Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 1173 – 1198 (2024)
www.ejabf.journals.ekb.eg

Comparative Analysis of Proximate, Amino Acid and Mineral Content of Two Fresh Water Fish Species, *Wallago attu* and *Heteropneustes fossilis* and their Quality Restoration with the Use of Additives During Cold Preservation

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ARTICLE INFO

Article History:
Received: May 16, 2024
Accepted: Sept. 21, 2024
Online: Sep. 29, 2024

Keywords:
Additives,
Amino acid,
Catfish,
Cold preservation,
Nutrition

ABSTRACT

The study investigated the effect of cold preservation on the nutritional composition of two fish species and the role of additives in restoring their quality. Fish samples were stored at 4±1°C for varying durations (0, 24, 48, 72, and 96 hours) and were analyzed. Results showed that proximate composition values progressively decreased with longer storage, while carbohydrate content significantly (P<0.05) increased. Fresh samples had the highest values for all parameters except carbohydrates, which showed the lowest values after 96 hours. Amino acid concentrations (essential and non-essential) did not significantly differ between fresh and stored fish. Leucine was the most abundant amino acid in the *Wallago attu*, with tryptophan being the least. In *Heteropneustes fossilis*, leucine and glutamic acid were the highest, while tryptophan and glutamine were the lowest. Mineral content generally declined with storage, except for calcium and zinc in *Wallago attu* and zinc and iron in *Heteropneustes fossilis*. Additives helped maintain nutritional value and extended fish shelflife during cold storage. However, significant nutrient loss occurred after an extended storage, which could be mitigated with additives. Cold storage remains a viable method for preserving fish taste and nutrition for a limited time.

INTRODUCTION

Fish is a vital source of animal protein and is widely regarded as a nutritious option, providing essential proteins and other nutrients for maintaining a healthy body **(Andrew, 2001)**. It plays a crucial role in human nutrition, supplying around 20% of protein intake for a third of the world's population, especially in developing countries. **(Bene *et al*., 2007)**. Fish and fish products are the most important sources of animal protein in the human diet. It comprises all the ten essential amino acids in desirable quantities for human consumption. Fish protein is extremely rich in such amino acid as methionine, lysine and low in tryptophan compared to mammalian protein **(Nowsad, 2007)**. The high nutritional value of fish is mainly related to their readily digested proteins which are an excellent source of EAA **(Sanchez-Alonso *et al*., 2007)**.

Proximate composition is a term usually used in the field of nutrition, mainly for components including moisture, protein, fat, ash, and carbohydrates, which are expressed as content percentage **(Hussain *et al.*, 2018; Shekarabi *et al*., 2020)**. In general, the proximate composition of the fish body indicates the fish quality. Amino acids are basic building-blocks of proteins, fundamentally amino acids are joined together by peptide bonds to form the basic structure of protein **(Peter, 1998)**. The amino acid profile is a critical factor since certain amino acids cannot be synthesized by the human body and must be obtained through diet **(Alina & Ovidu, 2007)**. These amino acids are key in determining the nutritional value of meat. Fish is a rich source of protein containing essential amino acids such as lysine, methionine, cysteine, threonine, and tryptophan **(FAO, 2011)**, all of which play vital roles in human health. Fish muscle provides all the necessary nutrients required for maintaining the human body.

Growing human population in many countries has also increased the demand for fish food. To meet the ever-increasing demand for fish, aquaculture has expanded very rapidly and is now the fastest growing food producing industry in the world **(Samim *et al*., 2019)**. One of the major problems facing the fisheries industries is the spoilage of fish. In the tropical countries such as India, hot climate favors the rapid growth of bacteria which leads to the spoilage of fishes and deterioration of its quality, which in turn decrease the capital gain **(Whittle, 1997)**.

Rigor mortis is a phenomenon which causes stiffness in the muscle after death which results in the rigidity. The progress of rigor mortis depends on several factors such as species, size, age, season, handling pressure, temperature, among others **(Hossain *et al*., 2013)**. The process of rigor mortis is very important for post-harvest preservation and the processing of fish. An excellent, simple, cost-effective method for short-term fish preservation after harvest is cold storage. Fish must be preserved in order to extend its shelf life and avoid spoiling. Moreover, fish should be preserved to maintain their freshness for an extended period of time while minimizing the loss of their nutritional content, flavor, texture, and digestibility. Therefore, among different methods of fish preservation, cold storage is one of the methods which can be used to preserve fish with a minimal change in its quality. Every preservation method has its own limitations and has different impact on the nutritive value of fishes.

Refrigeration and freezing inhibit microbial growth and reduce enzymatic and chemical deterioration by providing low temperatures. However, despite slowing numerous harmful processes, undesirable reactions involving lipids and proteins can still occur, leading to negative changes in the nutritional and sensory properties of the food. Some disadvantages of frozen storage include freezer burn, product dehydration, rancidity and drip loss, and this deterioration increases as duration of storage increases **(Roopma *et al*., 2013)**. Therefore, along with the use of food preservation methods, it is also aimed to extend the shelflife of the fish species with the addition of some additives

to maintain their quality. Additives have been approved for their antimicrobial and antioxidant properties. Antioxidants are compounds that increase shelf life by delaying rancidity and preventing bad taste and odor in fish. The use of antioxidants in the food industry is to prevent or reduce spoilage, maintain the desired quality, and increase the shelf life **(Chaleshtori *et al*., 2013)**. Turmeric extract contains phenolic compounds such as folic acid and protocatechuic acid **(Siripatrawan & Noipha, 2012)**. The most important group of secondary metabolites in the lemon includes flavonoids and other compounds, such as phenolic acids, coumarins, carboxylic acids, amino acids, and vitamins.

The Asian stinging catfish (*Heteropneustes fossilis*) is a freshwater fish found in Southeast Asian countries including Bangladesh, India, Pakistan, Thailand, and Sri Lanka. It is a high valued and very popular fish species in Bangladesh due to being highly palatable, nourishing, and tasty **(Kohinoor *et al*., 2012)**. Again, another type of freshwater catfish that is in high demand as a food with excellent nutritional content is *Wallago attu*. It is a highly valued and popular fish in Bangladesh, known for its palatability, nutritional content, and taste **(Kohinoor *et al*., 2012)**. It is also in high demand in countries like Sri Lanka, Thailand, Pakistan, and India. These catfish species are not only prized for their taste and commercial value but also for their nutritional and therapeutic benefits. Despite their popularity, limited data exist on their nutritional profile and the effects of storage. Therefore, the present study aimed to evaluate the nutritional profile of these fish species under cold storage conditions.

