A SEMINAR PAPER ON

Physicochemical and Microbial Changes of Some Freshwater Fishes during Ice Storage

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A SEMINAR PAPER

ON

PHYSICOCHEMICAL AND MICROBIAL CHANGES OF SOME FRESHWATER FISHES DURING ICE STORAGE[1]

BY

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ABSTRACT

The study was designed to investigate the effect of low temperature preservation on the physicochemical and microbial profile of some fresh water fish muscle stored in ice for several days. The proximate composition and microbiological analyses were examined at different time interval on muscle of freshwater fishes like Catla (*Gibelion catla*), Rui (*Labeo rohita*) and Common carp (*Cyprinus carpio*) during storage. According to physical characteristic analysis Catla, Rui and Common carp were acceptable for 10 days, 14 days and 12 days, respectively. On the basis of chemical composition analysis, those species are excellent in quality up to 10 days. The microbial count increased gradually during the period of storage. The coliform count also followed the same trend during the storage. Thus, a significant quality loss was observed in fish during ice storage. However, the present iced conditions retained the fish under acceptable microbial conditions for human consumption up to 10th to 15th day beyond which it became unfit for human consumption. The storage time can be increased by proper icing and maintaining proper ration of ice and fish.

Keywords: *Gibelion catla*, *Labeo rohita*, *Cyprinus carpio*, physicochemical and microbial load.

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CHAPTER I
INTRODUCTION

Bangladesh is a south Asian country located in between Latitude 20˚34´ and 26˚39´ north and longitude 80˚00´ and 90˚41´ East. It is considered one of the most suitable regions for fisheries in the world, with the world's largest flooded wetland and the third largest aquatic biodiversity in Asia after China and India. In Bangladesh, there are about 260 species of freshwater fishes. Fish supplements about 60% of Bangladeshi people’s daily animal protein intake (DoF, 2016). The fisheries sector plays a very important role in the national economy, contributing 3.69% to the Gross Domestic Product (GDP) of the country and 22.60% to the agricultural GDP (FRSS, 2016) and 5% of export earnings. Total fish production in Bangladesh in 2014-2015 was reported to be 3,684,245 MT, of which 1,023,991 MT (27.79%) were from inland open waters, 2,060,408 MT (55.93%) from inland closed waters and 599,846 MT (16.28%) from marine fisheries. In 2015-2016, Bangladesh was the 5th in world aquaculture production, which accounted for half of the country’s total fish production (55.15%) (DoF, 2016). In 2014-2015, total fishery production of Bangladesh was 3,684,245 metric tons, of which 1,023,991 metric tons was obtained from inland capture fisheries, 2,060,408 metric tons from inland aquaculture and 599,846 metric tons from marine water production (FRSS, 2016).

As fish is highly perishable commodity, better utilization calls for deeper understanding on the composition, spoilage process and stability to different processing conditions. Both quality and safety of the food will be major challenge faced by humankind in recent time. The deterioration of quality of both wild and farmed fish species is mainly due to action of intrinsic enzymes and microbes (Hsieh and Kinsella 1989; Pigott and Tucker 1987). To prevent the spoilage of fish, some form of preservation is necessary. There are many different fish preservation methods. These are the Chilling, Freezing, Drying, Canning, Smoking, preparing value added products etc. During the period of preservation, the fish is kept as ‘fresh’ as possible, with minimum losses in flavor, taste, odor, form, nutritive value, weight and digestibility of flesh. This preservation should cover the entire period from the time of harvest of fish to its sale at the retailer’s counter. Among all these preservation methods chilling is the easiest and low cost preservation method which is done just after the death of fish to delay the spoilage. Chilling of fish is a process by which temperature of a fish is reduced close to 0ºC, but not below freezing point of water. Cooling by means of ice is called icing, which slows down the multiplication of micro-organisms. Icing delays both biochemical and bacteriological process in fish and consequently prolongs the storage life of fish and fish products. Quality of chilled product depends mainly on the initial quality of raw fish. A reduction of storage temperature by 5–6 ºC halves the rate of biochemical reactions and doubles the shelf life of fish (Mjelda and Urdahl, 1974). Proper icing keeps the fish in an acceptable condition for reasonable periods. The shelf life of fish preserved in ice varies depending on the species, method of capture, location of fishing grounds and size of fish (Lima dos Santos et al., 1981). The storage life of iced fish is usually affected by initial microbial load of the fish and storage temperature (Church, 1998). However, the main merit of the preservation by ice is that it provides the maximum possibility of preserving the natural nutritional and functional properties of the fish.

According to, the supply of fresh fish, short term preservation by using ice or any chilling medium is more practical and economical. It is well known that lowering the body temperature will help to extend the keeping quality of fish (Pigott and Tucker, 1990). It is commonly recognized that fish after death goes through the following stages: pre-rigor, rigor mortis and resolution of rigor mortis, while autolysis and microbial spoilage occur throughout these stages. The lower the temperature of cold storage is, the longer the period that the fish stays in pre-rigor stage, thus extending the shelf life of the product (Yamamoto et al., 1966 and Bito et al., 1983). The use of ice is possibly the simplest method of preserving the fish catch and benefits of ice usage in the tropics are perhaps even greater than in the colder zone. The advantages of icing are ice melt-water helps to wash away surface bacteria and contaminants, ice melt-water keeps the surface of fish wet that prevents dehydration and preserves the glossy appearance, ice melt-water in contact with the fish is a good conductor of heat that facilitates cooling, ice can be transported from place to place and is, in effect, a method of portable refrigeration, ice melts at 0 ºC it will not freeze the fish but automatically controls the temperature at the ideal chill level, ice is relatively cheap compared with other means of preservation (Clucas and Ward, 1996). Therefore, the present study was aimed to compare the organoleptic, chemical and microbial characteristics of freshwater fish stored in ice.

The present study was conducted with the following objectives:

- ✓ To understand the effect of ice storage (0 ºC) on the biochemical, physical, and microbiological properties of freshwater fish.
- ✓ To determine the extended shelf life (consumable time) of different freshwater fish stored in ice.

