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Original Article

Post Mortem Changes in Snake Headed Fishes (Taki, *Channa punctatus* and Shol, *Channa striatus*) During Storage at Room Temperature (28 to 30 °C) and in Ice

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A B S T R A C T

Studies were conducted on the post-mortem changes in freshwater snake headed fishes (Taki, *Channa punctatus* and Shol, *Channa striatus*) stored at room temperature (28 to 30°C) and in ice to examine the progress of rigor-mortis, changes in muscle pH and shelf life of the fishes by testing the organoleptic, biochemical and bacteriological parameters. Rigor started within 1h after death of fishes at both storage temperatures but it lasted long in ice stored fish Rigor-index reached to the maximum value of 82.14% and 85.13% within 8 h after death in Taki and Shol, respectively at room temperature while in ice stored condition values reached to the maximum of 89.09% within 9 h in Taki and 89.52% within 10hrs in Shol. With the lapse of storage time rigor-index increased gradually. The rigor relaxed up to 12.65% and 15.65% within 40 h of ice storage in Taki and Shol, respectively. The pH of fish muscles were 6.91 and 6.87 in Taki and Shol, respectively just after catch which decreased gradually with the progress of storage time at both storage temperatures. In another experiment, the quality changes in Taki and Shol, during 32 days of ice storage (in an insulated box) were evaluated. Score of organoleptic characteristics observation showed that, Taki and Shol were in acceptable conditions until 28 days in ice. The values for TVB-N, peroxide and NPN also showed that, the fishes remained acceptable state for the similar period. The myofibrillar protein solubility of muscle of the experimental fishes decreased from 86.32±0.012% and 88.24±0.006% to 32.48±0.012% and 33.69±0.006% within the same storage time. The initial bacterial loads were 7.50×10^3 and 6.20×10^3 CFU/g in Taki and Shol, respectively which increased to 9.23×10^8 and 8.9×10^8 CFU/g at the end of the 32 days of storage indicating that the fishes were organoleptically rejected after that period.

Introduction

The inland fisheries resources of Bangladesh are among the richest in the world with only China and India producing more inland fish than Bangladesh. Situated in the delta of the enormous Brahmaputra, Ganges and Meghna river system, the country's water, climatic and soil conditions are highly favorable to inland fisheries and aquaculture. Among the freshwater fish species Taki (*Channa punctatus*), Shol (*Channa striatus*) are very important for commercial purpose. These fishes bear high market price and are delicious, nutritious and popular to the consumers. Considering these points, it is necessary to take some steps for their proper marketing and also need to take initiatives to maintain premium quality of the fishes during transportation. Either domestic consumption or value added product preparation activities for export in the international market, good quality raw materials are essential prerequisite, because once the quality of the raw material gets deteriorated there is no way to improve the quality by any means of preservation. This implies entire management of fisheries chain at national or enterprise level. Fish is extremely perishable food stuff. After death of fish, the brain control as well as blood circulation ceases which results in an inability to resynthesize ATP and fail to transport various materials essential for living cells. The death-stiffening of the muscle tissue, referred as rigor mortis, occurs sooner or later and

glycolysis forms some organic acids to decrease in pH values. After passing the rigor stage the muscle tissue loses the stiffness and amino acids and other low molecular weight compounds are formed by autolytic process. By utilizing these compounds microorganisms grow and subsequently attack high molecular weight compounds. During the spoilage by microorganisms, some specific putrefactive substances such as trimethylamine and histamine are formed depending on fish species (Nowsad, 2007). When glycogen is degraded to lactic acid, the pH of the muscle tissue begins to fall from initial physiological values of 7.2 to 7.4 to the ultimate postmortem pH of 6.0 or less (5.6 for tuna, bonito and mackerel). Rate of pH decrease considerably depends on initial glycogen content of meat. Higher the glycogen content, lower is the pH values (Nowsad, 2007). The major source of ATP supply to the muscle fibres is lost after death of fish, since glycogen can no longer be oxidized completely to CO_2 and H_2O. After death, for some time- ATP is produced by anaerobic glycolysis and maintained at a definite level in the muscle by active creatine kinase (Nowsad, 2007).

In determination of quality of fish during ice storage some parameters such as freshness test, pH, TVB-N value, peroxide value, NPN and bacterial loads are usually determined. A considerable number of studies have been conducted on the stability of myofibrillar proteins and on the post-mortem quality changes in fish under various storage conditions. Such information has been accumulated over many years on most fishes of the temperate region. However, the scientific and practical knowledge on the keeping qualities of tropical or sub-tropical fishes is very limited. In recent years, the importance of storage temperature and pH for the shelf –life of fish and quality of fish muscle proteins have been realized. Therefore, considering these facts the present study has been carried out to observe the post mortem changes take place in the freshwater snake headed fishes (Taki, *C. punctatus* and Shol, *C. striatus*) during storage at room temperature (28 to 30°C) and in ice in an insulated box.

