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Comparative Evaluation of Change in Organoleptic, Proximate and Vitamin Content of Freshwater Fish Species, *Heteropneustes fossilis* at Various Duration of Low Temperature Preservation

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ABSTRACT

The present study was conducted to evaluate the effect of low temperature preservation on the sensory characteristics, proximate and vitamin content of freshwater fish species, *Heteropneustes fossilis.* Samples prepared from the fish were kept in low temperature for different duration and nutritional parameters were analyzed. The values of proximate composition of the sample showed the highest content for the fresh sample and the values tend to decreased with increasing storage duration except for carbohydrate. Among all the proximate parameters, moisture, protein, lipid and ash showed a content decreased during storage. However, carbohydrate showed the opposite trend, increased with storage duration. Again, among all the fat soluble vitamins, sample showed the highest value for vitamin D and lowest for vitamin E. Vitamins also showed the declining trend with increasing storage duration. From the present results, it can be stated that fresh fish sample had the highest nutrient content, therefore they should be preferably consumed over preserved fishes or if preserved, it should be for a shorter duration of time so that its nutritional content retains the same.

Keywords *Heteropneustes fossilis,* Low temperature, Nutrition, Preservation.

INTRODUCTION

Fish is a healthy food and is a major player in human nutrition, ensuring about 20% of protein intake to a third of the world's population which is more evident in developing countries (Bene *et al.* 2007). Proteins have multiple functions in the body, including growth and maintenance of tissues. The biochemical and mineral composition of the whole body of the fish indicates its quality. Therefore, the assessment of the fish's proximate composition is important to know its nutritive value, and its better processing and preservation (Mridha *et al.* 2005). The predominant parts of fish are additionally divided into four categories, namely, protein, carbohydrate, lipid, and moisture. The chemical composition is historically used as an indicator of the dietary value, in addition, due to the fact of the physiological circumstance of fish and its habitat (Aberoumad and Pourshafi 2010).

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Fish proximate composition is of great interest in aquaculture because it affects fish appetite, growth and the efficiency of food utilization. It also affects other aspects of fish biology and ecology, including reproduction, survival, and energy value to predator (Breck 2014). However, other than proximate parameters, micro nutrients like vitamins and minerals are also present in the fish muscle. The micro nutrients fulfil the hidden hunger of human population and prevent many disorders due to deficiency of such micro nutrients (Mohanty *et al.* 2016). Fish provide a good source of readily digested high quality animal protein, fat, mineral and vitamins specially vitamin A, D and E. Fat-soluble vitamins act as essential nutrients in important biological processes in the human body. Vitamin A, also called retinol, which controls photoreception and regulates gene expression. Vitamin D_3 (cholecalciferol) promotes and enhances the absorption and metabolism of calcium and phosphorus in our body. α-Tocopherol is the vitamin E compound with the highest biological activity, which acts as an antioxidant, protecting membrane structures, essential fatty acids, and vitamin A from oxidation (Sau *et al.* 2004 and Paul *et al.* 2005).

Asian stinging catfish, is a freshwater fish of Heteropneustidae family of Siluridae order and found in Southeast Asian countries including Bangladesh, India Pakistan, Thailand, and Sri Lanka. It is a high valued and very popular fish in Bangladesh due to be considered as a highly palatable, nourishing, and tasty (Kohinoor *et al.* 2012). It is well preferred by consumers because of its less fat, less spine, and high digestibility (Khan *et al.* 2003). The species is not only recognized for its delicious taste and market value but also highly esteemed from nutritional and medicinal properties of view (Chakraborty and Nur 2012). The fish was previously captured from natural water sources, but due to overfishing, the natural supply ceased and become insufficient to meet consumer need. The principal components of the fish muscle include water, protein and fat while the minor components include carbohydrates, minerals and vitamins, and extractives, such as, sugars, free amino acids and nitrogenous bases (FAO 2010).

One of the major problem faced by the fishery industries is spoilage of fish after harvesting. In the tropical countries such as India, hot climate favors the rapid growth of bacteria which leads to spoilage of fishes and deterioration of its quality which in turn also decrease the capital gain (Whittle 1997). So, it requires a proper preservation method to maintain shelf-life of the fish during storage, transportation and marketing to ensure safe food to the consumers (Salma *et al.* 2021). Therefore, shelf life extension can be achieved by various preservation methods, viz. salting, brining, smoking, icing, glazing, refrigeration and freezing. Since, in our country, fish in a fresh state is not always available due to seasonal fishing and the far location of major fishing grounds from cities and consuming centers, the freezing of fish becomes an update method of long term preservation (Roopma *et al.* 2013). However, the conditions of raw material before processing are a very important factor for the quality and shelf life of the final product (Arason *et al.* 2014). If the fish is not fresh or is of low quality before processing, the final product quality is compromised.