CHAPTER II
METHOD AND MATERIALS

This seminar paper is exclusively a review paper. It has been prepared by reviewing the various articles published in different Books, Proceedings, Abstracts, Review papers, Journals, MS thesis, Ph.D. Dissertation etc. available in the library of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, including Bangladesh Fish Research Institute, Bangladesh Fisheries Development Corporation, DoF, etc. or anywhere else. I prepared this paper in consultation with my learned major professor, and other concerned experts. The necessary thoughts, ideas, facts and findings has been collected through internet searching and incorporated with the body of the seminar. In addition to that constructive and valuable suggestions of the experts were included, as and when necessary, in preparing this paper. Mostly secondary data have been adopted in this paper.

CHAPTER III
RESULT AND DISCUSSION

Physicochemical and microbial changes of Catla (*Gibelion catla*)

Physical characteristics

The average length and weight of Catla (*Gibelion catla*) used for ice storage study was 29.79 ± 2.98 cms and 366.02 ± 114.77 grams respectively. The longer shelf life of fresh fish stored in ice could be attributed to the low temperature of ice that hinders microbial growth. Uchoi et al. (2011). The natural/normal fresh color of the fish became discolored during storage. The pigments may have been oxidized or affected by some other factors. It is known that lipids convert to peroxides, aldehydes, ketones and lower aliphatic acids when they are oxidized Sowicz et al. (2004). Although hydro-peroxides are tasteless, they can cause brown and yellow discoloration of the fish tissue Bataringanya (2007). Some browning discoloration also occurs in fatty fishes due to hydrolytic changes between fats and proteins (MANAGE 2008). These facts may therefore also explain the observed color changes of the fish gills in this study. Texture became harder at the beginning due to stiffening of the muscle during rigor mortis process. The concave appearance of the eyes and scales becoming loose could be as a result of prolonged storage in ice earlier reported by Uchoi et al. (2011)

Chemical composition analysis

Moisture content

The moisture content in ice stored Catla (*Gibelion catla*) increased slowly from 77.9 ± 0.16% to 81.7 ± 2.14 % till the end of 22nd day (Table 1). The moisture content increased during the ice storage study for 22 days. Similar results were observed by Perigreen et al. (1987) in common murrel (*Channa striatus*), Indira et al. (2010) in common carp (*Cyprinus carpio*) and Sravani (2011) in rohu (*Labeo rohita*) stored in ice. The increase in moisture content may be due to the absorption of ice melt water by the fish muscle.

Protein content

During ice storage, the protein content decreased from an initial value of 17.03 ± 0.04% (0 day) to 16.02 ± 0.02% at the end of 22nd day (Table 1). Total protein content showed a significant decrease over the entire period of storage. Protein content was highest in the fresh fish. At the end of storage study (22nd day), the protein content decreased, mainly due to the loss of soluble protein components. Decrease in protein content was also observed in spotted seer during ice storage reported by Shenoy and James (1974). Suvanich et al. (2000) reported a decrease in protein content in minced channel cat fish stored at 4˚C. Devadasan and Nair

(1977) also reported decrease in protein fraction in fish muscle during ice storage. Similar results were also obtained by Reddy and Srikar (1991b) in pink perch, where the decrease in total protein content has been attributed to the leaching out of water-soluble protein components and dilution effect caused by water uptake. Similar observations were made by Sarma et al. (1994) in pink perch and oil sardine, Indira et al. (2010) in *Cyprinus carpio*, Meenakshi et al. (2010) in *Cyprinus carpio* and Chomnawang et al. (2007) in hybrid cat fish.

Lipid content

During ice storage, the lipid content decreased from 3.51 ± 0.18 % (0 day) to 0.4 ± 0.05 % at the end of 22[nd] day (Table 1). A decrease in the content of crude lipid has been observed during the ice storage. The fat content decreased from 3.51 ± 0.18% to 0.40 ± 0.05%. Gould (1996) also observed decrease in lipid content due to lipid breakdown in Brazilian freshwater fish stored at refrigeration temperature. Similar results are reported by Reddy and Srikar (1991a) and Sarma et al. (1994) in pink perch, Meenakshi et al. (2010) in *Cyprinus carpio*, Chomnawang et al. (2007) in hybrid cat fish, Kolakowska et al. (1996) in Baltic herring and Sravani (2011) in rohu (*Labeo rohita*) stored in ice.

Table 1: Changes in the moisture, lipid, ash and protein content of ice stored Catla (*Gibelion catla*)

Storage period (days)	Moisture %	Lipid %	Ash %	Protein %
0	77.9 ± 0.16[a]	3.51 ± 0.18[d]	0.70 ± 0.08[a]	17.03 ± 0.04[j]
2	79.5 ± 0.35[ab]	3.33 ± 0.12[d]	0.86 ± 0.03[ab]	16.76 ± 0.02[i]
4	79.7 ± 0.00[ab]	3.06 ± 0.04 [cd]	1.04 ± 0.25[abc]	16.72 ± 0.00[h]
6	79.8 ± 0.12[ab]	2.66 ± 0.09[bcd]	1.05 ± 0.03[abc]	16.68 ± 0.01[h]
8	80.2 ± 0.12[b]	2.41 ± 0.09 [bcd]	1.15 ± 0.01[abc]	16.49 ± 0.02[g]
10	80.3 ± 0.94[b]	1.77 ± 0.85[abc]	1.15 ± 0.18[abc]	16.45 ± 0.01[f]
12	80.4 ± 0.24[b]	1.64 ± 0.68[abc]	1.28 ± 0.08[abc]	16.37 ± 0.02[e]
14	80.5 ± 0.08[b]	1.53 ± 0.009[ab]	1.25 ± 0.43[abc]	16.31 ± 0.00[d]
16	80.5 ± 0.24[b]	1.50 ± 0.94[ab]	1.23 ± 0.14[abc]	16.24 ± 0.01[c]
18	80.8 ± 0.21[b]	1.41 ± 0.53[ab]	1.42 ± 0.34[bc]	16.20 ± 0.01[c]
20	81.3 ± 0.26[b]	0.90 ± 0.42[a]	1.35 ± 0.17[bc]	16.12 ± 0.0[2b]
22	81.7 ± 2.14[b]	0.4 ± 0.05[a]	1.53 ± 0.08[c]	16.02 ± 0.02[a]

Source: Abarna (2012)

* Each value is represented as arithmetic mean ± SD of n = 3.