Materials and Methods

Materials

Live snake headed fishes (Taki, *C. punctatus* and Shol, *C. striatus*) were purchased from nearby K. R. market and immediately brought to the laboratory of the Department of Fisheries Technology, Bangladesh Agricultural University. After cranial spiking the samples were kept in ice (0°C) and at room temperature (28 to 30°C). Total body length of Taki and Shol used for the determination of rigor index at room temperature were 14.22 and 34.16 cm, respectively and in iced condition13.97 and 29.08 cm, respectively.

Analytical methods

Determination of rigor index

"Rigor index" of the fish was measured (plate 1 and 2) according to the method described by Bito *et al*. (1983) and used as a parameter of rigor tension. Briefly, immediately after catch and cranial spiking of fish was placed on a horizontal table in such a way that half of its body (tail part) kept out of the table. At selected time intervals, rigor index was calculated by the following formula:

$$\text{Rigor Index (\%)} = \frac{D_0 - D}{D_0} \times 100$$

Where D_o and D represent the distances of the base of caudal fin from horizontal line of the table at the start of the experiment and at subsequent storage periods, respectively.

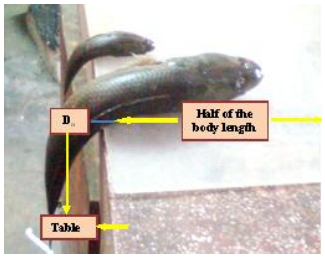

Plate 1. Determination of "Rigor-index" of fish

Plate 2. a. Taki/Lata (*Channa punctatus*) b. Shol (*Channa striatus*)

Measurement of muscle pH

During the determination time of rigor index 2 g of the ordinary muscle was dissected from the dorsal part of the sample and homogenized with 10 ml of distilled water in a blender. After adjusting the temperature, the electrode of the pH meter was put on the tube and the pH was measured by the pH meter (Modle 250 pH/ISE).

Organoleptic assessment

A large number of schemes have been proposed for sensory evaluation of various types of fish. Among them, the guidelines and methods given here using score on the organoleptic characteristics of fish as described by EC freshness grade for fishery products (Howgate*et al.*, 1992) which is shown in Table 1 and 2.

Table 1.Grading of fresh fish.

Grade	Points	Degree of freshness
A	<2	Excellent/Acceptable
B	2 to <5	Good/Acceptable
C	5	Bad Rejected

Table 2. Determination of defect points.

Sl. No.	Characteristics of whole fish		Defect characteristics	Defect points	Grade
1	Odor of neck when broken	a)	Natural odor	2	Acceptable
		b)	Faint or sour odor	5	Reject
2	Odor of gills	a)	Natural odor	1	Excellent
		b)	Faint sour odor	2	Acceptable
		c)	Slight moderate sour odor	3	Acceptable
		d)	Moderate to strong sour odor	5	Reject
3	Color of gills	a)	Slight pinkish red	1	Excellent
		b)	Pinkish red or brownish red., some mucus may be present	2	Acceptable
		c)	Brown of gray color covered with mucus	3	Acceptable
		d)	Bleached; thick yellow slime	5	Reject
4	General appearance	a)	Full bloom; bright; shining; iridescent	1	Excellent
		b)	Slight dullness and loss of bloom	2	Acceptable
		c)	Definite dullness and loss of bloom	3	Acceptable
		d)	Reddish lateral line; dull; no bloom	5	Reject
5	Eyes	a)	Bulging with protruding lens; transparent eye cap	1	Excellent
		b)	Slight clouding of lens and sunken	2	Acceptable
		c)	Dull, sunken, cloudy	3	Acceptable
		d)	Sunken dye covered with yellow slime	5	Reject
6	Slime	a)	Usually clear, transparent and uniformly spread but occasionally may be slightly opaque or milky	1	Acceptable
		b)	Becoming turbid opaque and milky, with marked increase in amount of slime present in skin	2	Acceptable
		c)	Thick, sticky, yellowish greenish in color	5	Reject
7	Consistency of flesh	a)	Firm and elastic	1	Acceptable
		b)	Moderately soft and some loss of elasticity	2	Acceptable
		c)	Some softening	3	Acceptable
		d)	Limp and floppy	5	Reject

Proximate composition analysis

AOAC (1990) method was followed for bio-chemical analysis. Homogeneity of the samples was maintained by using a blender. For each analysis of proximate composition, triplicate samples were used for each analysis.

