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Proximate biochemical composition and nutritional status of *Puntius sophore* (Hamilton 1822) in fresh and ice preserved condition of the river Bhima of Pune district (M.S.) India

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Abstract: **The present study was carried out to determine proximate biochemical composition of *Puntius sophore*, such as moisture, ash, protein, lipid, carbohydrate and pH in fresh fish samples were collected from the river Bhima of Pune district (M.S.) India. The changes in proximate biochemical compositions in ice preserved condition of the fishes for 7 days and 14 days period were also investigated for to know the freshness and nutrional state of fish. The fish comprised of** 71.62 ± 0.04 % **moisture,** 3.99 ± 0.07 **% ash** 16.85 ± 1.13 **% protein,** 5.62 ± 2.02 **% lipid,** 1.92 ± 0.12 **% carbohydrate and** 6.24 ±0.05 pH **in fresh condition. The amount of protein and lipid content reduced while amount of moisture, ash, carbohydrate and pH increased in 7 days and 14 days ice preserved condition.**

***Keywords*: *Puntius sophore*, Protein, Lipid, River Bhima, ice preservation.**

1. INTRODUCION

The nutritional quality of fish is of paramount importance. Today, there is an ever increasing awareness about health foods and fish is finding acceptance for its special nutritional qualities. Proximate composition of fish varied widely from species to species and even within the same species from one individual to another. This individual variation is normally due to some factors such as size, age, season, sex and geographical location [1]. Knowledge of the biochemical composition of different fish species finds application in several areas like processing and preservation of fish, which will help a processing technologist to define the optimum processing and storage condition as well as to develop fish and fishery products [2].

A study on the quality characteristics of these fish will help to understand the nutritive, biochemical quality and consumable quality of these fishes. The assessment of the proximate composition of the fish is not only important to know its nutritive value, but also for its better processing and preservation [3].

Fishes are good source of protein and lipid, which are essential for development of human body. Fish is highly perishable. So, a processing or storage method of fish is vital factor in fish consumption. Common methods used for fish preservation are icing, freezing, salting, roasting sun drying, frying etc. in icing, fishes are preserved in ice, at this low temperature the microbial and biochemical activities decreases. So, we cannot expect the nutritional quality as we get in fresh condition. The nutritional status changes with preservation period and various preservation methods [4].

To evaluate nutritional and freshness quality, it is essential to gather information on biochemical composition. There are some information on biochemical and nutritional studies on some freshwater fish species [5], [6]. In addition, considerable studies have been made on seasonal variation in the chemical composition of *Puntius gonionotus* [7].

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Bhigwan is one of the major fish landing centers located on river Bhima of Pune district of Maharashtra. It is significant to study the nutritive value of *Puntius sophore* because it is preferred by local people either in fresh or ice preserved from. The present investigation was carried out in order to evaluate the proximate biochemical composition and nutritional quality of commonly used small fresh water fish species *Puntius sophore* in fresh and ice preserved condition for 7 days and 14 days period.

2. MATERIALS AND METHODS

Fish samples collection:

Small fresh water fish species *Puntius sophore* (Hamilton, 1822) used for the present study were collected in fresh condition in polythene bags from river Bhima at Bhigwan Tq; Indapur of Pune district (M.S.) India, during the month of July to December 2017 and transported in an insulated box to the laboratory. In the laboratory fishes were killed and used for sample preparation.

Sample Preparation:

In the laboratory, fishes were washed thoroughly with running tap water and adherent water was removed with blotting paper. The average length and weight of individual fish were recorded using electric balance which are represented in Table 1 and stored in insulated boxes in alternating layer of ice-fish-ice. The boxes were kept in ambient temperature (28.5 – 32^0C). Sampling for biochemical analysis was done every seven days during period of ice preservation. During sampling fish were collected from different locations of box and fishes were descaled with sharp blade. The dorso-lateral portion muscle was collected and bones, entrails and gills were removed from fish muscle to make a representative sample for further biochemical analysis.

TABLE 1: The average length and weight of *Puntius sophore*. (Mean± SE)

Fish	Length (cm)	Weight (gm)
Puntius sophore	8.64 ± 0. 52	9.84 ± 1. 48

For analysis the proximate composition of fresh fish was done before icing and each experiment was conducted with three replicates.

