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Comparative Evaluation of Shelf Life and Quality of Freshwater Fish Species, *Wallago attu* at Different Duration of Low Temperature Preservation

Najma Khatun [a++] and Devajit Basumatari [a#*]

[a] Department of Zoology, Cotton University, Guwahati-781001, Assam, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author NK designed the study, performed the experiments, analyzed the results and wrote the final draft of the manuscript. Author DB conceptualized, supervised and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to evaluate the shelf life and quality of the fish at different duration of low temperature preservation. The fish was subjected to sensory evaluation to assess the shelf life and later biochemical and microbial analysis was carried out at an interval of 24 hours upto 96 hours of

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[++] *Research Scholar;*
[#] *Associate Professor;*
**Corresponding author: Email: devajitbasumatari@cottonuniversity.ac.in;*

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storage duration. Results of sensory evaluation showed that the fish had an acceptable value of 8.56±0.23 in fresh condition, which slightly changes to 8.5±0.00, 8.1±0.34, 7.8±0.45 and 7.4±0.23 in 24, 48, 72 and 96 hours of storage respectively. Total volatile basic nitrogen (TVBN) analysis showed an increase level from 4.32±0.05 in fresh to 4.34±0.02 with increase in storage duration upto 96 hours without any significant change. The vitamin analysis showed a decreasing trend for storage samples. Among all the fat soluble vitamins, fresh sample showed the highest value for vitamin A which is 62.41±0.5 and lowest value 1.16±0.06 was found for vitamin E. Vitamins showed significant decrease in content during storage. The microbial analysis for Total Plate Count (TPC) increased gradually from 2.38±0.22 in fresh to 2.90±0.51 in 96 hours of storage duration without showing any significant difference. However both *Escherichia coli* (EC) and Coliform Count (CC) were found absent in both fresh and storage samples. The results of the study showed that, the sensory characteristics, TVBN and microbial count of the fish remains significantly unchanged upto 96 hours of storage. However, results of vitamins showed that Vitamin A and D significantly changed at the end of storage duration while vitamin E and K remain significantly unchanged during the overall storage period. Thus, the biochemical and microbial analysis stated that the preserved fish samples are healthy for human consumption but upto a specific storage period. Overall, it is well recommended to consume fish in fresh condition and for stored fish, not more than 96 hours in the refrigerator in order to preserve the better quality and more benefits for human health.

Keywords: Biochemical; fish; microbial content; shelf life; TVBN; vitamins.

1. INTRODUCTION

"Fish provides a good source of readily digested high quality animal protein, fat, mineral and vitamins specially vitamin A, D and E" [1]. "It is available in the tropics and has been widely accepted as a good source of other elements needed for the maintenance of a healthy body" [2]. "Micronutrients like vitamins and minerals are also present in the fish muscle which fulfil the hidden hunger of human population and prevent many disorders which occurs due to their deficiency" [3]. "So, biochemical evaluation is necessary to ensure the nutritional value as well as eating quality fish" [4]. "Although the fish muscle have great nutritional value so the consumption can be increased by improving the palatability through flavor, smell, color, appearance, juiciness and tenderness" [5].

"Sensory assessment of fish has always played a key role in quality and freshness evaluation in fish industry" [6]. "Fish consumers expect a product that is safe and has good appearance, odor, taste and texture and their decision to purchase a fish product is based first on appearance, followed by flavor and then texture" [7]. "Although fish is highly nutritious, it is one of the rapidly perishable food because of its high moisture content. The high ambient temperature of our country favours the rapid microbial growth, hence there is a need to properly handle and preserve the fish, so as to increase its shelf life which can be achieved by various preservation methods, e.g., salting, brining, smoking, icing, glazing, refrigeration and freezing" [8].