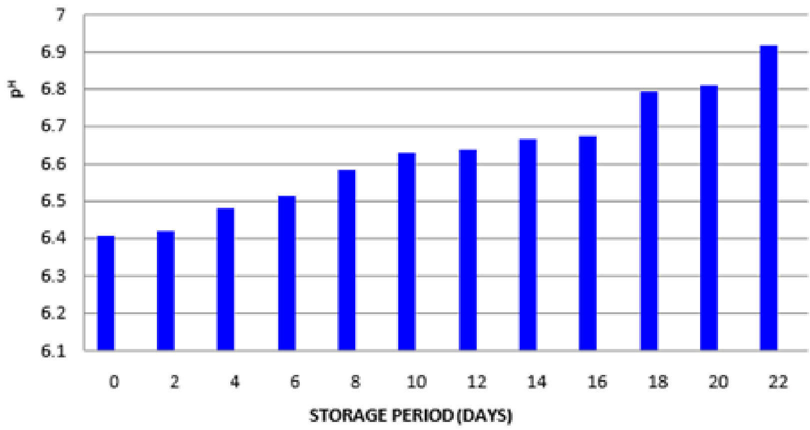
[abcdefghijkl] Means followed by the same superscript with in a column are not significantly different ($p > 0.01$).

Ash content

The ash content fluctuated throughout the storage study period. The results of ash content are presented in the (Table 1). The ash content of iced Catla obtained during the entire iced storage period did not show any particular trend. During ice storage, the ash content ranged between 1.27 ± 0.06 % to 1.78 ± 0.02 %. Sravani (2011) also observed that the ash content did not show a uniform trend during storage of rohu at frozen temperature. She observed that the ash content ranged from 0.97 ± 0.02 % to 1.28 ± 0.12 %. These findings are in accordance with the findings of Raju et al. (1998) in silver carp.

pH value

During ice storage, the pH of Catla (*Gibelion catla*) muscle increased from an initial value of 6.40 ± 0.06 to 6.91 ± 0.00 at the end of 22nd day (Figure 1). In the present study, pH of ice stored fish increased initially up to 30 days and later decreased till 60 days. The pH value of fish once again increased during further period of storage. The initial increase and decline in value of pH may be correlated with changes in total viable count. These results are similar to the observations made by Raju et al. (1998) in silver carp where the value of pH decreased during the initial 30 days followed by significant increase during later part of the storage due to production of volatile base compounds, as indicated by the increase in total plate count. Similar results were obtained by Tokur et al. (2006) in trout, Hulya et al. (2008) in *Gadus* spp., *Mugil* spp. and *Engraulis* spp. and Obemeata et al. (2011) in tilapia stored at low temperature where in the decrease in pH is attributed to the fact that fermentation of carbohydrate to acid was occurring.



Source: Abarna (2012)

Figure 1: Changes in pH of Catla (*Gibelion catla*) meat during ice storage.

Total volatile base nitrogen (TVB-N) & Trimethylamine nitrogen (TMA-N)

During ice storage, the TVB-N content increased continuously from 2.03 ± 0.04 to 4.60 ± 0.14 mg/100 gram of meat (Table 2). During storage, the TMA-N content increased from 2.00 ± 0.06 to 4.13 ± 0.04 mg/100 gram of meat (Table 2). Volatile base nitrogen indicates the production of ammonia, mono-di and trimethylamine nitrogen and is found in the common pattern of spoilage. Increase in the content of TVB-N significantly correlated with the decrease in overall acceptability scores in the present study ($P < 0.01$). Similar results were obtained by Chakrabarti (1984) in Indian major carps, Magnusson et al. (2006) in cod, Sarma et al. (1998) in pink perch and oil sardine, Raju et al. (1998) and Asgharzadeh et al. (2010) in silver carp, Hulya et al. (2008) in *Gadus euxinus*, *Mugil cephalus* and *Encrasicholus*.

Estimation of TMA-N is generally recommended for assessing the quality of iced fish. The low TMA-N levels recorded in the present study could be related to the composition of the microbial flora and the relatively low pH values encountered during storage. The optimum pH for activity of the bacterial TMAO reducing enzymes has been reported to be 7.2-7.4 (Castell and Snow, 1949 and Elinor et al., 1985). Similar results were recorded by Sarma et al. (1998) in pink perch and oil sardine and Hulya et al. (2008) in *Gadus euxinus*, *Mugil cephalus* and *Encrasicholus*.

Table 2: Changes in TVB-N and TMA-N content of Catla (*Gibelion catla*) during ice storage

Storage period (days)	TVB-N (mg/100g)	TMA-N (mg/100g)
0	2.03 ± 0.04^{a}	2.00 ± 0.06^{a}
2	2.70 ± 0.14^{b}	2.33 ± 0.52^{ab}
4	2.96 ± 0.04^{bc}	2.47 ± 0.65^{abc}
6	2.98 ± 0.17^{bcd}	2.83 ± 0.53^{abc}
8	3.18 ± 0.07^{bcd}	2.98 ± 0.17^{abc}
10	3.20 ± 0.64^{bcd}	3.00 ± 0.37^{abc}
12	3.26 ± 0.20^{bcd}	3.11 ± 0.18^{bc}
14	3.49 ± 0.01^{cd}	3.16 ± 0.04^{bcd}
16	3.51 ± 0.10^{cd}	3.19 ± 0.12^{bcd}
18	3.58 ± 0.00^{cd}	3.32 ± 0.16^{bcd}
20	3.71 ± 0.28^{d}	3.45 ± 0.06^{cd}
22	4.60 ± 0.14^{e}	4.13 ± 0.04^{d}