Estimation of Moisture:

The moisture content of fish tissue was determined in oven-dry method [8]. The initial weight of the samples were taken and dried in an oven at about 105°C for about 8 to 10 hours until a constant weight was reached and cooled in desiccators and weighed again. The difference in weight was taken as moisture content. The percentage of moisture content was calculated by the following equation

$$\text{Percentage of Moisture (\%)} = \frac{\text{Wet weight of Tissue} - \text{Dry weight of Tissue}}{\text{Wet weight of tissue}} \times 100$$

Estimation of ash content:

Porcelain crucibles were heated at 500 ^{o}C in Muffle Furnace and weighed. Total 5 gm of dry sample was each taken from each fish in the crucibles and allowed to vaporize at 500-600 ^{o}C temperature until getting grey to white residue. Crucibles were kept in to the desiccators to cool and removed and then kept at room temperature for few minutes. The process of heating, desiccating and weighing was repeated till obtaining the constant weight. Final weights of the crucible were taken and deducted from the previous weight. The ash content was expressed on the dry weight basis by using formula:

$$\text{Percentage ash content (\%)} = \frac{\text{Mass of Ash content}}{\text{Mass of weight sample}} \times 100$$

Estimation of Protein content:

The total protein content was estimated using Lowry method [9]. 10 mg of sample, 1ml NaOH was added for protein extraction in water bath for 30 minutes. Thereafter, it was cooled at room temperature and neutralized with 1 ml HCL.

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The extracted sample was centrifuged at 2000 rpm for 10 minutes and 1 ml of the sample was further diluted with distilled water (1/9 v/v). From the diluted sample, 1 ml was taken and treated with 2.5 ml of mixed reagent (carbonatetartrate-copper) and 0.5 ml of Folin's reagent. After 30 minutes, sample absorbency was read at 750 nm using spectrophotometer.

Estimation of Lipid content:

Lipid content was estimated by the method of Folch [10]. 10 mg of dried sample was homogenized in 10 ml of chloroform-methanol mixture (2/1 v/v). The homogenate was centrifuged at 2,000 rpm. The supernatant was washed in 0.9 % KCl solution to remove the non-lipid contaminants and allowed to separate. The upper phase was discarded and. the lower phase was allowed to dry in an oven. Then the weight was calculated and recorded. The lipid content was expressed as percentage by the following formula:

$$\text{Lipid \%} = \frac{\text{Weight of the lipid}}{\text{Wet weight of tissue}} \times 100$$

Estimation of Carbohydrate content:

Total carbohydrate content was estimated by the phenol sulphuric acid method [11]. 10 mg. of dried tissue sample was treated with 2 ml of 80 % sulphuric acid and was allowed to digest for about 20 - 21 hours at room temperature. Two ml of 5 % phenol reagent followed by 5 ml of concentrated sulphuric acid were added to the digested sample and was allowed to cool. Absorbency was measured at 490 nm. D glucose was used as the standard carbohydrate. The percentage of carbohydrates was calculated as follows

$$\text{Carbohydrate \%} = \frac{\text{Standard value x Optical density}}{\text{Weight of the tissue taken}} \times 100$$

Estimation of pH:

1 gm of fish sample was minced with 10 ml of distilled water and pH was measured by using digital pH meter [12].

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) available in MS-Excel 2007 to evaluate the effect of ice preservation on muscles during storage (0, 7 and 14) days. Means were compared using Duncan's test[13]. All data were expressed as means standard error (Mean ± SE) and the significance level was set at $P<0.05$.

3. RESULTS AND DISCUSSION

Fish is an important part of a healthy diet because they are considered to be an excellent source of high value protein and essential nutrients. The Proximate biochemical compositions of *Puntius sophore* in fresh condition and ice preserved condition at 7 Days and 14 Days are shown in (Table 2) and (Fig. 1).

TABLE 2: The Proximate biochemical compositions of *Puntius sophore* in fresh condition and ice preserved condition at 7 Days and 14 Days. (Mean± SE)

Condition Duration	Proximate Biochemical Composition					
	Moisture (%)	**Ash (%)**	**Protein (%)**	**lipid (%)**	**Carbohydrate (%)**	**pH**
Fresh 0 day	71.62^{a}±0.04	3.99^{a} ±0.07	16.85^{a} ± 1.13	5.62^{a} ± 2.02	1.92^{a} ± 0.12	6.24^{a}±0.05
ice preserved 7 days	73.33^{a}±0.14	4.59^{a} ± 0.13	14.92^{a}± 1.07	4.71^{a}± 1.10	2.24^{a} ± 0.17	6.48^{a} ±0.02
14 days	77.42^{b} ±0.37	4.92^{a} ± 0.17	12.28^{b}± 0.63	3.29^{b}± 1.02	2. 32^{a}± 0.31	6.68^{b} ±0.04