"Among various preservation techniques, freezing is one kind where fish would preserve for longer period of time" [9]. "Frozen storage is an important method for processing of fish. However, when fish are stored in frozen state, they necessarily lose quality mainly due to changes in muscle integrity, proteins and lipids. These mean that fish, if necessary, should be stored for a short period of time to retain the taste and provide both the protein and fat at optimal level. Traditionally, refrigeration and freezing are the most popular cold treatments, used to maintain tissue quality and considered as very useful food preservation processes" [10].

"The refrigeration process 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 temperature" [11]. "Stored fish in the fridge is likely to undergo several processes such as decomposition, proliferation of microorganisms and redox generations" [12]. "It is important to analyze the quality of fish muscle that are less frequently analysed prior to their processing and storage. Such information can help to preserve the quality especially during

post- harvest processing and storage of fish which otherwise could affect the level of moisture, protein and fat contents" [13].

"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 updated method of long term preservation. Refrigeration and freezing provide low temperature thus, inactivating the microbial growth and there by reducing enzymatic and chemical deterioration. Some disadvantages of frozen storage include freezer burn, product dehydration, rancidity and drip loss and this deterioration increases as duration of storage increases" [8]. "Total volatile basic nitrogen (TVBN) is an important characteristic for the assessment of quality in food products and appears as the most common chemical indicators of fish spoilage" [14, 15]. "It is a group of biogenic amines formed in non-fermented food products during storage" [16]. "The combined total amount of ammonia (NH3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile basic nitrogen (TVBN) content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for the freshness of fish" [15].

"Wallago attu (Bloch and Schneider, 1801) is a commercially important fish having high protein contents and taste. It is one of the large freshwater catfish found in India, Sri Lanka, Pakistan, Nepal, Bangladesh, Burma, Thailand, Vietnam, Kampuchea, Malay Peninsula, Afghanistan, Sumatra and Java" [17,18]. "Irrespective of human preferences, however, the fish is an extremely perishable food item, as it begins to spoils soon after death" [19]. The rapid growth [20] and high nutritional quality of its flesh [21] encourage "investigation into the aquaculture potential of this excellent food fish. Hence, Fish deterioration or spoilage is one of the greatest problems affecting the fishing industry. Reductions in the nutritional values of fish caused by the processing and preservation methods have long been of interest and concern". Despite the fact that several studies have been carried out on the effect of storage conditions on the nutritional quality of fish, [22] no data have been reported yet on the effect of refrigerated storage duration on the nutritional characteristics. Therefore, the present study was conducted to evaluate the effect of low temperature preservation on the shelf life and quality of the fish species.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fish samples were purchased from the Uzan bazar fish market of Guwahati, Assam, India, in the early hours of the day (Fig. 1). The weight and length for the fish samples ranges from 1000-3000 gram and 30-60 cm respectively. The samples were freshly collected by the fisherman from the Brahmaputra Riverine system, Uzan bazar ghat, Guwahati (Fig. 2). A large sized container with water was used for carrying the fishes (sample size=50) to the laboratory within a short period of time. Fishes were divided randomly into 5 groups. One group containing ten numbers of fish were separated to analyze them in fresh condition. Other fishes were then grouped into four categories for storage in low temperature with each group having 10 number of fishes.