MATERIALS AND METHODS

Sample collection

Fish species (Fresh) were collected from the Brahmaputra Riverine System (landing site at Uzan bazar Ghat), Guwahati in the early hours of the day (Fig. 1).

Sample preparation and preservation

Fresh fish were collected and thoroughly washed with tap water, followed by rinsing with distilled water. Scales and fins were removed, and a significant portion of the dorsal muscle was excised **(Akter *et al*., 2020)**. To ensure representation of various body sections, six or seven slices were randomly selected for analysis **(Al-Reza *et al*., 2015)**. The fish pieces were then placed in airtight zip-lock bags and were stored at 4±1°C. Biochemical analyses were conducted at 0, 24, 48, 72, and 96 hours of cold storage (Fig. 1).

(a)

(b)

(c)

(d)

(e)

(f)

Fig. 1. Collection, preparation and preservation of sample of

(a, c and e) *Wallago attu* and **(b, d and f)** *Heteropneustes fossilis*

Sensory analysis

Sensory characteristics i.e. appearance, color, odor and overall acceptability were evaluated by a trained panel of 20 members using 9-point hedonic scale according to standard procedure **(Peryam, 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, 2018)**.

Proximate composition analysis

Proximate composition such as moisture, protein, lipid and ash content analysis were carried out by following the methods of **AOAC (2016)**. The fish samples were subjected to the following analyses:

Moisture content

About 20-30 grams of fresh sample were weighed. The sample was then dried in an oven at 105°C for 24 hours in order to remove the moisture to obtain a constant weight. Then moisture contents were calculated by 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

Approximately 2.0g of the sample was placed in a Kjeldahl flask, and 10.5g of catalyst (digestion mixture) along with 25ml of concentrated sulfuric acid (H_2SO_4) were added to digest the sample. The mixture was heated at 80-100°C for 2-3 hours until a clear bluish-green solution was obtained. After cooling to room temperature, the solution was diluted to 250ml with distilled water. For distillation, 10ml of the digested sample was taken, and 10ml of 40% sodium hydroxide (NaOH) was added. The sample was distilled, and ammonia was absorbed in 10ml of boric acid in a conical flask. The distillate was then titrated with N/100 hydrochloric acid (HCl) until a light pink endpoint was reached. Total crude protein was calculated by using the following formula:

$$\text{N (\%)} = \frac{0.14 \times (\text{Titration final} - \text{blank}) \text{ reading} \times \text{Strength of HCl (0.01)}}{\text{Weight of sample (g)}} \times 100$$

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

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

Lipid content

Approximately 4-5g of dry sample was placed in an extraction thimble, which was then inserted into the hollow space of a Soxhlet apparatus. A pre-weighed oil flask was fitted to the extractor, and ether was added for the extraction process, which lasted 8-

10 hours. After the extraction, the thimble and flask containing crude fat were removed and dried in a hot air oven at 100ºC for 1 hour. After cooling, the flask was weighed, and the total crude lipid content was calculated using the following formula **(Mishra, 2021)**:

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

Ash content

Approximately 2g of moisture free sample was weighed into an empty pre weighed silica crucible and kept in a muffle furnace, which was then heated at 550-600°C for 6hrs till the sample became completely white or grayish white. The furnace was turned off to cool, and then the sample was taken out and weighted again. The ash content was calculated as follows:

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

Carbohydrate content

The determination of the carbohydrate content of the product was obtained by difference, as 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)

Amino acid analysis

Amino acid analysis was done by using HPLC following the method given by **Ishida *et al*. (1981)**. At first, the sample was taken in a tube and digested with 6N hydrochloric acid at 110º C for 24 hours. After complete digestion, the sample was neutralized with 6N NaOH and was derivatized. The derivatized samples were injected in high performance liquid chromatography (HPLC) and the individual amino acids were detected using a fluorescence detector. The amino acids were identified and quantified by comparing with the retention times and peak areas of standards amino acids.

Mineral analysis

Mineral analysis was carried out by using an atomic absorption spectrometry **(Mphande & Chama, 2015)**. At first, the sample was taken and heated in an oven at

105°C for 6 - 8 hours. 2.5 grams of sample was transferred into digestion flasks and 25ml of HNO_3 was added and placed on a hot plate at 120°C till the sample was digested. The solution was then allowed to cool down and 10ml of 70% $HClO_4$ was added. Again, the solution was boiled till it became colorless. The solution was then cooled, and diluted to 100ml with deionized water. The residual analysis of minerals was determined in all treatments using an atomic absorption spectrophotometry **(AOAC, 2016)**.

Preparation of additives

In the present study, two additives turmeric (*Curcuma longa*) and lemon (*Citrus limon*) were used for quality restoration. For that, fish samples were divided into four groups to store in cold temperature for 4 days. First NE (Sample preserved with no extract), second, TE (Sample preserved with turmeric extract), third LE (Sample preserved with lemon extract) and fourth TE+LE (Sample preserved with turmeric and lemon extract).

Turmeric extract preparation:

Turmeric rhizomes were purchased from the market and powdered. Using the Soxhlet method, an alcoholic extract was prepared by placing 100g of turmeric powder in the thimble of the Soxhlet extractor. Then, 600ml of 70% alcohol was added to the flask. As the alcohol was heated, it circulated through the turmeric powder, extracting its compounds and returning to the flask as a thick liquid. After extraction, the solvent was removed, and the turmeric extract was collected in autoclaved jars, sealed with aluminum foil, and stored until further use **(Sahne *et al*., 2016; Mugahi *et al.*, 2022)**.

Lemon extract preparation

Lemon extract was purchased from the market, and a 1.5% solution (1.5g in 100ml distilled water) was prepared for each treatment, with triplicates **(Mugahi *et al.*, 2022)**. Fish samples were fully immersed in the solution for 30 seconds, then immersed again for an additional 30 seconds. After treatment, all samples were placed in sterile plastic containers and stored separately in a refrigerator at 4°C **(Jeon, 2022)**.

Statistical analysis

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

RESULTS

1. Results of sensory evaluation

In the present study, sensory characteristics of fish sample during cold storage are represented in the Table (1). Results showed a steady decline in sensory characteristics with increasing the storage time. However, the highest value was found for fresh fish samples compared to cold preserved fish samples. According to the current investigation, cold storage samples remain good in quality scores at the end of 96 hours of storage significantly ($P<0.05$).