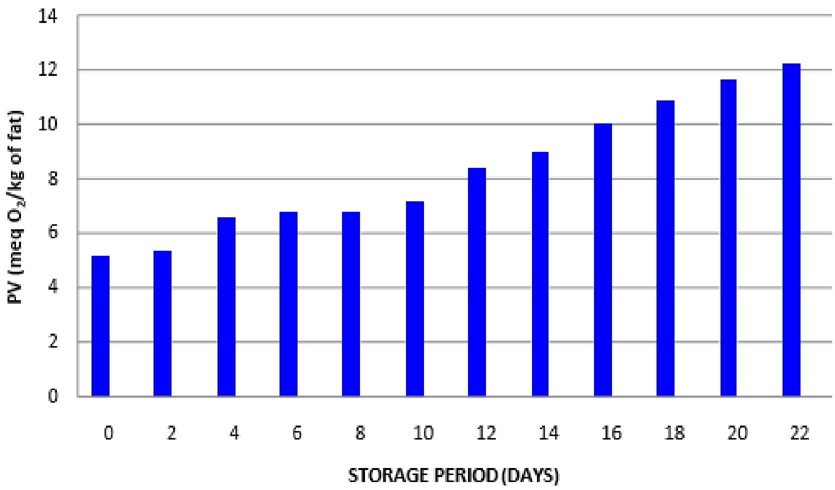
Source: Abarna (2012)

*Each value is represented as arithmetic mean ± SD of n = 3.

^{abcde} Means followed by the same superscript with in a column are not significantly different ($p > 0.01$).

Peroxide Value (PV)

The peroxide value increased with increase in ice storage period and formed a linear relationship with storage days. During ice storage, the peroxides value increased from 5.2 ± 0.56 meq O_2/kg of fat to 12.2 ± 0.12 meq O_2/kg of fat (Figure 2). In the present study, the PV increased steadily over the entire period of storage. The slow increase of PV may be due to preservative effect of low storage temperature. An increase in PV during low temperature storage has been observed by Srikar et al. (1993) in salted mackerel and pink perch where the higher content of PV was attributed to the higher rate of lipid oxidation. Similar results were found by Sarma et al. (1998) in pink perch and oil sardines, Aubourg and Medina (1999) in cod and haddock, Keyvan (2008) in *Rutilus frisi* (kutum fish), Hulya et al. (2008) in *Gadus euxinus*, *Mugil cephalus* and *Encrasicholus*, Makri et al. (2009) in *Sparus aurata*, Asgharzadeh et al., (2010) in of silver carp. The increase in PV during low temperature storage is explained by Erickson (1997) and Sikorski and Kolakowska (2000) as a result of the presence of pro-oxidant enzymes (lipoxygenases, peroxidases) and chemical pro-oxidant molecules (hemoproteins and metal ions).

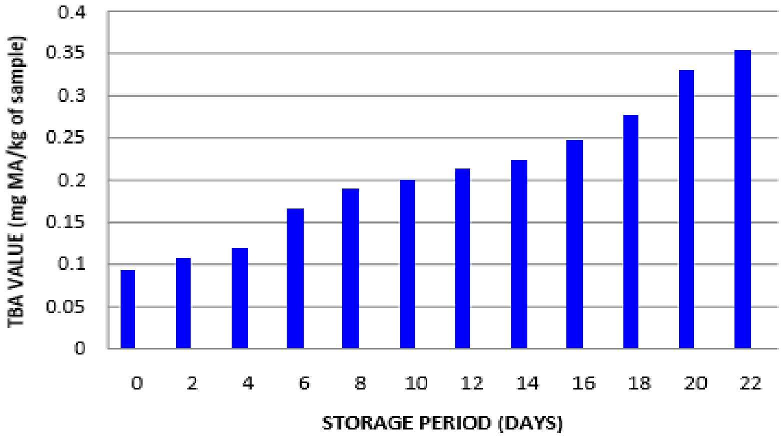


Source: Abarna (2012)

Figure 2: Changes in the PV of ice stored Catla (*Gibelion catla*).

Thiobarbutric Acid (TBA)

The TBA content which is considered as a secondary lipid oxidation product increased throughout the storage study. During ice storage, TBA content increased to 0.35 ± 0.01mg of malonaldehyde/kg of sample from an initial value of 0.09 ± 0.00 mg of malonaldehyde /kg of sample (Figure 3). Increase in TBA values during low temperature storage has been noticed in the present study throughout the period. These results are in agreement with the observations of Aubourg and Medina (1999) in low temperature stored cod and haddock. Similar results were also observed by Keyvan (2008) in *Rutilus frisi* (kutum fish), Hulya et al. (2008) in *Gadus euxinus*, *Mugil cephalus* and *Encrasicholus* in three different styles (like whole, gutted and filleted). In gilthead sea bream Makri et al. (2009) observed an increase in values of TBA till 30 days of low temperature storage and then decreased as the TBA reactive substances were prone to interact with biological constitutes present in muscle leading to decrease. Asgharzadeh et al. (2010) revealed similar results in silver carp.

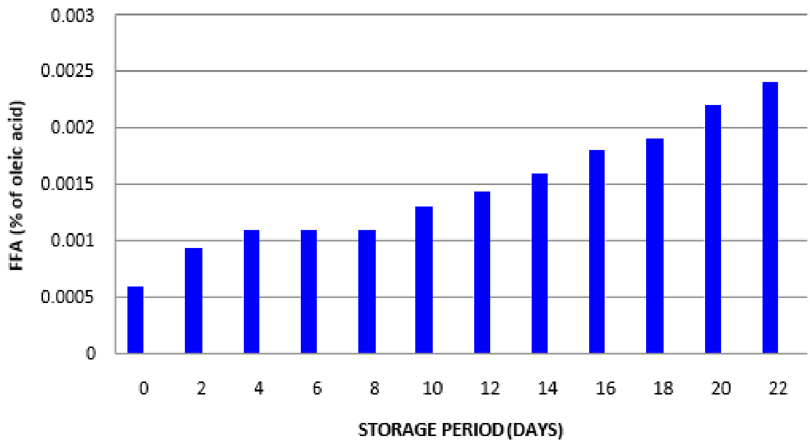


Source: Abarna (2012)

Figure 3: Changes in TBA value of ice stored Catla (*Gibelion catla*).