(Means with different superscripts in the same column are significantly different at $P<0.05$)

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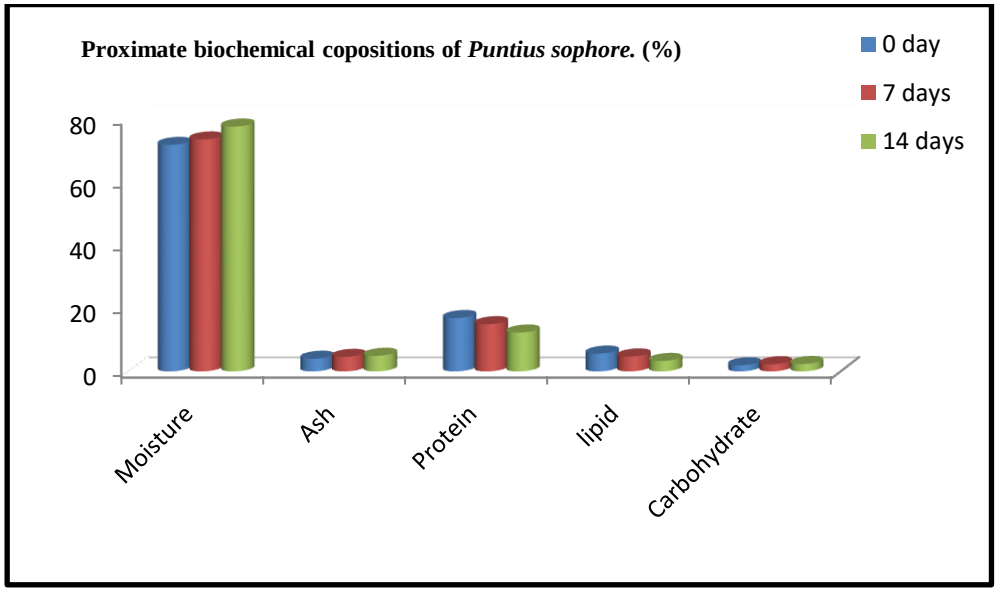

Fig 1: Showing the proximate biochemical compositions of *Puntius sophore* of 0, 7 and 14 days ice preservation.

Moisture content:

During the present course of investigation, in fresh condition the moisture content of the *Puntius sophore* species recorded was 71.62 ± 0.04 %. After 7days and 14 days of ice preserved condition, the amount of moisture recorded was 73.33 ± 0.14 % and 77.42 ± 0.37 % respectively. After 7 days and 14 days of ice preserved condition, the amount of moisture slightly increased significantly ($P<0.05$). Present study has affinities with the findings of [14].They found the moisture content 72.55 ± 1.79 % in *Puntius sophore* in fresh condition and 75.16 % in 10 day's ice preserved condition. On contrary to the results of present study, [4] have found that an increasing trend in moisture content in *Puntius sp*.

[15] have reported that after twenty days of freezing , the amount of moisture for *Mystus tengara, Mystus cavasius, Mystus gulio* increased from 76.12% to78.02%, 75.35% to 77.25%and 76.03% to 78.23% respectively. [16] have reported a similar trend during iced storage of cultured *Labeo rohita*. The higher moisture content of the ice stored fish was probably associated with the uptake of water during ice storage [17].

But present study has considerably differed from the result found by [14]. This deviation may occur due to variation in size, processing method, temperature, season etc [18]. Moreover there is an inverse relation between size and moisture content of the fish [6].

Ash content:

In the present study, the ash content recorded was 3.99 ± 0.07% in the fresh samples while increased to 4.59 ± 0.13 % and 4.92 ± 0.17% in ice preserved fish samples after 7 days and 14 days respectively. The ash content has shown slight increase of no significant importance ($P>0.05$). These results are in agreement with [14]. They observed that the ash content 5.72 ± 0.25% in fresh fish *Puntius sophore* , While [19], [20] both of them observed that the ash content decreased with storage time of *Mystus seenghala* and of Tilapia at the end of the eight weeks of freezing. Ash in fish muscle contains nutritionally important minerals. The increase in ash content was affected with mineral percentage in fish muscle, physiological parameters and nutrition

Protein content:

The protein content of the present study fish species *Puntius sophore* recorded that 16.85 ± 1.13 % in fresh condition. After 7 days and 14 days of ice preserved condition, the amount of protein content recorded was 14.92 ± 1.07% and 12.28 ± 0.63% respectively. In ice preserved condition protein content reduced significantly. According to statistical findings, there were significant differences ($p<0.05$) between the fresh fish and ice preserved fish samples after 7and 14 days of preservation period.