Table 1. Sensory analysis of fish muscle in cold storage (4°±1°C) (Mean ± SD)

Sample	Duration in hours	Sensory quality attributes					
		Appearance	Flavor	Odor	Juiciness	Texture	Overall acceptability
Wallago attu	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	7.4±0.8	8±0.33	6.5±0.21	8±0.25	7.5±0.12	7.48±0.45
	96	7±0.00	7.5±0.21	6±0.23	7±0.21	7.5±0.45	7±0.23
Heteropneustes fossilis	0	9±0.00	8.5±0.12	8±0.34	9±0.53	8.5±0.32	8.6±0.12
	24	9±0.56	8.5±0.34	8±0.00	8.8±0.5	8.5±0.34	8.56±0.23
	48	8.5±0.45	8.5±0.00	7.5±0.34	8.5±0.23	8±0.00	8.2±0.21
	72	7.8±0.32	7.5±0.11	7±0.16	8±0.65	7.5±0.12	7.56±0.00
	96	7±0.00	7.5±0.23	7±0.12	7.5±0.32	7±0.00	7.2±0.32

2. Results of proximate composition

2.1 Proximate results of Wallago attu

The nutritional composition of *Wallago attu* at fresh (0 hour of storage) condition was observed as 78.24% moisture, 16.52% protein, 3.24% crude fat, 1.47% ash, and 0.53% carbohydrate. All the values gradually decreased with increasing the hours of storage duration, except for carbohydrates which showed significantly increasing trend

with increasing storage hours. However, the decreased values are non-significant, except for protein which showed a significant decrease in 96 hours of cold storage (Table 2).

Table 2. Proximate composition (expressed in %) of *Wallago attu* in both fresh and cold storage conditions. Each value is represented as the Mean± SD of n=3. Different means followed by different superscripts in a particular row differs significantly

Parameter (%)	0 hour	24 hours	48 hours	72 hours	96 hours
Moisture	78.24 ± 0.41^{a}	78.13 ± 0.49^{a}	77.94 ± 0.32^{a}	77.72 ± 0.48^{a}	77.53 ± 0.30^{a}
Crude protein	16.52 ± 0.20^{a}	16.49 ± 0.13^{a}	16.43 ± 0.12^{a}	16.32 ± 0.09^{a}	16.17 ± 0.03^{b}
Crude fat	3.24 ± 0.34^{a}	3.17 ± 0.29^{a}	3.10 ± 0.40^{a}	2.94 ± 0.27^{a}	2.83 ± 0.77^{a}
Ash	1.47 ± 0.20^{a}	1.46 ± 0.26^{a}	1.45 ± 0.22^{a}	1.43 ± 0.23^{a}	1.41 ± 0.30^{a}
Carbohydrate	0.53 ± 0.28^{a}	0.75 ± 0.30^{a}	1.08 ± 0.73^{a}	1.59 ± 0.60^{a}	2.06 ± 0.04^{b}

2.2 Proximate results of Heteropneustes fossilis

The nutritional values of *Wallago attu* at fresh (0 hour) condition was observed as 78.39% moisture, 18.47% protein, 1.25% crude fat, 1.37% ash, and 0.52% carbohydrate. The values for all parameters decreased non-significantly with increasing the cold storage duration, except for carbohydrates, which significantly increased over time (Table 3).

Table 3. Proximate composition (expressed in %) of *Heteropneustes fossilis* in both fresh and cold storage conditions. Each value is represented as the Mean± SD of n=3. Different means followed by different superscripts in a particular row differ significantly

Parameter	0 hour	24 hours	48 hours	72 hours	96 hours

(%)					
Moisture	78.39±0.78[a]	78.20±0.69[a]	78.01±0.20[a]	77.91±0.95[a]	77.69±0.48[a]
Crude Protein	18.47±0.38[a]	18.35±0.43[a]	18.23±0.73[a]	17.68±0.25[b]	17.54±0.14[b]
Crude fat	1.25±0.16[a]	1.23±0.35[a]	1.18±0.35[a]	1.12±0.45[a]	0.86±0.06[b]
Ash	1.37±0.38[a]	1.36±0.39[a]	1.27±0.82[a]	1.14±0.53[a]	1.03±0.64[a]
Carbohydrate	0.52±0.11[a]	0.86±0.09[b]	1.31±0.20[c]	1.15±0.07[d]	1.28±0.03[e]

3. Amino acid analysis

3.1 Amino acid composition of Wallago attu in different duration of cold storage

In *Wallago attu*, leucine is the most abundant essential amino acid, while tryptophan is the least concentrated. The effect of cold storage indicates that, with increasing the duration, the concentrations of total and individual amino acids decline compared to the fresh (0 hour) sample, although these changes are not significant. Among the non-essential amino acids, aspartic acid has the highest concentration, while cysteine has the lowest. Similar to the essential amino acids, the concentrations of total and individual non-essential amino acids also decline with longer storage duration, but again, the changes are not significant (Table 4).

Table 4. Essential and non-essential amino acids (expressed in g/100g in dry weight) found in *Wallago attu* at fresh and different hours of cold storage conditions. Each value is represented as the Mean± SD of n=3

Essential amino acid (gm/100gm)	0 hour	24 hours	48 hours	72 hours	96 hours
Histidine	2.14±0.03	2.14±0.34	2.11±0.50	2.04±0.33	1.98±0.54
Isoleucine	3.48±0.99	3.42±0.94	3.44±0.79	3.42±0.81	3.42±0.10
Leucine	8.56±0.56	8.56±0.76	8.52±0.74	8.48±0.04	8.48±0.81
Lysine	7.28±0.40	7.24±0.39	7.22±0.45	7.18±0.57	7.13±0.78
Methionine	3.46±0.20	3.46±0.70	3.38±0.12	3.37±1.01	3.32±0.38
Phenylalanine	2.65±0.54	2.51±0.31	2.51±0.16	2.50±0.47	2.50±0.56