Free Fatty Acid (FFA)

The free fatty acid content values showed a linear relationship with storage period (Figure 4). The FFA values increased from an initial value of 0.0006 ± 0.00% of oleic acid to 0.0024 ± 0.00% of oleic acid on 22nd day of storage. FFA, resulting from lipid hydrolysis, accumulates during low temperature storage and accelerates quality deterioration (Saeed and Howell, 2002).



Source: Abarna (2012)

Figure 4: Changes in FFA content of ice stored Catla (*Gibelion catla*).

In the present study hydrolysis of fish lipids in iced condition increased gradually during storage, forming a good linear correlation. Similar results were obtained by Srikar et al. (1993) and Sarma et al. (1994) where the lower increase in FFA may be attributed to the slower rate of lipid oxidation. Similar observation was made by Aubourg et al. (2004) in horse mackerel, Aubourg et al. (2005) in *Scomber scombrus* and Stodolnik et al. (2005) in *Scomber scombrus*. Munoz et al. (2006) showed an increase in the content of FFA of lipids extracted from iced carp fillets stored up to 30 days. Similar results were observed by Keyvan

(2008) in *Rutilus frisi* (kutum fish) and Makri et al. (2009) in gilthead seabream (S*parus aurta*). The result of the present study implies that lipolytic enzymes were active in the muscle of iced fish throughout the storage period.

Microbiological load analysis

Total plate count (TPC)

During ice storage, the total plate count of mesophilic bacteria decreased continuously in the fish sample. *Staphylococcus aureus* counts also have shown a similar trend during the ice storage. *Escherichia coli* and Faecal *Streptococci* were absent throughout the storage period (Table 3). The total plate count increased during low temperature storage up to 30 days and later decreased during further period of storage. *Staphylococcus* spp. showed a decreasing trend during the low temperature storage up to 30 days. The counts were not detectable during subsequent storage. Nickelson et al. (1980) observed a similar trend in the black drum, sand trout and tilapia. The increase in TPC was due to the utilization of NPN matter during storage (Jhaveri and Constantinidens, 1982 and Reddy et al., 1997). The same can be speculated in the present study also. In case of *Psychrophiles*, the count increased continuously. On the contrary, the Sulphur producing bacteria increased up to 30 days and decreased gradually during further period of storage.

Table 3: Changes in Total plate count in Catla (*Gibelion catla*)

Storage period (days)	Total Plate Count (cfu/gm)	
	24 hrs	**48 hrs**
0	7.16×10^2 (2.84)	7.16×10^2 (2.85)
2	6.90×10^2 (2.83)	7.10×10^2 (2.85)
4	4.30×10^2 (2.63)	4.40×10^2 (2.64)
6	3.10×10^2 (2.49)	3.15×10^2 (2.49)
8	2.66×10^2 (2.42)	2.72×10^2 (2.43)
10	2.50×10^2 (2.39)	2.56×10^2 (2.40)
12	1.94×10^2 (2.28)	2.10×10^2 (2.32)
14	1.28×10^2 (2.10)	1.42×10^2 (2.15)
16	7.6×10^2 (1.88)	1.20×10^2 (2.07)
18	0.54×10^2 (1.73)	0.68×10^2 (1.83)
20	0.44×10^2 (1.64)	0.56×10^2 (1.74)
22	0.28×10^2 (1.44)	0.38×10^2 (1.57)

Source: Abarna (2012)

Physicochemical and microbial changes of Rui (*Labeo rohita*)

Physical characteristics

Table 4 shows the changes in the sensory characteristics and gives the average score of the iced fish in the hedonic scale. The muscle had sweet taste and muddy odor. The muscle was soft. The muddy odor was noticed in many fishes collected from ponds. The soft texture is characteristic of freshwater fishes. The changes in the gills, eyes and muscle gave an indication of the extent of changes and the condition of the fish. In storage with ice the muddy odor decreased and spoiled weedy odor took its place. The gills changed from bright red to pink, bleached, brown and finally to dark color. After 7 days storage the gills became slimy, color change was noticed in the muscle by 14 days storage and yellow discoloration developed at the belly portion on 14th day. Organoleptically the muscle was in acceptable condition up to 14 days.

Table 4: Physical characteristics of Rui (*Labeo rohita*) during ice storage

Days of storage	Fresh Fish	Iced fish	Score
0	Pre-rigor, muddy odor	Sweet, soft, slight muddy taste, white meat	8.5 ± 0.4
7	Muscle soft, mixed odor of seaweed and mud, gills brown with mucous, color of meat changed to dull gray	Slight muddy odor, soft and slightly pasty, slightly sweet, gills became slimy.	6.0 ± 0.72
14	Eyes opaque and sunken, red color around the eyes, brown gills, soft muscle, scales intact, overall appearance satisfactory. Yellow discoloration in the belly portion.	Muscle soft and moderately pasty, dull color, slight muddy taste, yellow discoloration developed at the belly portion.	4.6 ± 0.55
21	Dark gills, decayed odor, sunken and opaque eyes, pink color around the eyes, soft and pasty muscle.	Pasty, decayed odor, spoiled	2.0 ± 0.75

Source: Josefh et al. (1988)

Chemical analysis

Moisture Contents

Perusals of (Table 5) showed that initially on day 0, moisture content was found to be 84.74±0.1% and it decreased significantly to the value of 80.84±0.09% on 21st days of storage at low temperature. The results depicted that there was 1.08%, 2.70% and 4.60% decrease in moisture content on 7th, 14th and 21st day respectively. These results are in accordance with Alasalvar et al. (2002), Orban et al. (2002) and Bekelvik et al. (2005) who reported a decrease in total moisture content in sea bass (*Dicentrarchus labrax*) fillets during frozen storage. This decrease in moisture content was attributed to the sublimation of ice in frozen storage and drip loss during thawing process by them. Kandeepan & Biswas (2007) reported a decrease of 1.57% in total moisture content in buffalo meat after seven days of frozen storage. In contrary to the results of present study, Zamir et al. (1998) in crab; Bao et al. (2007) in Arctic Charr (*Salvelinus alpinus*) and Siddique et al. (2011) in *Puntius* sp. found an increasing trend in moisture content. Zamir et al. (1998) attributed this increase to the loss of water holding capacity of tissue.