Present study has resemblance with the findings of [21] who reported that the protein content 17.86 ± 0.54% in *Puntius sophore* in 0 day and 15.88± 0.28% in 30 days freezing duration. The present results are similar with [22]. They recorded

that average protein content of Nuna *tengra* fish was around 16.81% in its fresh condition and after eight days of its frozen condition it was 14.21%, which indicates slight deterioration of protein level in frozen condition. The alteration of protein was probably linked with the increase of water holding capacity of the fish muscle during ice storage [17]. The reduction in protein level is connected with denaturation of fish protein that is associated with freezing [23].

Lipid content:

The lipid content of the present study fish species *Puntius sophore* found that 5.62 ± 2.02 % in fresh condition. . But in ice preserved condition lipid content reduced slowly. After 7 days and 14 days of ice preserved condition, the amount of lipid content recorded was 4.71 ± 1.10% and 3.29 ± 1.02% respectively. There was a significant difference ($p<0.05$) in lipid content between fresh and ice preserved fish that was preserved for 7 and 14 days. Present study has affinities with the findings of[14]. They found the lipid content 4.49 ± 0.34% in *Puntius sophore* in fresh condition and 4.78% in 10^{th} day's ice preserved condition.[22], have reported that the average lipid content of *Nuna tengra i*s about 6.28% in fresh state and after eight days in frozen condition it was found to be around 5.09%. Similarly, the decreasing lipid trend has been reported by [24] in *Wallago attu*. [25] have observed that there is an inverse relation between lipid content of fish and its freezing time. A gradual decreasing trend was observed in lipid content of the fish during ice preservation duration. This could be a result of the presence of prooxidant enzymes, lipoxygenases, peroxidases and chemical pro-oxidant molecules namely, hemoproteins and metal ions [26].

Carbohydrate content:

In the present study, the carbohydrate content was found to be 1.92 ± 0.12 % in fresh samples and increased to the value 2.24 ± 0.17 % and 2.32 ± 0.31 % in 7 and 14 days of ice preservation. There was comparatively slow increase in carbohydrate between fresh and during ice preserved samples. There was no significant difference ($p>0.05$) in carbohydrate content between fresh and ice preserved fish that was preserved for 7 and 14 days.

pH value:

In the present study, the pH was found to be 6.24 ±0.05 in fresh samples and increased to the value 6.48 ±0.02 and 6.68 ±0.04 in 7 and 14 days of ice preservation. There was comparatively slow increase in pH between fresh and during ice preserved samples. There was significant differences ($p<0.05$) between fresh fish and ice preserved fish that was preserved for 7 and 14 days. In present study, increase in pH during iced period was associated with the state of rapid spoilage of the fish. [27] Have showed that the increase in pH was higher in the 4 °C stored sample of Tilapia, indicating that biochemical and microbial changes are occurring faster in 4 °C stored fish. [28] Have reported that slightly increased pH in *Catla catla* from 6.50 to 6.79 when stored at chilled temperature (-2 to -4 °C). The change in pH of fish muscle is usually good index for quality assessment. The increase in pH is caused by the enzymatic degradation of fish muscle.

A classification of the fish depending on the basis of protein and oil content According to [1] (table 3). Based on to the present results, the classification of analyzed fish species under study *Puntius sophore* can be included under the category B, the oil content recorded was 5. 62 % and the maximum protein content recorded was 16.85 %. From the present findings, it could be concluded that *Puntius sophore* can be referred to as medium oil- high protein, bony fish. The fish is nutritionally good for consummation in fresh condition.

TABLE 3: Classification of the analyzed fish under study depending on the basis of protein and oil content (According to Stansby, 1962)

Category	Type	Oil Content (%)	Protein Content (%)
A	Low oil- high protein	Less than 5	15-20
B	Medium oil- high protein	5-15	15-20
C	High oil – low protein	More than 15	Less than 15
D	Low oil- very high protein	Less than 5	More than 20
E	Low oil- low protein	Less than 5	Less than 5

4. CONCLUSION

The results of this study showed that the better quality of fish found in fresh and after 7 days and 14 days of ice preservation. The icing of fresh fish leads to decrease in protein %, lipid %, and increase in moisture % ash % and pH value compared with fresh fish sample. The increases of pH value for ice preserved fish samples make more exposure for the decomposition. Recommendation is eating fresh fish which is most benefit for human health.

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