Threonine	6.32±0.94	6.30±0.65	6.28±0.36	6.24±0.62	6.22±0.54
Tryptophan	0.23±0.11	0.23±0.09	0.23±0.10	0.21±0.11	0.21±0.11
Valine	2.83±0.32	2.81±0.14	2.81±0.33	2.78±0.19	2.72±0.52
∑EAA	**36.95**	**36.67**	**36.50**	**36.22**	**35.98**
Alanine	6.37±1.01	6.40±0.75	6.38±0.35	6.38±0.51	6.36±0.90
Arginine	3.84±0.28	3.82±0.16	3.82±0.58	3.76±0.19	3.76±0.17
Asparagine	3.23±0.47	3.23±0.48	3.20±0.30	3.20±0.54	3.14±0.35
Aspertic acid	7.92±0.50	7.88±0.34	7.87±0.22	7.84±0.33	7.82±0.53
Cysteine	1.02±0.13	1.01±0.06	1.01±0.19	1.02±0.25	1.01±0.23
Glutamic acid	6.32±0.26	6.32±0.60	6.28±0.28	6.26±0.30	6.26±0.49
Glutamine	1.41±0.16	1.41±0.17	1.39±0.58	1.36±0.41	1.34±0.48
Glycine	2.17±0.63	2.15±0.37	2.16±0.25	2.16±0.53	2.14±0.39
Proline	2.63±0.31	2.63±0.59	2.61±0.30	2.61±0.16	2.57±0.39
Serine	4.26±0.54	4.23±0.68	4.21±0.62	4.19±0.63	4.19±0.92
Tyrosine	2.42±0.21	2.39±0.45	2.37±0.33	2.37±0.98	2.34±0.69
∑NEAA	**41.59**	**41.47**	**41.30**	**41.15**	**40.93**

3.2 Amino acid composition of Heteropneustes fossilis in different duration of cold storage

In *Heteropneustes fossilis,* leucine is the most abundant essential amino acid, while tryptophan is the least concentrated. The effect of storage duration reveals a non-significant decline in the concentrations of total and individual amino acids. Cold storage did not result in considerable changes in the amino acid content of *H. fossilis*. Among the non-essential amino acids, glutamic acid is the most prevalent, while glutamine has the lowest concentration. Similar to the essential amino acids, the concentrations of total and individual non-essential amino acids also decline non-significantly with increased storage duration, with no substantial changes observed in the amino acid content of *H. fossilis* (Table 5).

Table 5. Essential and non- essential amino acids (expressed in g/100g in dry weight) found in *Heteropneustes fossilis* at fresh and different hours of cold storage conditions. Each value is represented as the Mean± SD of n=3

Essential amino acid (gm/100gm)	0 hour	24 hours	48 hours	72 hours	96 hours
Histidine	3.81±0.60	3.80±0.86	3.80±0.37	3.78±0.22	3.76±0.51
Isoleucine	5.43±0.54	5.43±0.66	5.41±0.56	5.41±0.09	5.40±0.39
Leucine	8.05±0.77	8.01±0.60	8.01±0.58	8.01±0.29	8.01±0.74
Lysine	3.87±0.61	3.92±0.44	3.86±0.23	3.85±0.13	3.84±0.46
Methionine	1.35±0.10	1.32±0.26	1.31±0.26	1.31±0.11	1.30±0.53
Phenylalanine	6.14±0.83	6.12±0.24	6.12±0.64	6.10±0.78	6.10±0.58
Threonine	5.85±0.23	5.85±0.53	5.83±0.47	5.82±0.13	5.82±0.45
Tryptophan	0.42±0.04	0.40±0.09	0.40±0.03	0.38±0.08	0.38±0.05
Valine	5.76±0.01	5.73±0.37	5.73±0.13	5.72±0.33	5.70±0.12
∑EAA	**40.68**	**40.58**	**40.47**	**40.38**	**40.31**
Alanine	5.97±0.34	5.94±0.25	5.94±0.34	5.92±0.07	5.92±0.41
Arginine	1.53±0.28	1.50±0.18	1.48±0.16	1.48±0.04	1.46±0.06
Asparagine	4.16±0.07	4.16±0.43	4.13±0.15	4.13±0.17	4.10±0.11
Aspertic acid	7.46±0.51	7.41±0.36	7.41±0.11	7.40±0.26	7.40±0.46
Cysteine	0.37±0.14	0.37±0.09	0.35±0.07	0.35±0.19	0.35±0.05
Glutamic acid	12.72±0.48	12.72±0.44	12.68±0.28	12.67±0.19	12.64±0.17
Glutamine	0.24±0.01	0.24±0.09	0.21±0.07	0.21±0.03	0.20±0.01
Glycine	8.76±1.07	8.74±0.53	8.73±0.12	8.71±0.01	8.70±0.02
Proline	0.68±0.03	0.68±0.06	0.65±0.09	0.64±0.09	0.64±0.12
Serine	6.22±0.24	6.26±0.12	6.21±0.44	6.21±0.05	6.20±0.43
Tyrosine	0.43±0.16	0.43±0.11	0.42±0.02	0.42±0.09	0.42±0.05
∑NEAA	**48.54**	**48.45**	**48.21**	**48.24**	**48.03**

4. Mineral analysis

4.1 Mineral content of Wallago attu in different duration of cold storage

Among the macro minerals, calcium (Ca) is the most abundant, while magnesium (Mg) is present in lower concentrations. In the case of micro minerals, zinc (Zn) is less concentrated than iron (Fe). Mineral concentrations show significant differences over various storage durations although some remain stable (Table 6). Calcium, despite its high concentration, does not exhibit significant changes during the storage period, similar to zinc. In contrast, other minerals such as potassium (K), sodium (Na), magnesium (Mg), and iron (Fe) demonstrate significant variations, with the most pronounced changes observed after 48 hours of cold storage (Table 6).

4.2 Mineral content of Heteropneustes fossilis in different duration of cold storage

Among the macro minerals, sodium (Na) is the most abundant, while magnesium (Mg) is present in lower concentrations. In the case of micro minerals, the concentration of zinc (Zn) is lower compared to iron (Fe). Minerals exhibit slight significant differences in their concentrations during storage, although some remain constant as storage duration increases (Table 7). The variations observed in mineral concentrations among the fish species could be attributed to the availability of these inorganic elements in their aquatic environment and the fish's ability to absorb them.

In the case of *Heteropneustes fossilis*, the concentrations of microminerals (Zn and Fe) do not show significant differences with increasing the duration of cold preservation. However, the concentrations of macro minerals (Ca, K, Na, and Mg) begin to differ significantly starting from 48 hours of cold storage, with these changes continuing up to 96 hours of storage (Table 7).