Total Volatile Base Nitrogen (TVB-N)

The Total Volatile Base Nitrogen (TVB-N) was determined according to the methods given in AOAC (1990). The results were expressed as mg of TVB-N/100 g sample according to the following equation:

$$\text{Amount of TVB - N (mg/100g) sample} = \frac{\text{ml titrant} \times 0.014 \times \text{normality of acid}}{\text{Sample weight}} \times 100$$

Non-protein nitrogen (NPN) estimation

Total Non-protein nitrogen (NPN) in freshwater snake headed fish samples were determined by the following procedure as was done by Konusu *et al.*, 1974.

The NPN content of the sample was calculated by using the following formula:

$$\text{Total NPN (\%)} = \frac{0.014 \times \text{ml of titrant} \times \text{strength of titrant}}{\text{Sample weight}} \times 100$$

Peroxide value estimation

The method for the determination of peroxide value was described by Egan *et al*. (1981) and adopted from Wood and Aurand (1977). The peroxide value was calculated as follows:

Peroxide value = 2(S-B)/W, m. eq/kg of oil.

Where, 'S' is sample titre, 'B' is blank titre and 'W' is weight of sample oil in g.

Changes in the protein solubility

Proteins are polymer of amino acids. Myofibrils are a part of muscle protein. Myofibrillar protein solubility indicates the denaturation rate of protein.

Preparation of myofibrils

Myofibrils were prepared from ordinary muscles immediately after excision according to Perry and Grey (1956) with slight modification and the procedure is shown in (Figure 1).

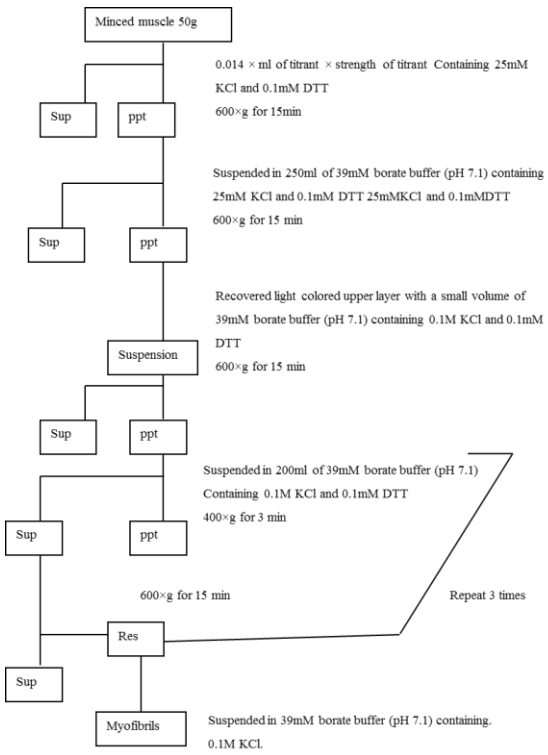

Figure 1. Preparation of fish myofibrils

Myofibrllar protein solubility

Two ml of myofibrillar suspensions (5mg/ml) were homogenized with 2ml of 1M KCl plus 100mM phosphate buffer (pH 7.0) using a homogenizer. The homogenate was allowed to stand at refrigerated temperature (4^0C) overnight. The suspension was centrifuged for 30min at 900×g in cold condition. The protein in supernatant was determined by Biuret method (Gornall *et al.*, 1949).

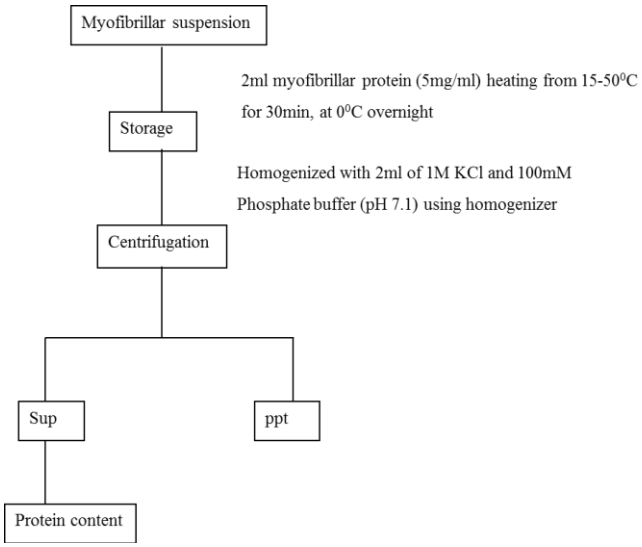

Figure 2. Determination of solubility of muscle myofibrils

Aerobic plate count

Aerobic plate count of the samples was done by spread laboratory as per direction of Cowan and Steel's Manual for the Identification of Medical Bacteria (edited by Barrow and Felthham, 1993) and with the help of DIFCO, Manual of Dehydrated Culture Media and Reagents, 9th edition, 1964. Number of bacteria per gram of the fish sample (CFU/g) was calculated by using the following formula:

$$\text{Aerobic plate count (APC) in CFU/g of fish} = \frac{C \times D \times 10 \times V}{S}$$

Where,

C = Number of colonies found
D = Dilution factor
V = Volume of original sample
S = Weight of sample in grams
CFU = Colony forming unit.

