the same relation. The protein content was 18.95% when the freezing time was eight days in the experiment of the Nurullah et al., [20]. On another experiment, protein content was 17.89% in the freezing time of five days interval in the experiment of the Siddique et al., [30] and the present study was conducted at every three days interval. So the present study supports the finding of others. There might be changes due to the time interval. Deviation may occur due to the food habits, during spawning period as they kept fast at that time and migration etc [35]. Denaturation of fish protein that associates with frozen or preserved fish which is connected to the deterioration of protein [27]. The amount of protein content is high at dried condition of *Puntius sophore* was $3.610.23 \text{ mg/g}$ due to the loss of the amount of water that is the moisture content of the fish. This result indicates that except moisture all other components are higher in dry matter basis than moisture basis [29].

Moisture content varies from species to species and temperature to temperature and at different time duration of its preservation [30]. Siddique et al., [30] found that the average moisture content of *Puntius sophore* was 75.40% and after 5 days interval of freezing it was 78.125 in freezing condition. Khan et al., [13] reported the moisture varies because of species variation, size, processing method, temperature, season etc. There is an inverse relation between fat and moisture content has an inverse relation with size of the fish [11]. However, the present experiment has not been sufficiently equipped with various variable and less sample size. Therefore, further comprehensive analyses are necessary for drawn of logical conclusion.

IV. CONCLUSION

The present findings are very much suggestive of that the protein content between the fresh and refrigerated condition remain unchanged. But, significant alteration of maximum protein under sundried condition has been evident. Also, an inverse relationship could be drawn between the moisture and protein content in this investigation. Therefore, it could well be recommended that sun drying method is effective for the storage fish in terms of protein content.

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