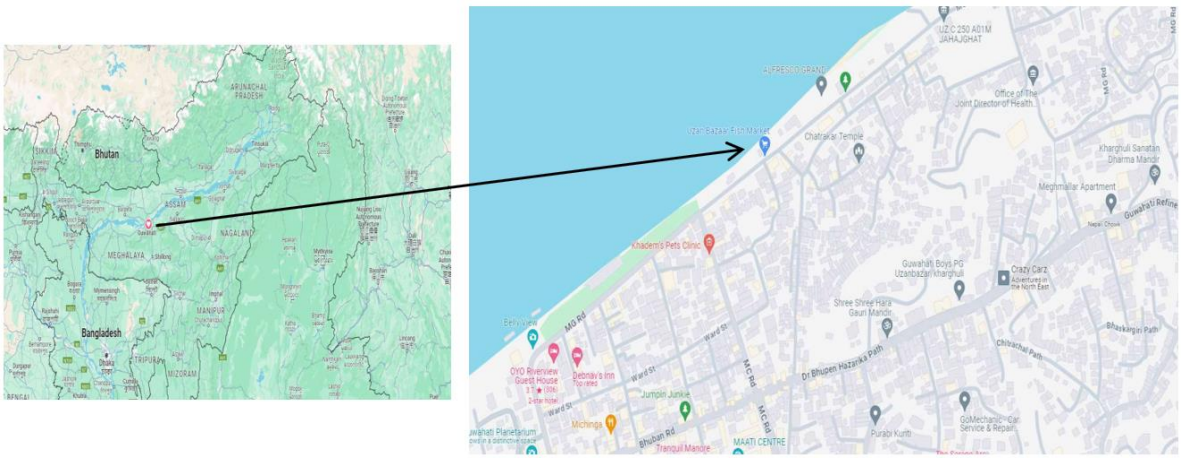

Fig. 1. Map showing the Uzan bazar fish market, Guwahati, Assam, India

Fig. 2. Collected fish species (*Wallago attu*)

2.2 Sample Preparation and Preservation

After collection, fish samples were clean with tap water, fins were removed and eviscerated and fish muscle was collected for sample preparation from the fish dorsal region. The fish samples were divided into groups: one group was fresh which was not subjected for preservation and other groups are preserved samples which were kept in low temperature (4℃±1) for preservation at different duration of time (24, 48, 72 and 96 hours). After that, the fresh sample was analyzed for biochemical and microbial content. Again, the same way the preserved samples were analyzed at an 24 hours interval upto 96 hours of storage period.

2.3 Sensory Analysis

Sensory characteristics i.e. appearance, color, odor and overall acceptability were evaluated by a panel of 20 members using 9-point hedonic scale according to standard procedure [23] 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). After that, the panel of evaluators were given another set of questionnaires for preference test, and asked them to give points in numbers to determine the acceptability of sensory characteristics in fresh and storage duration [24]. The limit of acceptability was 4 for all the samples. High score indicated good quality and vice versa [25].

2.4 Total Volatile Basic Nitrogen (TVBN) Analysis

TVBN was determined according to the standard procedure [26] by using Conway micro diffusion unit. For preparation of extract, approximately 2 gm of sample was taken with 4% TCA in a 50 ml beaker and were homogenized. The mixture was then left for 30 minutes and filtered. The filtrate was stored for analysis. Further, three Conway units were taken and the edge of the outer ring of each unit was sealed using a sealing agent. Now, 1 ml of boric acid solution was added to the inner ring of each unit using micropipette and 1 ml of the sample extract was added into the outer ring with 1 ml of K_2CO_3 solution and closed with a clip. The solutions in the units were then mixed gently, to prevent any solution mixing of both the ring. Now, the units were placed in an incubator at 37°C for 60 mins and the covers of the units were removed and the inner ring solution (a green color) was titrated with 0.02 N HCl using a burette until the green color solution turned to pink. An average titrate volume of HCl was found from the results of three titrations for each sample. For each sample, the TVBN values were calculated.

TVBN (mg/100gm) =

$$\frac{(\mathrm{Vs}-\mathrm{Vb})\times 0.14\times \text{volume of extract}\times 100}{\text{Volume of sample taken}\ \times \text{weight of sample}}$$

Where, Vs = Titrate value of 0.01 N NaOH for the sample (ml)
Vb = Titrate value of 0.01 N NaOH for the blank (ml)

2.5 Vitamin Analysis

Vitamins were examined using the AOAC described methodology. High Performance Liquid Chromatography was used to assay the fat-soluble vitamins, Retinol (Vitamin A), Cholecalciferol (Vitamin D), Tocopherol (Vitamin E), and Phylloquinone (Vitamin K). Approximately 30g of fish tissue was ground with anhydrous sodium sulfate and the oil was extracted using a 2:1 chloroform:methanol ratio following the addition of BHA as an antioxidant

[27]. The procedure suggested by Sankar *et al.* (2010) was followed for sample preparation [28] and High Performance Liquid Chromatography was used for vitamin analysis [29].