Traditionally, refrigeration and freezing are the most popular cold treatments, used to maintain tissue quality and considered as very useful food preservation processes (Hematyar *et al*. 2017). It can preserve the freshness of food for a short period. However, the proliferation of microorganisms, as well as the generation of enzymatic activity, will not be stopped. Generally, the decomposition process is slowed down at low temperatures (Ashie *et al.* 2009). Some of the deterioration still occurs in the stored food, during which the freezing rate and temperature fluctuation are affecting the extent of quality loss (Pourshamsian *et al.* 2012). Although many damaging processes are inhibited by such low temperature storage methods, but the undesirable reactions associated with lipids and proteins are shown to occur, leading to the detrimental changes in nutritional and sensory properties. Some disadvantages of frozen storage include freezer burn, product dehydration, rancidity and drip loss and this deterioration increases as duration of storage increases. The measurement of the proximate profiles is often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Watermann 2000). Therefore, the present study was conducted to compared the proximate and mineral contents of the fish species in fresh and various duration of low temperature preservation.

MATERIALS AND METHODS

Sample collection

Asian stinging catfish, *Heteropneustes fossilis* of approximately same length and weight were collected from Uzan bazar fish market, Guwahati, Assam, India and brought to the laboratory in live condition. Sample was prepared as per the procedure of Salma *et al.* (2021). A large sized plastic drum with water was used for transporting 50 fishes in laboratory within few minutes of harvest. Ten number of fishes were randomly separated from them to analyze the proximate compositions in fresh condition. Other fishes were then grouped into four categories for storage where each group having 10 fishes.

Sample processing and preservation

The samples of different groups were then washed separately with tap water before rinsing with distilled water, then cut into slices. The slices were separately packed, labeled and stored in the refrigerator for 24, 48, 72 and 96 hours respectively at 4°C±1 for further nutritional analysis (Lin *et al.* 2020 and Samim *et al.* 2019).

Organoleptic evaluation

Sensory characteristics i.e. appearance, color, odor and overall acceptability were evaluated by a trained panel of 5 members using 9-point hedonic scale according to standard procedure (Peryam and Pilgrim 1957) as Like extremely (9), Like very much (8), Like moderately (7), Like slightly (6), Neither like nor dislike (5), Dislike slightly (4), Dislike moderately (3), Dislike very much (2), Dislike extremely (1). The limit of acceptability was 4 for all the samples. High score indicated good quality and vice versa (Chudasama *et al.* 2018).

Proximate analysis

The various groups of preserved fish samples were examined for their moisture, protein, lipid, ash, and carbohydrate content following the protocol given by AOAC (2016). The methods used for analyzing proximate composition was as follows:

Moisture content

Sample of about 20-30 gram of fresh fish was taken and weighed and then dried at 100-105°C for 24 hrs to remove the moisture and weighed again. Then moisture content was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Wet sample weight (g)} - \text{Dry sample weight (g)}}{\text{Wet sample weight (g)}} \times 100$$

Protein content

A Kjeldahl flask was taken with two grams (2.0 g) of the sample with 10.5 g of the digestion mixture (catalyst) and 25 ml of concentrated sulfuric acid (H_2SO_4). The samples were then heated for two to three hours at 80 to 100°C for digestion. The flask had a clear solution of the sample which is bluish green color. After cooling the clear solution at room temperature distilled water was added to make it up to 250 ml. Approximately, 10 ml of the digested sample was taken for distillation. Subsequently, 10 ml of 40% sodium hydroxide solution (NaOH) were added to the distillation flask and allowed to distill for a short time. A conical flask was used filled with 10 ml of boric acid, which served as an indicator and absorbed the ammonia that was distilled out. N/100 hydrochloric acid was used to titrate the obtained distillates. The pale pinkish color served as an indicator of the finish line. Total crude protein was calculated by using the following formula:

$$\text{N (\%)} = \frac{0.14 \times (\text{Titration final} - \text{blank}) \text{ reading} \times \text{Strength of HCl (1.01)}}{\text{Weight of sample (g)}} \times 100$$