Table 6. Mineral analysis (expressed in mg/100g in dry weight) of *Wallago attu* at different hours of cold storage conditions. Values are represented as Mean± SD. Different means followed by different superscripts in a particular column differ significantly

Storage hours	Macro minerals				Micro minerals	
	Calcium	**Potassium**	**Sodium**	**Magnesium**	**Zinc**	**Iron**
0	772.52±0.91^{a}	246.76±0.34^{a}	143.83±0.49^{a}	83.49±0.39^{a}	2.38±0.15^{a}	3.85±0.17^{a}
24	772.29±0.05^{a}	246.63±0.84^{a}	143.62±0.68^{a}	82.63±0.72^{a}	2.29±0.23^{a}	3.67±0.16^{a}
48	771.50±0.58^{a}	245.94±0.41^{a}	143.25±0.78^{a}	78.64±0.60^{b}	2.25±0.05^{a}	3.55±0.56^{a}
72	771.22±0.67^{a}	245.56±1.07^{a}	138.75±0.63^{b}	78.26±0.36^{bc}	2.17±0.13^{a}	3.46±0.49^{b}
96	771.02±0.67^{a}	244.23±0.46^{b}	137.91±0.28^{b}	77.31±0.43^{cd}	2.12±0.10^{a}	3.45±0.58^{bc}

Table 7. Mineral analysis (expressed in mg/100g in dry weight) of *Heteropneustes fossilis* at fresh and different hours of cold storage conditions. Values are represented as Mean± SD. Different means followed by different superscripts in a particular column differ significantly

Storage hours	Macro minerals				Micro minerals	
	Calcium	**Potassium**	**Sodium**	**Magnesium**	**Zinc**	**Iron**
0	164.82±0.74^{a}	162.26±0.42^{a}	223.64±0.14^{a}	103.31±0.61^{a}	1.48±0.16^{a}	2.34±0.12^{a}
24	163.70±0.98^{a}	162.23±0.94^{a}	223.31±0.73^{a}	102.52±0.43^{a}	1.47±0.09^{a}	2.26±0.22^{a}
48	163.10±0.67^{a}	156.44±0.22^{b}	221.43±0.06^{b}	102.32±0.33^{a}	1.47±0.05^{a}	2.26±0.08^{a}
72	162.71±0.80^{b}	156.11±0.67^{bc}	220.35±0.27^{c}	101.62±0.33^{b}	1.46±0.22^{a}	2.27±0.22^{a}
96	161.81±0.46^{bc}	157.26±0.47^{cd}	220.09±0.84^{cd}	101.05±0.69^{bc}	1.47±0.23^{a}	2.27±0.12^{a}

5. Impact of Additives

5.1 Effects of additives on moisture content

Fresh sample of *Wallago attu* and *Heteropneustes fossilis* showed a mean value for moisture as 78.24± 0.41 and 78.39± 0.78, respectively. After 96 hours of storage, the mean values decreased non-significantly to 77.53±0.30 for one fish species and 77.69±0.48 for the other. The results indicated a progressive restoration in the quality of the samples when different additives were used for both fish species. Among the additives tested, the combination of turmeric extract and lemon extract (TE+LE) demonstrated the greatest shelf life extension compared to samples with turmeric extract (TE) or lemon extract (LE) used separately during cold preservation. However, no significant differences were observed among all the values recorded (Table 8 & Fig. 2).

Table 8. Moisture content of the selected fish species in fresh and 96 hours of cold storage conditions (with or without additives). Data are presented as mean ± SD. Different means followed by different superscripts in a particular row differ significantly

Sample	**Fresh (FS)**	**96 hours of storage**			
		Without additives	**With additives**		
		NE	**TE**	**LE**	**TE+LE**
WA	78.24±0.41^{a}	77.53±0.30^{a}	77.8±0.30^{a}	77.75±0.19^{a}	78.1±0.24^{a}
HF	78.39±0.78^{a}	77.69±0.48^{a}	78±0.10^{a}	77.94 ± 0.17^{a}	78.31±0.12^{a}

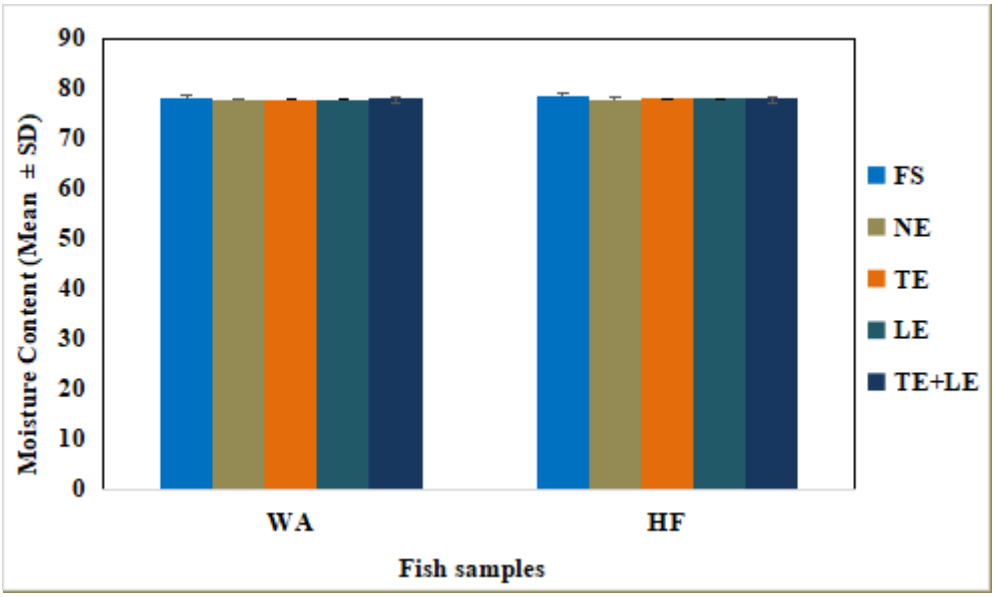

Fig. 2. Moisture content of the fish species in different cold storage conditions

5.2 Effects of additives on protein content

The fresh fish sample of *Wallago attu* exhibited a protein content of 16.52±0.20, which significantly decreased to 16.17±0.03 after four days of cold storage. Samples preserved with turmeric extract (TE) showed no significant difference compared to those with normal extract (NE). However, the samples with lemon extract (LE) maintained their protein content, showing no significant difference from the fresh sample. Notably, samples with the combination of turmeric and lemon extract (TE+LE) demonstrated a better quality restoration, with values remaining almost equivalent to the fresh samples.

In contrast, the fresh sample of *Heteropneustes fossilis* displayed a protein content of 18.47±0.38, which significantly changed after 96 hours of storage. The results indicated that the protein content could be restored with the addition of turmeric and lemon extracts during cold preservation. For all samples with additives, there were no significant differences compared to the fresh samples (Table 9 & Fig. 3).