Protein content

Perusals of (Table 5) revealed that the highest protein content (15.93±0.04%) was recorded for fresh (before using ice) fish samples and the least protein content (13.06±0.04%) was recorded for fish sample stored for 21 days at low temperature storage. A significant percent decrease ($p \leq 0.05$) was found in total protein content i.e. 5.77%, 11.23% and 18.01% on 7^{th}, 14^{th} and 21^{st} day of storage respectively (Table 5). In support of present findings, Bekelvik et al. (2005) in sea bass (*Dicentrarchus labrex*) and Siddique et al. (2011) in *Puntius* sp. reported significant decrease in protein content during low temperature storage. They attributed this protein loss due to the leaching effect of amino acids and water soluble protein leaching out with melting ice. According to Xiong (1997), Zamir et al. (1998) and Saeed & Howell (2002) proteins exposed to oxidizing environments are very susceptible to chemical modification, such as amino acid destruction, peptide scission and formation of protein-lipid complexes that results in decrease in protein content.

Lipid Content

The results shown in the (Table 5) revealed that the lipid content decreased significantly ($p \leq 0.05$) from day 0 i.e. 3.86±0.03% to 3.00±0.03% on 21^{st} day. There was 5.44%, 15.80% and 22.27% decrease in total lipid content on 7^{th}, 14^{th} and 21^{st} day respectively during the storage of 21 days at low temperature (Table 5). In favor of present findings Arannilewa et al. (2005) in Tilapia; Peter et al. (2010) in Alaska Pollack (*Theragra chalcogramma*) and Siddique et al. (2011) in *Puntius* sp. found a significant loss in total lipid content when stored at low temperature. These workers attributed this loss due to oxidation of lipid that is the major cause of deterioration of fish.

Ash content

Result shown in (Table 5) revealed that the ash content decreased significantly from 1.79±0.01% on day 0 to 1.36±0.03% on 21^{st} day of storage at low temperature. These results are in agreement with Beklevik et al. (2005) while working on sea bass fillets; Okoyo et al. (2009) on Nile perch and Emire et al. (2009) on Tilapia, (*Oreochromis niloticus*) reported a decrease in total ash content during its frozen storage. But Arannilewa et al. (2005) observed that the ash content remained almost the same throughout the 60 days of frozen storage of tilapia. However, Khan et al. (2005) reported a strong correlation between the storage period and ash content ($r=0.819$, $p \leq 0.0002$) in blue mussels (*Mytilus edulis*) during storage in ice.

The decrease in ash and moisture content was attributed to the drip loss during thawing process by Beklevik et al. (2005).

Table 5. Proximate composition (wet weight basis) of raw fish muscle of Rui (*Labeo rohita*) stored at low temperature during 21 days of storage

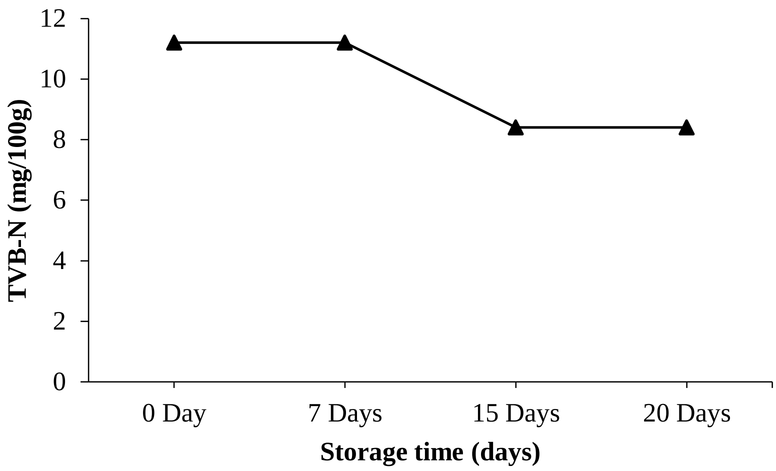
Days	**0 Day**	**7th Day**	**14th Day**	**21st Day**
Total protein (%)	15.93±0.04	15.01±0.02	14.14±0.03	13.86±0.03
Total lipid (%)	3.86±0.04	3.65±0.02	3.25±0.04	3.00±0.03
Moisture	84.74±0.1	83.82±0.02	82.64±0.02	80.84±0.09
Ash	1.79±0.01	1.69±0.012	1.57±0.02	1.36±0.03

Source: Gandotra et al. (2012)

Mean±SD with different superscripts in a row differs significantly (P<0.05)

TVB-N Content

It was noticed that the TVB-N value did not increase during iced storage of *Labeo rohita*, but showed a decrease after 15 days of storage (figure 5). TVB-N is the production of ammonia, mono-di and trimethylamine nitrogen and is found in the common pattern of spoilage. Increase in the content of TVB-N significantly correlated with the decrease in overall acceptability scores in the present study ($P < 0.01$). Similar results were obtained by Chakrabarti (1984) in Indian major carps, Magnusson et al. (2006) in cod, Sarma et al. (1998) in pink perch and oil sardine, Raju et al. (1998) and Asgharzadeh et al. (2010) in silver carp, Hulya et al. (2008) in *Gadus euxinus*, *Mugil cephalus* and *Encrasicholus*.



Source: Josefh et al. (1988)

Figure 5. TVB-N changes during iced storage of Rui (*Labeo rohita*).