2.6 Microbiological Analysis

The microbiological profile was determined according to standard method [30]. Ten gm of sample were mixed with 90 ml of 0.9% sodium chloride solution in a sterile manner, and the mixture was then used for the total count of bacteria in a particular culture medium. 1 ml of each dilution was then cultured in the plate count agar (PCA) medium using the pour plate method. The cultured samples were then incubated for 48 hours at 37° C in order to determine the total count of bacterial load at 7°C and for 7 days to identify specific bacteria. Following the incubation period, the colony was counted, and the total count was determined using cfu/g [31].

2.7 Statistical Analysis

Mean and standard deviations were calculated for all the parameters and significant differences between the samples were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. The data analyses were performed using SPSS software (IBM SPSS Statistics 20.0).

3. RESULTS AND DISCUSSION

3.1 Sensory Characteristics Evaluation

The results of sensory characteristics showed that the fresh fish sample had the highest acceptability score which was 8.56±0.23 in terms of all the sensory characters. The score gradually tends to declined with increase in storage duration i.e 8.5±0.00, 8.1±0.34, 7.8±0.45 and 7.4±0.23 in 24, 48, 72 and 96 hours of cold temperature preservation respectively (Table 1, Fig. 3). The values showed no significant difference upto 96 hours of storage ($p>0.05$).

However, the changes that observed were not significantly different among the preserved samples. The results indicated that cold preserved fish samples tend to remain same in quality scores at the end of 96 hours of storage.

3.2 TVBN content

In the study, it was found that the fresh fish sample (*Wallago attu*) had TVBN content as 4.32 ± 0.05 mg/100 gm. The value slightly changes to 4.32 ± 0.02, 4.33 ± 0.04, 4.33 ± 0.02 and 4.34 ± 0.02 in 24, 48, 72 and 96 hours of cold storage respectively. The TVBN content of the fish muscle stored in cold temperature upto 96 hours was increased from 4.32 ± 0.05 to 4.34 ± 0.02 mg/100 gm (Table 2, Fig. 4). However, no significant changes had observed between fresh and stored fish samples.

If the TVBN value reaches 30 mg/100g most authorities would consider the fish to be stale, whilst at 40 mg N/100 g the fish is regarded as unfit for consumption. The level of TVBN for white fish is generally considered to be fresh if the TVBN is less than 20 mg/100 gm sample according to the Codex Alimentarius Committee proposed in 1968. Fish and fish products is unfit for human consumption when exceeding the value (TVBN) 30 mg /100g of meat [32]. Again, chemical evaluation revealed acceptable results with significant differences in examined fish, total volatile basic nitrogen (TVN) was 12.23 for Bizz to 16.41mg N/100gm for Silver carp [33]. These results were higher as compared to the values found in the present study. This may be due to various factors such as handling and storage at inadequate temperature and light; as some shops are exposed to sun light [34].

Table 1. Sensory analysis of the fish (*Wallago attu*) at fresh and low temperature preservation (4°±1°C). Datas are presented as Mean ± SD

Duration of storage (in hours)	Sensory quality attributes					Overall Accept-ability
	Appearance	Flavour	Odor	Juiciness	Texture	
0	9±0.00	8.8±0.34	7.5±0.45	9±0.11	8.5±0.21	8.56±0.23
24	9±0.11	8.5±0.23	7.5±0.54	9±0.00	8.5±0.22	8.5±0.00
48	8.5±0.12	8.5±0.52	7±0.21	8.5±0.13	8±0.71	8.1±0.34
72	8±0.8	8±0.33	7±0.21	8.5±0.25	7.5±0.12	7.8±0.45
96	7±0.00	7.5±0.21	7±0.23	8±0.21	7.5±0.45	7.4±0.23