Table 9. Protein content of the selected fish species at fresh and 96 hours of storage condition (with or without additives). Data are presented as mean ± SD. Different means followed by different superscripts in a particular row differ significantly

	Fresh	**96 hours of storage**

Sample	(FS)	Without additives	With additives		
		NE	TE	LE	TE+LE
WA	16.52±0.20[a]	16.17±0.03[b]	16.17±0.06[b]	16.20±0.14[a]	16.23±0.43[a]
HF	18.47±0.38[a]	18.38±0.88[b]	18.43±0.09[a]	18.36±0.08[a]	18. 40±0.05[a]

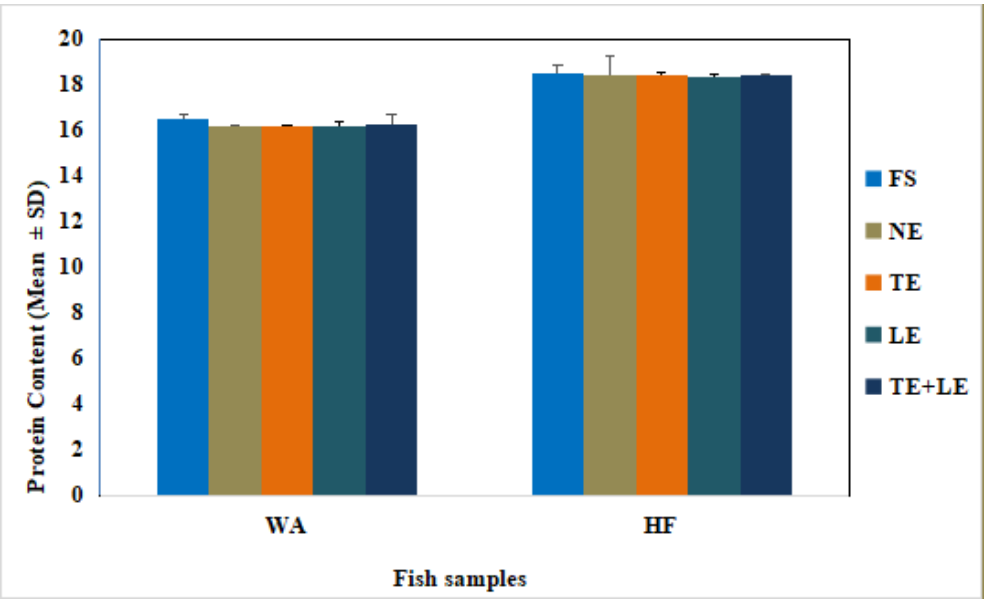

Fig. 3. Protein content of the fish species in different cold storage conditions

5.3 Effects of additives on Lipid content

The fresh fish sample of *Wallago attu* exhibited a mean lipid content of 3.24±0.34, which non-significantly decreased to 2.83±0.29 after 96 hours of cold storage. The addition of various additives and their combinations effectively increased the shelflife of the sample. In comparison, the fresh sample of *Heteropneustes fossilis* showed a mean lipid content of 1.25±0.03, which also non-significantly decreased to 1.14±0.22 after 96 hours of storage. The trend observed in the lipid content for *Heteropneustes fossilis* mirrored that of *Wallago attu*, indicating similar effects of the additives on lipid preservation (Table 10 & Fig. 4).

Table 10. Lipid content of the three selected fish species at fresh and 96 hours of storage condition (with or without additives). Data are presented as mean ± SD. Different means followed by different superscripts in a particular row differ significantly

Sample	Fresh	96 hours of storage

	(FS)	Without additives	With additives		
		NE	TE	LE	TE+LE
WA	3.24±0.34[a]	2.83±0.29[a]	2.83±0.04[a]	2.81±0.09[a]	2.82±0.06[a]
HF	1.25±0.03[a]	1.14±0.22[a]	1.13±0.04[a]	1.14±0.02[a]	1.16±0.02[a]

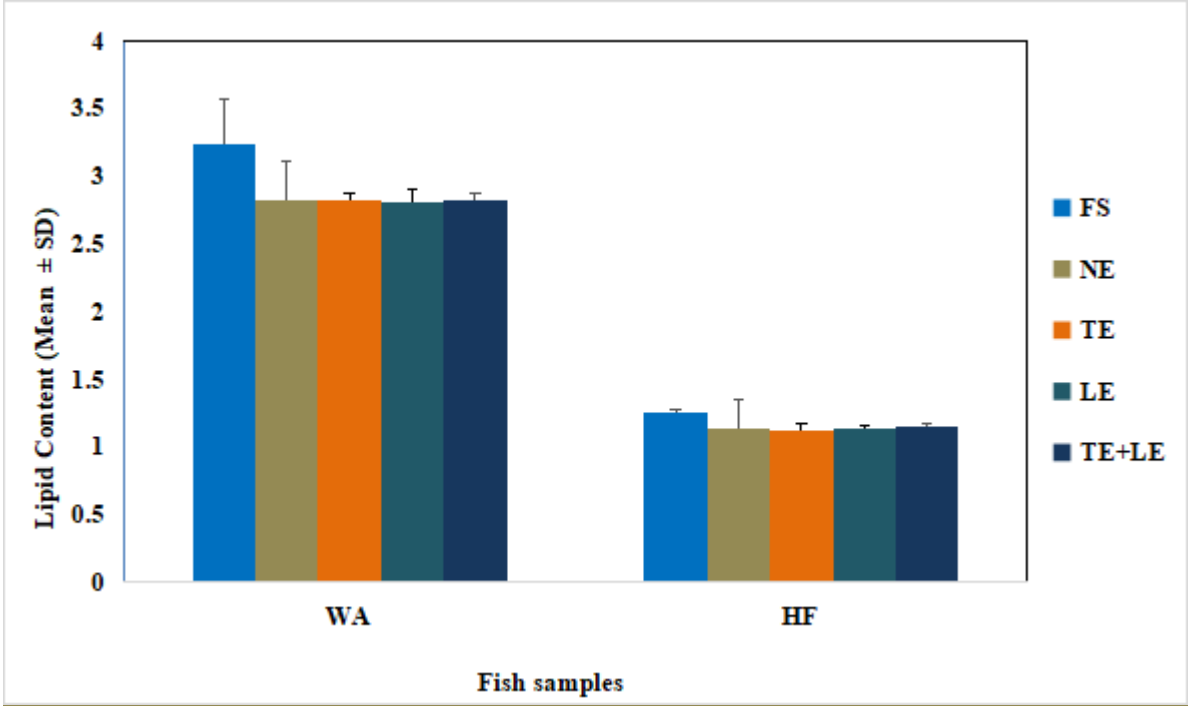

Fig. 4. Lipid content of the fish species in different cold storage conditions

5.4 Effects of additives on ash content

The fresh samples of *Wallago attu* and *Heteropneustes fossilis* exhibited mean ash values of 1.47±0.20 and 1.37±0.04, respectively. After 96 hours of cold storage, the mean ash values non-significantly decreased to 1.41±0.30 for *Wallago attu* and 1.03±0.30 for *Heteropneustes fossilis*. The results indicated a progressive restoration in the quality of the samples with the addition of various additives for both fish species. Notably, the combination of turmeric extract (TE) and lemon extract (LE) demonstrated the most significant shelf life extension compared to samples with TE or LE added separately. However, no significant differences were found among all the values measured (Table 11 & Fig. 5).