Microbiological load analysis

We can determine the freshness and quality of fish before and after low temperature storage, by the determination of total plate count (TPC), Coliform count (CC) and *Psychrophillic* count (PC). The results presented in (Table 6) depicted that the total plate count (TPC) increased from initial load of 2.04±0.2 log cfu/g to 5.10±0.2 log cfu/g after 21 days of iced storage. The above results clearly revealed that the microbial growth was more rapid with increasing storage period. It has been found in the present studies that the TPC on 21st day of storage (5.10±0.2 log cfu/g) was within the permissible limit of 6 log cfu/g (ICMSF, 1986). Similarly, Bao et al. (2007) found faster microbial growth in chilled than in super chilled samples of Arctic Charr under the effect of dry ice. Similar inhancement on total bacterial load in fish muscle during ice storage was reported by Obemeata et al. (2011). Ozogul et al. (2011) also reported a significant statistical increase ($p \leq 0.05$) in the Initial total viable counts of whole gutted common sole (*Solea solea*) over the storage period of 24 days. Lawire (1998) attributed the growth of microbes to the growth promoting effect of moisture on microorganisms in fish muscle stored in ice and the less acid enzymatic reactions of fish flesh.

Table 6: Change in microbial count of fish muscle of Rui (*Labeo rohita*) during low temperature from 0 day to 21st day

Days of storage	0 Day	7th Day	14th Day	21st Day
TPC log cfu/g	2.04±0.2	4.01±0.11	4.30±0.07	5.10±0.02
CC log cfu/g	1.15±0.15	2.0±0.2	2.14±0.1	3.08±0.07
PC log cfu/g	2.15±0.2	3.30±0.04	4.45±0.1	5.96±0.05

Source: Gandotra et al. (2012)

Mean±SD with different superscripts in a row differs significantly ($P<0.05$)

The Coliform count increased exponentially with the increase in storage time. Perusals of (table 6) revealed that in 21 days of storage, it increased from initial value of 1.15±0.15 log cfu/g to final value of 3.08±0.07 log cfu/g, thus crossed the permissible limit i.e. 2.69 log cfu/g recommended by Goldenberg and Elliot (1973) after 14th day of storage.

The *Psychrophillic* count was also found to be increase from 2.15±0.2 log cfu/g on day 0 to 5.96±0.05 log cfu/g on final day of storage as shown in (Table 6), thus crossing the permissible limit i.e. 4.6 log cfu/g on 14th day as proposed by Cremer and Chepley (1977). During ice storage of Croaker (*Pseudotholitus senegalensis*), Ola and Oladipo (2004) found that *psychrophiles* increased in number and accounted for over 90% of the spoilage flora when fish were considered spoiled. The spoilage of fish during iced storage is influenced by *psychrophiles* irrespective of the initial bacterial flora according to their findings. Liu (2010) investigated that *Psychrophillic* bacteria grew exponentially from initial load of 3-4 log cfu/g, reaching 7.4 log cfu/g on day 13 and up to about 9.0 log cfu/g in tilapia fillets at the end of storage.

Physicochemical and microbial changes of Common carp (*Cyprinus carpio*)

Physical characteristics

These results showed that, the overall mean acceptability scores were found to decrease in the fish during the entire period of storage at low temperature. A negative correlation was observed between storage period and overall acceptability scores (Table 7). The overall acceptability of Common carp was acceptable for 12 days on comparing the scores with refrigerated storage period. The Anchovy fish had a shelf life of 6 days (Inanli et al., 2013).

Table 7: Changes in overall acceptability of Common carp during low temperature storage

Overall acceptability	
Storage period (Days)	**Scores**
0	9.21 ± 0.16^{g}
1	8.76 ± 0.17^{g}
3	8.41 ± 0.15^{fg}
5	7.64 ± 0.19^{f}
7	6.72 ± 0.15^{e}
9	5.26 ± 0.21^{d}
11	4.44 ± 0.18^{c}
13	3.50 ± 0.23^{b}
15	3.10 ± 0.11^{a}

Source: Bavitha et al. (2016)

^{abcdefgh} Means followed by the same superscript within a column are not significantly different (P > 0.01).

The initial moisture, protein, fat and ash content of Common carp fish were found to be 77.84 (±0.31) %, 16.95 (±0.10) %, 3.15 (±0.07) % and 1.17 (±0.03) % respectively. The proximate composition of the fish during the ice storage was given in Table 8. The moisture content in fish decreased significantly at 0.01% level (P <0.01) from 52.28 % to 48.46 %. An increase in the fat and ash content has been observed from 12.27 to 13.32 % and 1.51 to 2.12 % respectively (P<0.01). The increase in fat can be attributed to the decrease in moisture content as they are inversely proportional. Similar observation was found by the Ucak et al. (2011) during the refrigeration storage of mackerel fish burgers for 15 days. During storage, crude protein decreased significantly at (P<0.01). The decrease can be attributed to the leaching out of the water soluble nitrogenous components, during storage along with moisture. The ash content in the product was higher than the fresh fish. The remaining percentage of the proximate composition is thought to be due to carbohydrates (Tokur et al., 2006).

Table 8: Changes in the proximate composition* of Common carp during ice storage

Storage period (days)	Moisture%	Crude protein%	Fat%	Ash%
0	52.28±0.23[h]	6.47±0.04[g]	12.27±0.04[a]	1.51±0.02[a]
1	51.41±0.15[g]	6.36±0.03[f]	12.42±0.04[b]	1.55±0.02[a]
3	50.92±0.11[f]	6.15±0.03[e]	12.57±0.04[c]	1.63±0.01[b]
5	50.49±0.09[e]	6.00±0.06[d]	12.72±0.03[d]	1.69±0.02[bc]
7	50.15±0.06[de]	5.90±0.04[c]	12.92±0.02[e]	1.75±0.03[c]
9	49.84±0.09[d]	5.63±0.04[c]	12.97±0.02[fg]	1.83±0.01[d]
11	49.40±0.05[c]	5.43±0.04[b]	13.07±0.04[g]	1.90±0.01[e]
13	49.00±0.03[b]	5.19±0.01[a]	13.22±0.04[h]	2.01±0.02[f]
15	48.46±0.11[a]	4.92±0.04[a]	13.32±0.02[h]	2.12±0.03[g]

Source: Bavitha et al. (2016)

*Each value is represented as the mean±SD of n= 3. [abcdefgh] Means followed by the same superscript within a column are not significantly different (P > 0.01).