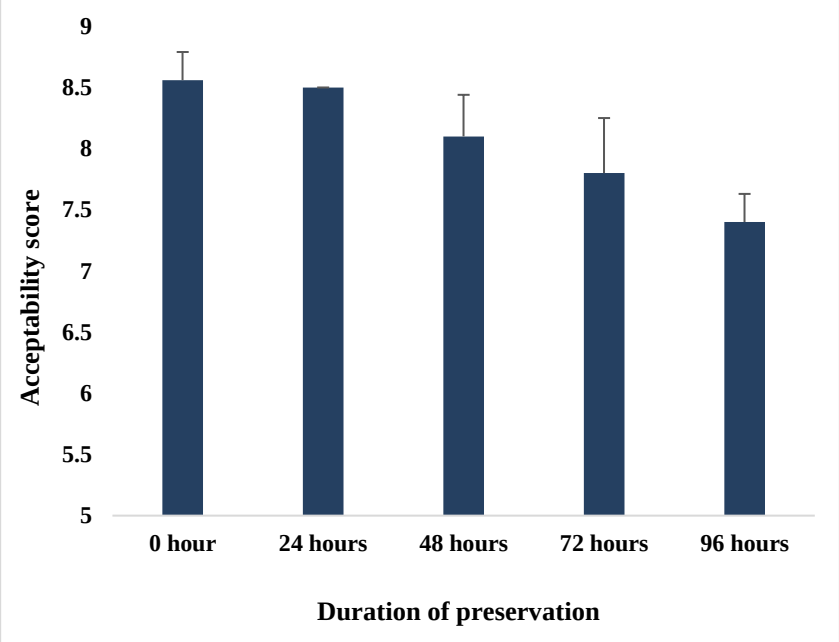

Fig. 3. Sensory analysis of the fish species at various storage duration

Table 2. TVBN content (mg/100gm) in the fish species at fresh and low temperature preservation. Datas are presented as Mean ± SD

Duration of storage	Concentration of TVBN
0 hour	4.32 ± 0.05
24 hours	4.32 ± 0.02
48 hours	4.33 ± 0.04
72 hours	4.33 ± 0.02
96 hours	4.34 ± 0.02