Table 11. Ash content of all the three selected fish species at fresh and 96 hours of storage condition with or without additives). Data are presented as mean ± SD. Different means followed by different superscripts in a particular row differ significantly

Sample	Fresh (FS)	96 hours of storage			
		Without additives	With additives		
		NE	TE	LE	TE+LE
WA	1.47±0.20[a]	1.41±0.30[a]	1.41±0.06[a]	1.42±0.03[a]	1.40±0.09[a]
HF	1.37±0.04[a]	1.03±0.30[a]	1.03±0.06[a]	1.04±0.11[a]	1.04±0.06[a]

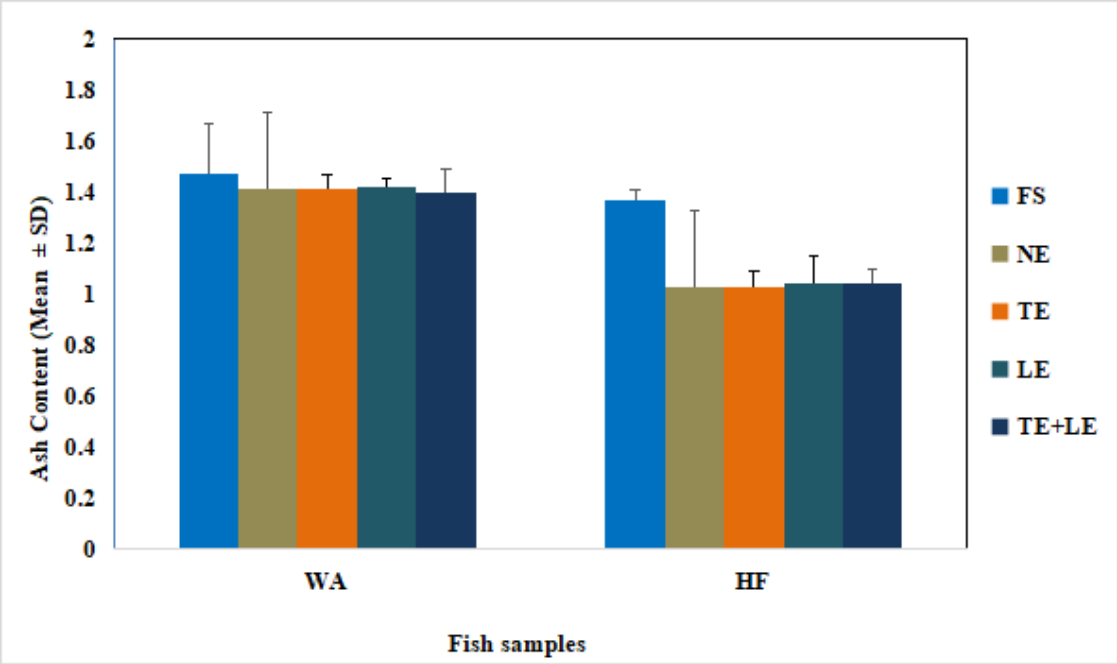

Fig. 5. Ash content of the fish species in different cold storage conditions

DISCUSSION

The two fish species investigated in the present study are highly preferred in the market by both fishermen and consumers in the region. The nutritional content of both catfish species exhibited varying values across all analyzed parameters. It is reassuring to note that both species contained high levels of nutrition, including crude protein and essential amino acids. This finding underscores their significance as a nutritional resource in a region where protein-rich foods are scarce. Consequently, fish, as a source of quality protein, can command a high market price **(Zuraini *et al.*, 2006; Jabeen & Chaudhry, 2011)**.

The percentage of moisture content is a good indicator of its relative contents of energy, proteins and lipids. The total moisture content found in *Wallago attu* decreased from 78.24±0.41% in fresh (0 hour) to 77.53±0.30% in 96 hours of cold storage. Similar result was also found by **Paul *et al. (*2018)**, where they found that the moisture content of *Wallago attu* was 74.25±0.42. Results of the present study was also supported by the findings of **Roopma *et al*. (2013)**, where they reported that the moisture content in the muscles of *Wallago attu* decreases from 81.66±0.03% on day zero to 74±0.05% on the 30th day during frozen storage (-12±2ºC) conditions. **Naher *et al.* (2018)** reported that the mean moisture content during the first week of frozen storage was 79±0.45%, which decreased to 75±0.01% by the end of the fourth week. Similarly, **Aberoumand (2013)** noted a decrease in moisture content, attributing this reduction to water condensation during frozen storage. In the case of *Heteropneustes fossilis*, the moisture content was 78.39±0.78% in fresh fish (0 hour), declining to 77.69±0.48% after 96 hours of cold storage. **Salma *et al.* (2021)** observed a comparable moisture content of 77.30% in *H. fossilis*, indicating a slight decrease in ice storage. This reduction may be attributed to moisture evaporation from the fish's surface during ice storage, influenced by factors such as relative humidity, chemical changes, and storage temperature. Additionally, **Gandotra *et al.* (2012)** reported a similar trend, noting a reduction in moisture content in *Labeo rohita* fillets stored at low temperatures for 21 days.

Fish protein is recognized as a unique source of animal protein due to its high content of myofibrillar protein and lower levels of stroma protein **(Salman *et al.*, 2021)**. Proteins are essential for hormonal and enzyme development and serve as a good energy source. In *Wallago attu*, the highest recorded protein content was 16.52±0.20% in fresh fish muscle, which significantly decreased to 16.17±0.03% after 96 hours of cold storage. Conversely, *Heteropneustes fossilis* exhibited a change in protein content from 18.47±0.38% to 18.93±0.88% after 96 hours of cold storage, although this variation was not statistically significant ($P \leq 0.05$) compared to the fresh sample.