The crude protein was calculated by multiplying 6.25 with the nitrogen percent.

$$\text{Crude protein (\%)} = \text{N (\%)} \times 6.25$$

Lipid content

A dry sample weighing around 4–5 grams was taken in an extraction thimble. The thimble was then inserted into the Soxhlet apparatus's hollow chambers. The

extractor was attached to an oil flask after it had been weighed. Then, ether was poured to the extractor and allowed for extraction for 8-10 hrs. After that, the flask only had crude fat which was removed along with the thimble when the extraction process was completed, and it was allowed to dry for an hour at 100°C and weighed. Total crude lipid was calculated by using the following formula (Mishra 2021).

$$\text{Fat (\%)} = \frac{\text{Weight of lipid (beaker containing lipid – empty beaker) in (g)}}{\text{Weight of sample in (g)}} \times 100$$

Ash content

An empty, pre-weighed silica crucible containing around 2 g of moisture-free sample was placed in a muffle furnace and heated at 550 and 600°C for six hours, or until the sample turned totally white or gray. After turning off the furnace, it was allowed to cool down, the sample was removed and weighed again. The ash content was calculated by using following formula:

$$\text{\% Ash} = \frac{\text{(Weight of crucible plus sample after ashing – Empty weight of crucible)}}{\text{Weight of the sample before adding}} \times 100$$

Carbohydrate content

Carbohydrate content of the sample was obtained by using the method described by Ihekoronye and Ngoddy (1985). This was done by subtraction of the sum of moisture, ash, protein and fat from the total weight of 100.

Carbohydrate (%) = 100 – (% moisture + % protein + % fat + % ash)

Vitamin content

Vitamins were measured using HPLC in accordance with the AOAC (2016) standard. Vitamins A, D, E, and K that are fat-soluble which were tested using High Performance Liquid Chromatography. After the addition of BHA as an antioxidant, approximately 25–30 g of fish muscle was ground with anhydrous sodium sulfate, and the oil was extracted using a 2:1 chloroform: Methanol ratio (Folch *et al.* 1957). Sample preparation was carried out following the method given by Sankar *et al.* (2010).

Statistical analysis

Datas were expressed as Mean ± SD and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test was performed to find out significant differences between the results obtained. The statistical analysis was performed using SPSS software.

RESULTS AND DISCUSSION

Organoleptic evaluation

The scores for sensory characteristics were gradually decreased (not significant) with increasing duration of low temperature storage and the overall quality of the fish was also comprised in sensory acceptability by the consumer. In the present study, sensory attributes of the fresh and stored samples were studied (Table1). The results indicated that cold stored fish remain good in quality scores at the end of 4th days of storage without any significant changes.

Table 1. Sensory analysis of fish (*Heteropneustes fossilis*) muscle at various duration of low temperature storage (4° ± 1°C) (Mean ± SD).

Sample	Duration in hours	Sensory quality attributes					
		Appearance	Flavor	Odor	Juiciness	Texture	Overall acceptability
Heteropneustes fossilis	0	9±0.00	9±0.21	7±0.42	8.5±0.23	8±0.12	8.3±0.19
	24	9±0.56	9±0.34	7±0.00	8.5±0.5	8±0.34	8.3±0.34
	48	8.5±0.45	8.5±0.00	6.5±0.34	8.5±0.23	8±0.00	8±0.20
	72	8.5±0.32	8±0.11	6.5±0.16	8.5±0.65	7.5±0.12	7.8±0.27
	96	8.5±0.53	7.5±0.73	6.5±0.42	7.5±0.32	7.5±0.57	7.5±0.51

*Values were expressed as Mean ± SD.

Table 2. Proximate composition (expressed in %) of fish (*Heteropneustes fossilis*) muscle at various duration of low temperature storage (4°±1°C).

Parameters	0 hr	24 hrs	48 hrs	72 hrs	96 hrs
Moisture	76.31±0.54[a]	76.14±0.21[a]	76.02±0.34[a]	75.68±0.65[a]	75.42±0.11[b]
Crude protein	17.31±0.26[a]	17.04±0.08[a]	17.01±0.73[a]	17.12±0.46[a]	16.76±0.02[b]
Crude fat	2.78±0.24[a]	2.76±0.43[a]	2.54±0.57[a]	2.56±0.5[a]	2.37±0.67[a]
Ash	2.15±0.32[a]	2.13±0.45[a]	2.24±0.51[a]	2.11±0.42[a]	2.03±0.67[a]
Carbohydrate	1.45±0.13[a]	1.93±0.34[a]	2.19±0.51[a]	2.53±0.21[b]	3.42±0.23[c]

*Values were expressed as Mean ±SD, Mean values with different superscript in a particular row differ significantly.