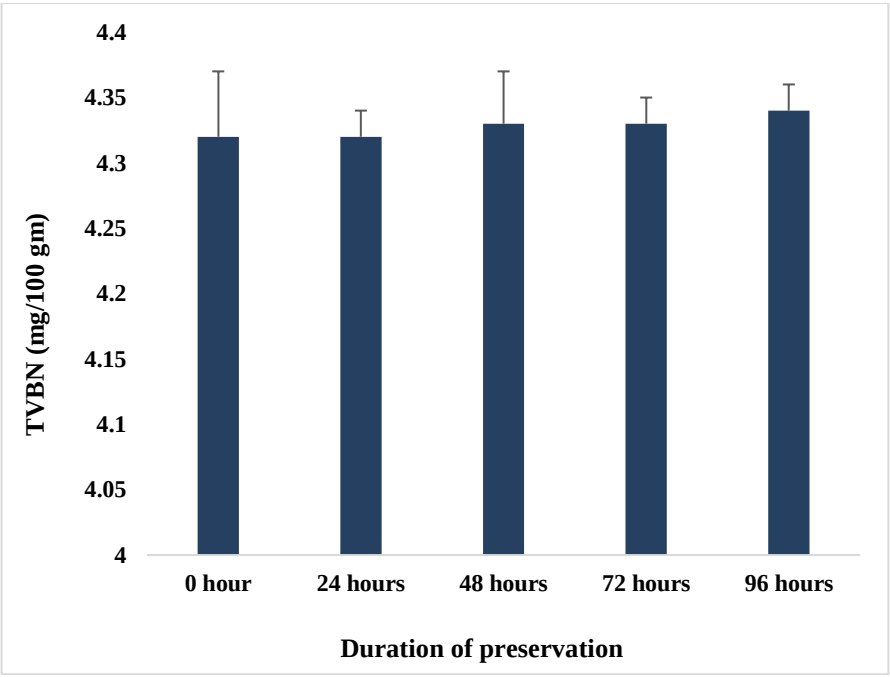

Fig. 4. TVB-N Content in the fish species at various storage duration

3.3 Vitamin Content

Results showed that, among all the fat soluble vitamins, the fish contain the highest concentration of vitamin A which is followed by others such as vitamin K, D and vitamin E. Vitamin A plays a vital role for normal vision and bone growth; its derivative retinoic acid regulates gene expression in the development of epithelial tissue [35]. In the study, Vitamin A was found to be 62.41± 0.5 in the fresh fish sample which changes to 62.06± 0.17, 61.6± 0.24, 61.39± 0.45 and 60.77± 0.85 after 24, 48, 72 and 96 hours of cold storage respectively. The values showed no significant difference till 72 hours. However, significant changes were observed in 96 hours of storage only (Table 3, Fig. 5).

The vitamin D activate the innate immune system whereas dampen the adaptive immune system [36]; in addition to its role in bone development. Again, the Vitamin D concentration was found to be 3.27± 0.23 in fresh while significant changes was observed as 2.24± 0.27 in 72 hours of storage (Table 3, Fig. 5). The values of Vitamin D showed no significant difference upto 48 hours. However, notable change was observed at 72 hours of storage.