Similar findings were reported by **Naher *et al.* (2018)**, who found a protein content of 16.15±1% in the first week of storage, which decreased to 14±0.08% by the fourth week of frozen storage. **Roopma *et al.* (2013)** observed a comparable trend, with the highest protein content of fresh (unfrozen) fish samples at 15.45±0.2%, while the lowest, at 10.14±0.015%, was noted in samples stored for 30 days under frozen conditions. Supporting these results, **Aberoumand (2013)** documented protein loss during frozen storage in various Iranian fish species, attributing this loss to protein denaturation and the escape of nitrogen as volatile bases and nitrogenous substances due to bacterial decomposition.

Additionally, **Gandotra *et al.* (2012)** reported a decrease in protein content from 15.93% to 13.06% after cold storage at -12°C for 21 days. The reduction in crude protein content during storage may result from protein degradation and the formation of various

volatile compounds, including total volatile bases, trimethylamine, ammonia, and hydrogen sulfide **(Eyo, 2001)**.

Lipids are essential food reserves that significantly influence the condition of fish, leading to the use of fat indices as a measure of the relationship between water and fat percentages **(Sinclair & Duncan, 1972)**. The current study indicates that the lipid content in the muscle of both catfish species does not change significantly over a storage duration of up to 96 hours. **Roopma *et al.* (2013)** observed a similar trend in their study on *Wallago attu* during frozen storage, noting a significant decrease in lipid content from 4.02±0.04% on day 0 to 2.36±0.03% on day 30 ($P \leq 0.05$). **Aberoumand (2013)** reported a decrease in total lipid content in various Iranian fish species during frozen storage, attributing this loss to lipid oxidation and hydrolysis. **Salma *et al.* (2021)** found comparable lipid content in fresh and stored samples of *Heteropneustes fossilis*, suggesting that reduced lipid oxidation occurs during cold storage. **Kyrana and Lougovois (2002)** also noted that lipid auto-oxidation was minimal during ice storage in the whole iced sea bass.

In the present work, ash content exhibited a non-significant decreasing trend with increased cold storage duration for both fish species. **Roopma *et al.* (2013)** similarly reported a total percent decrease of 4.72% on day 10, escalating to 10.13% by day 30 of frozen storage. These findings align with those of **Aberoumand (2013)** and **Gandotra *et al.* (2012)**, who noted reductions in ash content during storage. **Salma *et al.* (2021)** observed that while ash content did not fluctuate during storage, the variation in values was influenced by other component fluctuations. Conversely, carbohydrate content displayed an increasing trend with extended cold storage duration in both species, likely due to glycogen accumulation **(Sadhu *et al.*, 2020)**.

Fish muscle comprises a substantial amount of amino acids, with the amino acid profile of *Wallago attu* presented in Table (3). Among essential amino acids, *Wallago attu* has the highest concentrations of leucine and lysine, while tryptophan levels are at their lowest. A reduced availability of lysine can lead to mental and physical disorders, as it is crucial for synthesizing glutamate, a key neurotransmitter in the mammalian central nervous system **(Papes *et al*., 2001)**. Non-essential amino acids in *Wallago attu* include high levels of aspartic acid, glutamic acid, and alanine, with cysteine being the lowest. Similar findings have been reported for mackerel, particularly regarding glutamic acid concentration **(Hou *et al.*, 2011)**.

In *Heteropneustes fossilis*, leucine is the most abundant essential amino acid, with the lowest concentration of tryptophan. This species also contains high levels of glutamic acid and lower levels of glutamine among non-essential amino acids. Various researchers have noted that the primary amino acids in fish are aspartate, glutamate, and lysine **(Iwasake & Harada, 1985)**. The presence of significant amounts of essential amino

acids, such as lysine and leucine, in both catfish species may help supplement dietary protein deficiencies.

Fish meat serves as a rich source of minerals, with zinc (Zn), iron (Fe), and copper (Cu) being the most abundant microelements essential for normal metabolic processes within fish tissue **(Window *et al.*, 1987)**. The current study reveals that both fish species are mineral-rich, although the effects of cold storage vary between them. In *Wallago attu*, there is a significant decline in the concentration of all minerals with prolonged storage, except for calcium (Ca) and zinc (Zn), which remain stable by the end of the storage period. Conversely, in *Heteropneustes fossilis*, all macronutrients exhibit significant changes across different storage durations, while the micronutrients do not show significant differences.

The variations in mineral concentrations observed in this study align with the findings of previous research, indicating that differences in mineral element concentrations among fish species can result from the chemical forms of these elements and their availability in the local environment **(Window *et al.*, 1987)**. **Mazrouh (2015)** reported similar findings, noting maximum values of magnesium (Mg), calcium (Ca), and phosphorus (P) in fresh fish samples, with a decrease noted with prolonged freezing times. Furthermore, **Mphande and Chama (2015)** observed that all nutritional parameters, including calcium, potassium, and magnesium, were reduced across various fish species following freezing, with this reduction continuing as storage duration increased.

The results regarding the proximate composition of fish samples immersed in turmeric and lemon extracts are illustrated in Figs. (2-5). The findings indicate that immersion in these extracts leads to significant changes in the proximate composition of the fish muscle. The study demonstrates that the application of additives enhances the quality of fish samples during cold preservation. Similar research has shown that immersing carp fillets in extracts of turmeric, cinnamon, and lemon improves spoilage indices, including thiobarbituric acid, volatile nitrogen bases, and pH levels. These studies suggest that the compounds in the extracts help maintain nutritional value and extend the shelf life of fish by reducing spoilage indices during cold storage **(Mugahi *et al.*, 2022)**.

CONCLUSION

The primary aim of this study was to determine the optimal cold storage duration and assess its impact on the nutritional changes in two significant catfish species, *Wallago attu* and *Heteropneustes fossilis*, over various cold storage periods. The nutrient

profile of these fish species revealed a richness in essential amino acids and other nutrients vital for human health. Cold storage helps reduce spoilage by lowering bacterial activity, making fish safer for consumption. The findings demonstrated that most nutritional parameters decline as storage duration increases, indicating a negative impact on the nutritional composition of both species. To maintain optimal nutrient levels, particularly minerals, it is advisable to consume fish while it is still fresh or refrigerated for a limited time. Given that not all fish species are available throughout the year, freezing or cold storage becomes crucial for preserving their quality. Additionally, the study highlighted the importance of additives in extending shelf life during cold storage. The nutritional insights from this research can serve as valuable guidelines for dietitians, nutritionists, and researchers, enabling them to recommend fish as a healthful food option in daily diets. Importantly, the study concluded that at standard cold storage temperatures, fish samples can be preserved for specific durations without significant nutritional loss. For prolonged storage, the application of additives can help restore and maintain the quality of the fish during cold storage.

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