** On dry weight basis

Chemical composition analysis

The changes in pH, Peroxide Value (PV), Free Fatty Acid (FFA), Thiobarbutric Acid (TBA) and Total Volatile Base Nitrogen (TVB-N) values of Common carp during ice storage are shown in Table 9. The pH values of fish increased significantly (P <0.01) from 6.42 to 6.83. The observed increase in the pH was probably because of the microorganisms and enzymes release free oxygen and hydrogen, increasing hydroxyl ion concentration and thus causing a rise in pH (Turhan et al., 2001). During the initial storage period, PV of fish was found to be 6.12 $mEqO_2$/kg of fat and it increased to 9.97 $mEqO_2$/kg of fat at the end of the 5th day of storage and subsequently decreased to 7.86 $mEqO_2$/kg of fat, at the end of the 15 days of storage period. The decrease of the PV at the end of the storage may be owing to decomposition of hydro peroxides into secondary oxidation products and similar trend was also observed by the Yerlikaya et al. (2005) during the refrigerated studies of fish patties from anchovy. The FFA content increased from 0.19 to 0.75 % of Oleic acid. Increase in FFA results is attributed due to the enzymatic hydrolysis of esterified lipids (Hwang and Regenstein, 1993). A similar observation was made by Sowmya (2012) in Rohu and Sandhya (2014) in Mrigal during refrigerated storage of fish cutlets and fish fingers. TBA content showed an increasing trend in fish from the initial value of 0.88 mg MA/kg to 1.42 mg MA/kg by the end of storage period. The increase in TBA value during the ice storage of fish was significant at 0.01% level (P<0.01). Similarly, Inanli et al. (2013) observed an increasing trend of TBA results was observed in anchovy fish, from 0.85 to 1.25 mg MA/kg and also reported that, the reason for quite low TBA amounts obtained during the production and storage was the fact that the product consists of other ingredients along with fish. The TVBN content increased in fish from an initial value of 4.79 to 6.19 mg/100g sample, during 15 days storage period at low temperature. Inanli et al. (2013) reported that fish cake prepared from anchovy also showed an increase in the TVBN from 19.59 to 24.09 mg/100g of sample during the entire ice storage up to 12 days and also stated that, the value didn't exceed the limit during storage because the fish was not the only ingredient in the preparation.

Table 9: Changes in the biochemical parameters* of Common carp during ice storage

Storage period (days)	pH	PV (meqO2/kg fat)	FFA (% of Oleic acid)	TBA (mg MA/kg of sample)	TVBN (mg/100g of sample)
0	6.42±0.02^{a}	6.12±0.03^{a}	0.19±0.01^{a}	0.88±0.02^{a}	4.79±0.03^{a}
1	6.47±0.01^{ab}	7.28±0.03^{b}	0.23±0.01^{ab}	0.91±0.01^{ab}	4.88±0.01^{a}
3	6.51±0.01^{bc}	8.51±0.04^{e}	0.27±0.02^{bc}	0.96±0.02^{b}	5.02±0.02^{b}
5	6.57±0.01^{cd}	9.97±0.04^{i}	0.33±0.01^{c}	1.07±0.02^{c}	5.16±0.03^{c}
7	6.63±0.01^{de}	9.54±0.06^{h}	0.39±0.02^{d}	1.15±0.01^{d}	5.35±0.04^{d}
9	6.66±0.02^{ef}	9.31±0.04^{g}	0.46±0.01^{e}	1.21±0.02^{e}	5.56±0.04^{e}
11	6.71±0.03^{f}	8.70±0.07^{f}	0.55±0.03^{f}	1.30±0.02^{f}	5.76±0.02^{f}
13	6.78±0.02^{g}	8.22±0.04^{d}	0.62±0.01^{g}	1.38±0.02^{g}	5.94±0.03^{g}
15	6.83±0.02^{g}	7.86±0.04^{c}	0.75±0.03^{h}	1.42±0.02^{g}	6.19±0.02^{h}

Source: Bavitha et al. (2016)

*Each value is represented as mean±SD of n=3. ^{Abcdefgh} Means followed by the same superscript within a column are not significantly different (P > 0.01).

Microbiological load analysis

The initial TPC of fish cake was 9.50×10^{1} cfu/g of sample. At the end of 15 days of storage, the counts increased to 3.63×10^{4} cfu/g of sample shown in table 10. TPC analyses of fish did not exceed the maximum levels (7 Log. cfu/g of meat) of microbiological criteria for fresh and frozen fish given by the ICMSF (1978).

Table 10: Changes in the TPC in Common carp (*Cyprinus carpio*) during ice storage

Storage time	0 day	1 day	3 days	5 days	7 days	9 days	11 days	13 days	15 days
TPC	9.50×10^{1} (1.98)	2.29×10^{2} (2.36)	6.02×10^{2} (2.78)	1.04×10^{3} (3.02)	1.69×10^{3} (3.23)	3.25×10^{3} (3.51)	7.24×10^{3} (3.86)	1.47×10^{4} (4.17)	3.63×10^{4} (4.56)

Source: Bavitha et al. (2016)

CHAPTER IV
CONCLUSION

In Bangladesh, icing is mostly done in the period of marketing. Results of this study demonstrated that the quality of iced fish is decreasing day by day compared to the fresh fish but the rate of deterioration is slower than the fishes that are not preserved in ice. The physical appearances of iced fishes are not good as fresh fish. From viewing the proximate composition, it can say that these proximate compositions of iced fishes are lower than fresh fishes. The iced fishes are spoiled slowly during the storage period and safe for human consumption up to 10-15 days depending on the species, method of icing, ratio of icing, environment temperature etc. Therefore, it is necessary to maintain proper fish ice ratio (Ice: Fish 1:1 and 2:1 in winter and summer, respectively) in insulated ice box to preserve the fish for longer time.

CHAPTER V

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