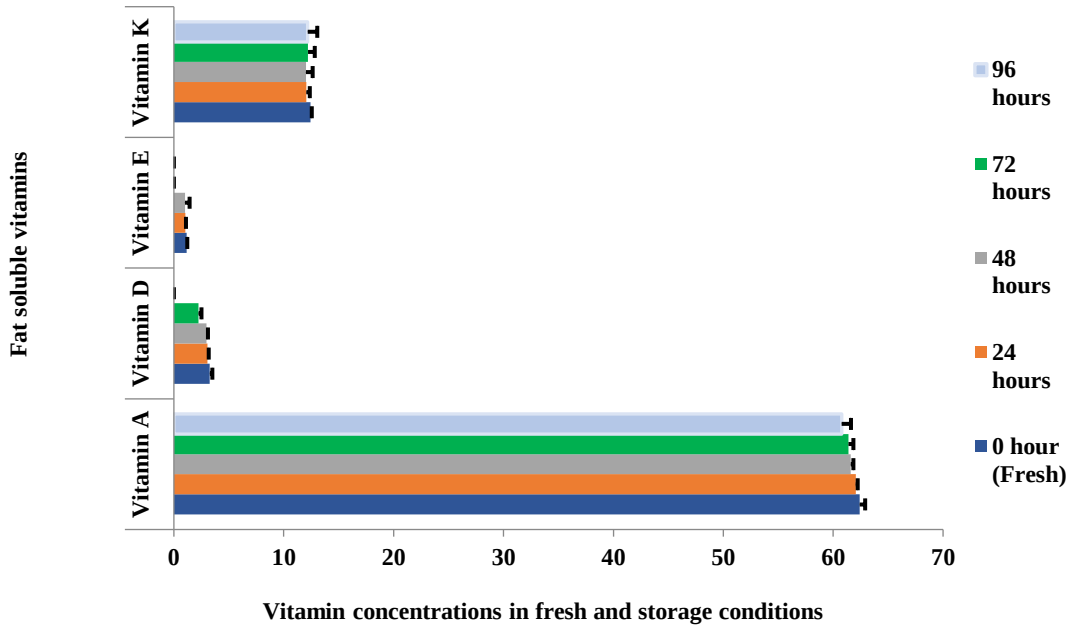

Fig. 5. Vitamin concentration in *Wallago attu* at various storage duration

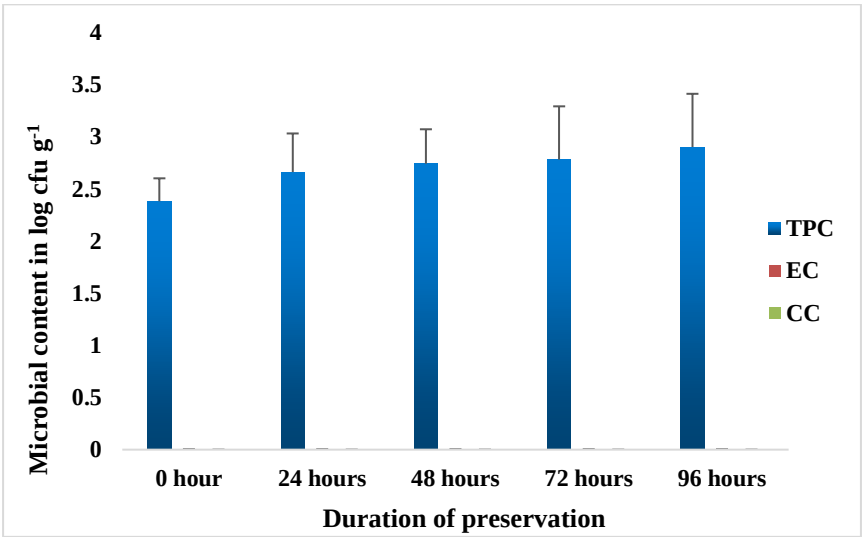

Fig. 6. Microbial count in *Wallago attu* at various storage duration.

Table 3. Vitamin content (expressed in mg/100gm) of *Wallago attu* at both fresh and low temperature preservation. Each value is represented as the Mean± SD. Different means followed by different superscripts in a particular row differs significantly

Vitamins	Storage duration				
	0 hour	24 hours	48 hours	72 hours	96 hours
A	62.41± 0.5[a]	62.06± 0.17[a]	61.6± 0.24[a]	61.39± 0.45[a]	60.77± 0.85[b]
D	3.27± 0.23[a]	3.04± 0.13[a]	2.96± 0.14[a]	2.24± 0.27[b]	BDL
E	1.16± 0.06[a]	1.04± 0.06[a]	1.01± 0.42[a]	BDL	BDL
K	12.43± 0.12[a]	12.05± 0.32[a]	12.02± 0.61[a]	12.21± 0.61[a]	12.18± 0.87[a]

**BDL: Below Detectable Limit*

Table 4. Microbial load of raw fish muscle of *Wallago attu* at both fresh and low temperature preservation (4±1°C)

Parameters (in log cfu/gm)	0 hour	24 hours	48 hours	72 hours	96 hours
Total Plate Count (TPC)	2.38±0.22	2.66±0.37	2.74±0.33	2.78±0.51	2.90±0.51
Escherichia coli Count (EC)	Absent	Absent	Absent	Absent	Absent
Coliform Count (CC)	Absent	Absent	Absent	Absent	Absent

***TPC:** Total Plate Count (log cfu/g); **E. coli Count** (log cfu/g) **CC:** Coliform Count (log cfu/g)*
In present study, it has been found that the TPC in preserved samples was within the permissible limit i.e. 2.90±0.51 log CFU/g as recommended by ICMSF [41] up to 96 hours. TPC for preserved fish muscle shows comparatively slow increment which is because of the significant water loss during storing and thawing process.

Vitamin E content was the lowest among all which was 1.16± 0.06 in the fresh sample which non-significantly changed to 1.01± 0.42 after 48 hours of cold storage. After 48 hours of storage duration, vitamin E content was found to be below detectable limit (BDL). Vitamin K content was present as 12.43± 0.12 in fresh sample which changed to 12.05± 0.32, 12.02± 0.61, 12.21± 0.61 and 12.18± 0.87 after 24, 48, 72 and 96 hours of storage respectively. However, no significant changes were observed in the values (Table 3, Fig. 5). Similar study was reported by other researchers in some other fish groups. Some workers reported that fresh sample of fish (*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 [37] and which was found different from our findings which may be because of the different fish group. Similar study was also reported in some important fish of Bangladesh [38].