Results for proximate composition

Moisture content

The moisture content was found to be 76.31±0.54 in fresh sample of *Heteropneustes fossilis* which significantly reduced to 76.14±0.21, 76.02±0.34, 75.68±0.65 and 75.42±0.11 after 24, 48, 72 and 96 hrs of low temperature preservation respectively. The values showed no significant difference upto 48 hrs of storage after that values started to decrease significantly (Table 2, Fig. 1). Similar results for moisture were also reported by other workers where they stated that the moisture content of fresh sample of the fish was 77.30%, which was decreased to 75.57% when stored in ice and stated that the moisture content was slightly decreased in ice storage which may be due to evaporation of moisture from fish surface in ice storage because of various factors like relative humidity, chemical changes, and storage temperature (Salma *et al.* 2021). Again, Gandotra *et al.* (2012) reported the moisture content in Labeo rohita stored at low temperature for 21 days and stated that the moisture content was 84.74±0.1% on the first day of storage and the value reduced to 80.84±0.09% on the last day (21) of storage at -12± 20ºC. Some also reported a higher moisture content in muscle of *Heteropneustes fossilis* and stated the content as 82.90% in the fresh fish sample (Lin *et al.* 2020).

Protein content

The fresh fish sample had a good amount of protein which was 17.31±0.26 and the value showed the same trend as moisture i.e. the protein content gradually reduces to 16.76±0.02 at the end of 96 hrs, which is statistically significant. The protein content found in others storage hours showed no significant difference with other groups (Table 2, Fig. 2). Similar work was also done by some other researchers and stated that the protein content is fresh fish was 15.04% which reduced to 15.34% in ice stored sample and the protein content in ice stored fish did not varied significantly (Salma *et al*. 2021). In support to the present findings, Gandotra *et al*. (2012) reported that protein content of *L. rohita* was 15.93% in fresh fish

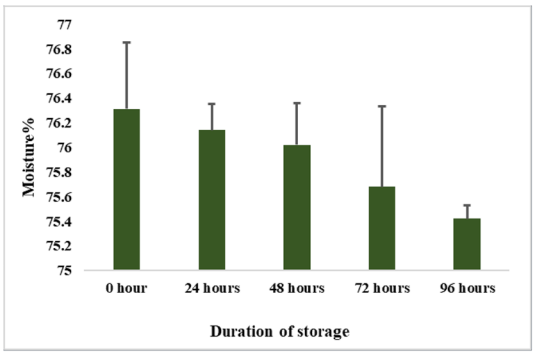

Fig. 1. Moisture content of the fish (*Heteropneustes fossilis*) muscle at different duration of low temperature storage.

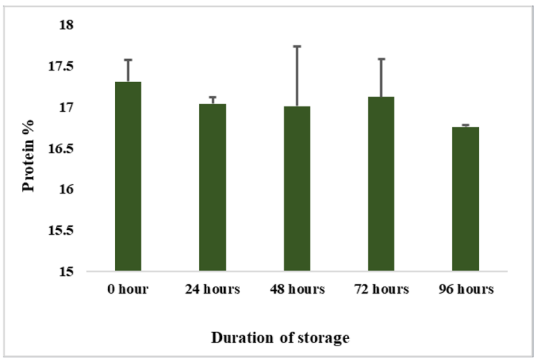

Fig. 2. Protein content of the fish (*Heteropneustes fossilis*) muscle at different duration of low temperature storage.

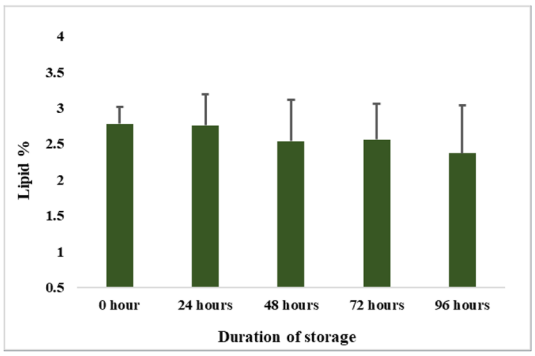

Fig. 3. Lipid content of the fish (*Heteropneustes fossilis*) muscle at different duration of low temperature storage.

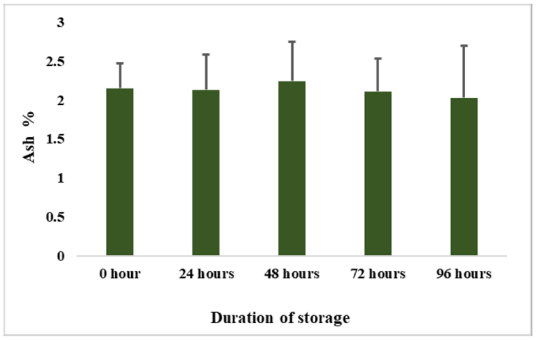

Fig. 4. Ash content of the fish (*Heteropneustes fossilis*) muscle at different duration of low temperature storage.

sample of *Labeo rohita* which get reduced to 13.06% for fish samples stored in cold storage at for 21st day at -12°C. Again Lin *et al.* (2020) reported 14.59% of protein content in the fish sample which was low as found in present study.

Lipid content

The lipid content was found to be 2.78±0.24 in fresh sample which again slightly changed to 2.76±0.43, 2.54±0.57, 2.56±0.5 and 2.37±0.67 after 24, 48, 72 and 96 hrs of low temperature storage respectively. All the changes found in different duration was statistically not significant (Table 2, Fig. 3). In similar to the current results trend, Gandotra *et al.* (2012) also reported that lipid content in *L. rohita* decreased during cold storage. However, others reported that the lipid content of *Heteropneustes fossilis* as 6.10% in fresh fish sample which is higher than the result found in present report and reported the change of lipid content to 6.30% which was similar to fresh because the lipid oxidation at cold condition was very slow. The reduction of lipid content in storage is attributed due to oxidation and formation of undesired and obnoxious chemical compounds (Salma *et al.* 2021). Some also reported the similar trend in other fish species. The lipid content found in fresh T*ilapia guineensis* was 14.50% which decreased to 8.50% in cold stored fish after four weeks (Obemeata and Christopher 2012). Again Lin *et al.* (2020) reported a very less amount of fat content in the fish sample which was 0.24%.

Ash content

The ash content was found to be 2.15±0.32 in the fresh fish sample which non significantly reduced in its content in different duration of storage. The ash content reduced to 2.03±0.67 after 96 hrs of storage (Table 2, Fig. 4). Similar results was reported by many other workers where they reported the changes of ash content during cold storage. The ash content in fresh fish muscle of *Labeo rohita* was found as 1.79±0.01 which declined to 1.36±0.03 on 21 days of storage at -12±2^{0}C (Gandotra *et al.* 2012). Again Lin *et al.* (2020) also reported 1.13% of ash content in fresh fish sample.

Carbohydrate content

The carbohydrate content was found to be 1.45±0.13 in fresh fish muscle which gradually changed with increasing storage duration and became 1.93±0.34, 2.19±0.51, 2.53±0.21 and 3.42±0.23 after 24, 48, 72 and 96 hrs of low temperature storage respectively. The values showed no significant difference upto 48 hrs of storage after that changes became significant when compared to sample of other groups (Table 2, Fig. 5). The increasing trend of carbohydrate was also reported by Sadhu *et al.* (2020) in *Labeo rohita* and stated that the fresh sample had 0.28± 0.003 which increased to 0.41±0.004 in storage at –20ºC for 30 days. Lin *et al.* (2020) reported 1.15% of carbohydrate content which was similar to our present study.

Results for vitamin content

Results showed that, among all the fat soluble vita-

Table 3. Vitamin content (expressed in mg/100 g) of fish (*Heteropneustes fossilis*) muscle at various duration of low temperature storage (4°±1°C).

	Storage duration				
Vitamins	0 hr	24 hrs	48 hrs	72 hrs	96 hrs
A	58.32 ±0.41[a]	58.26±0.5[a]	56.84±0.27[b]	54.80±0.21[c]	54.50±0.41[c]
D	163.42±0.5[a]	164.20±0.22[a]	163.96±0.26[a]	158.80±0.36[b]	158.36±0.05[b]
E	0.44±0.03[a]	0.41±0.01[a]	0.19±0.04[b]	BDL	BDL
K	12.38±0.04[a]	12.41±0.02[a]	12.26±0.12[a]	7.04±0.51[b]	6.29±0.07b

*Values were expressed as Mean ±SD, Mean values with different superscripts in a particular row differ significantly.