3.4 Microbial Content

The quality of the fish meat is largely dependent on its microbial contamination. The changes in TPC of both fresh and preserved fish samples were given below. The results showed that the fresh fish sample had a low value for TPC compared to the preserved samples. In the preserved fish muscle, the values for TPC increased from 2.38±0.22 log cfu/gm in fresh to 2.90±0.51 log cfu/gm in 96 hours of cold preservation which were within acceptable limits. In the preset study, it was found that the the value of total plate count (TPC) in the fresh fish sample was 2.38±0.22 log cfu/g which gradually increases with increase in storage duration. The changes were observed as 2.66±0.37, 2.74±0.33, 2.78±0.51 and 2.90±0.51 in 24, 48, 72 and 96 hours of cold storage respectively. The microbial count for *Escherichia coli Count* (EC) and Coliform Count (CC) was not detectable in both fresh and cold storage conditions (Table 4, Fig. 6).

Similar results were reported by others where they stated that the microbial count for Total Plate Count (TPC), Coliform Count (CC) and Psychrotrophic Count (PC) increased gradually from 2.18±0.02 log cfu/g, 2.02±0.04 log cfu/g and 2.43±0.03 log cfu/g on day 0 to 6.87±0.1 log cfu/g, 5.25±0.2 log cfu/g and 5.99±0.02 log cfu/g on day 30th respectively [8]. Again, it was reported that the Total plate count (TPC), Psychrophilic bacterial count and Psychrotrophic counts were within the acceptable limits for all types of fish (TPC valued 8.37 for Bizz to 25.80×105 Cfu/g for Silver carp, Psychrophils valued 6.83 for Bizz to 63.91 ×105 Cfu/g for silver carp and Psychrotrophs valued 7.7 for Bizz to 20.70×105 Cfu/g for Silver carp) but they varied in count [33]. Again, some results has been reported a lower microbial count in smoked catfish treated with ginger [39]. Again, Arannilewa *et al*., 2005 found an increase in Coliform count with the increasing storage period

in frozen Tilapia [40]. This increase in microbial count is attributed to growth promoting effect of moisture during cold storage. Ozogul *et al*., 2011 also reported a significant statistical increase in total viable counts of whole gutted common sole (*Solea solea)* over the storage period of 24 days [22].

4. CONCLUSION

Fish muscle contains all the nutrient components that is required for human body maintenance. The findings from the sensory evaluation suggested that the fish maintained a satisfactory level of quality for a duration of up to 96 hours of storage. An increase in levels of Total volatile basic nitrogen (TVBN) was observed as the storage duration lengthened, displaying a gradual upward trend with no significant variations. Examination of the vitamins unveiled a downward pattern in their content over the storage period, particularly noting a noteworthy decline in fat-soluble vitamins like vitamin A and D. The microbial assessment illustrated a progressive increase in Total Plate Count (TPC) throughout storage, while Escherichia coli (EC) and Coliform Count (CC) were undetected in both fresh and stored fish samples. The current findings showed that the nutritional values of the fish species are negatively affected by storage duration. Therefore, It was recommended in the research that fish should be consumed fresh to maximize benefits, and if stored, it is advised not to go beyond 96 hours in the refrigerator to uphold quality and ensure health advantages. Implementing appropriate techniques for handling and preservation, such as refrigeration, can aid in prolonging the fish's shelf life by retarding the decomposition process and impeding microbial proliferation. Critical parameters to monitor like TVBN, sensory assessment, vitamin content, and microbial analysis play a vital role in evaluating the quality and safety of fish during storage and establishing its shelf life.

Again, having information about these nutritional values of the fish will make it easier to grade fish, which will help to decide the market value of fish for consumers who are health conscious as well as the livelihood of the fishing community.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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