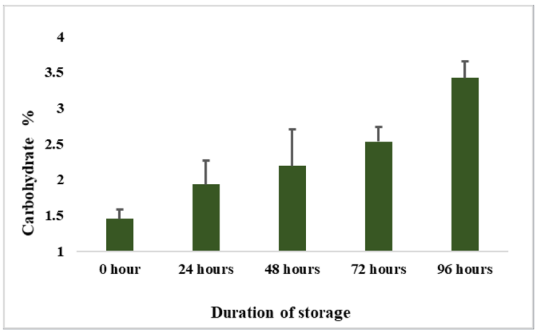

Fig. 5. Carbohydrate content of the fish (*Heteropneustes fossilis)* muscle at different duration of low temperature storage.

mins, vitamin D was present in highest concentration followed by vitamin A, K and vitamin E, which was lowest among all in the fresh fish sample. Vitamin A and E showed significant changes from 48 hrs of storage while the other two i.e. vitamin D and K showed changes from 72 hrs of storage duration. Vitamin A

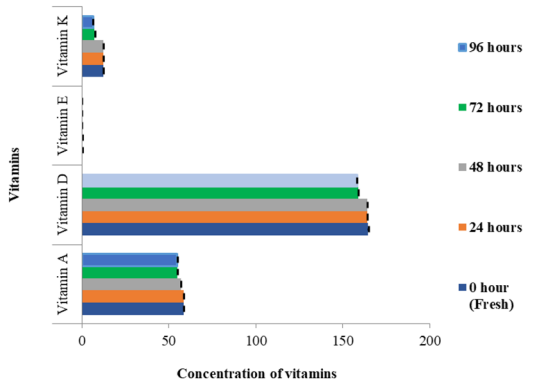

Fig. 6. Vitamin content (expressed in mg/100 g) of the fish (*Heteropneustes fossilis*) muscle at different duration of low temperature storage.

was found to be 58.32 ±0.41 in fresh sample which significantly decreased to 56.84±0.27, 54.80±0.21 and 54.50±0.41 in 48, 72 and 96 hrs of storage respectively. Vitamin D also showed the same trend with 163.42±0.5 in fresh while significant changes was observed as 158.80±0.36 and 158.36±0.05 in 72 and 96 hrs of storage respectively (Table 3, Fig. 6).

Vitamin E content was the lowest which was 0.44±0.03 in fresh sample which significantly changed to 0.19±0.04 in 48 hrs of storage. However, after 48 hrs of storage duration, vitamin E content was found to be below detectable level. Again, vitamin K content was present as 12.38±0.04 in fresh sample which gradually decreased to 12.41±0.02, 12.26±0.12, 7.04±0.51 and 6.29±0.07 after 24, 48, 72 and 96 hrs of storage respectively. However, significant changes was observed from 72 hrs before that values showed no significant difference (Table 3, Fig. 6). Similar results were reported by some other workers in other fish groups where they reported that fresh sample of *Labeo rohita* contain 4.22±0.47, 36.08±2.06, 0.54±0.02 and 0.41±0.03 I U/100 g fillet of Vitamin A, Vitamin D, Vitamin E and Vitamin K respectively (Paul *et al.* 2016).

CONCLUSION

Fish plays a very significant role in fulfilling the nutrient demand of poorer sections of people of the country. The study was carried out to estimate the changes of nutrition of fish muscle that occurs during low temperature storage and also to find out the appropriate storage duration for preservation of fish. The results of sensory evaluation upto 96 hrs of storage showed no significant changes in the fish samples. However, analysis of other nutritional parameters like

proximate composition and vitamin showed significant differences in their content in different storage duration. In proximate composition, the values found in fresh sample and samples stored upto 48 hrs show very less difference in composition but after that, considerable changes were observed in different parameters of proximate composition. Vitamin also showed the same trend i.e. with storage duration it starts to decrease in its content, which varied for different vitamins. The results of the present study tells that we can store the fish sample in low temperature but just for a limited duration of time with keeping its quality unimpaired. Therefore, such information can help to preserve the quality especially during post- harvest processing and storage of fish.

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