Results and Discussion

Rigor index

Rigor index of experimental fishes are presented in Table 3. The progress of rigor mortis was more rapid in fishes stored at room temperature than in ice. Rigor started 1 h after spiking and it reached to maximum value of 82.14 and 85.13% within 8 h after death in Take and Shol, respectively while fishes were stored at room temperature, On the other hand, rigor reached to the maximum value of 89.09% in Taki and 89.52% in Shol within 10 h of death while these fishes were stored in ice. The in rigor stage continued for about 3 h then the fish muscle started to relax in iced condition. The rigor relaxed up to 16.23 and 15.65% in 40 h in both fishes without emitting any foul odor. Several studies have revealed that the biochemical process leading to rigor-mortis is similar in the muscles of great variety of species of fish and different warm blooded animals but the onset and the rate of progress of rigor-mortis varies from species to species and temperature of storage medium plays important role. It has been reported that lower temperature delays the onset of rigor, that is, lengthens the pre-rigor period and also slows down the rate of progress of rigor-mortis (Poulter *et al.*, 1981; Iwamoto *et al.*, 1985, 1987, 1988; Iwamoto and Yamanaka, 1986; Amlacher, 1961 and Yamamoto and Noguchi, 1964). However, in the present study an early onset of rigor was observed at lower temperature in ice and a prolonged period of in-rigor was observed for Taki and Shol than that of at room temperature.

Table 3. Changes in rigor- index (%) of freshwater snake headed fishes (Taki, *Channa punctatus* and Shol, *Channa striatus)* at room temperature (28 to 30^{0}C) and in ice.

Time (Hours)	Rigor Index (%) at room temperature		Rigor Index (%) in ice	
	Channa punctatus	*Channa striatus*	*Channa punctatus*	*Channa striatus*
1	17.39	14.13	28.51	18.76
2	25.56	23.65	34.35	24.3
3	35.47	34.56	40.18	33.13
4	43.32	45.69	47.36	39.32
5	56.91	54.37	58.16	47.14
6	67.29	66.58	67.13	55.32
7	76.19	79.36	74.55	66.27
8	82.14	85.13	80.36	75.58
9	82.14	85.13	89.09	82.66
10	82.14	85.13	89.09	89.52
11	76.15	78.35	89.09	89.52
12	69.30	70.19	88.93	89.52
13	63.53	65.37	88.76	89.49
14	57.19	59.28	88.30	89.4
15	52.38	52.16	88.01	89.19
16	45.18	43.38	87.50	89.00
17	39.06	36.58	85.37	88.89
18	33.34	29.49	83.13	88.69
19	24.54	22.38	80.25	85.68
20	16.93	13.52	79.48	83.56
22			74.24	78.65
24			69.87	72.39
26			63.19	66.19
28			57.35	60.3
30			50.19	54.29
32			44.32	47.52
34			38.59	40.23
36			29.12	33.2
38			20.24	24.53
40			12.65	15.65

pH change

The changes in pH of experimental fishes stored at room temperature and in ice is presented in Table 4. Just after catch, the pH of the muscle found 6.91 and 6.87 in Taki and Shol, respectively which started to decline gradually with the lapse of storage time, regardless of the storage temperatures. In post-mortem muscle the rate in drop of pH is a function of ATPase activity (Scopes, 1974). Among two temperatures, the decrement in pH in muscle was much faster at room temperature and this change in rate might be due to the accelerated turnover of ATP at high temperature (Watabe *et al.*, 1991). The decrease in pH occurs at the initial stage of rigor might be due to the post mortem anaerobic formation of lactic whereas the increment in pH value at the later phase of storage at room temperature might case by the formation of basic compounds. After 20 h of storage, the ultimate pH were obtained 6.15 and 6.30 at room temperature and 6.67

and 6.70 in ice for Taki and Shol, respectively. The initial post-mortem pH varies with species, catching ground and season. The catching method does not seem to influence the final value of the post-mortem pH (Love, 1980). While the onset of rigor is quick, its duration is shorter and the values decline much faster than the rigor which develops slowly.

Table 4. Changes in muscle pH of freshwater snake headed fishes (*Channa punctatus* and *Channa striatus*) at room temperature (28 to 30°C) and in ice.

Time (Hours)	pH			
	At room temperature		In ice	
	Channa punctatus	*Channa striatus*	*Channa punctatus*	*Channa striatus*
0	6.91	6.87	6.85	6.87
2	6.82	6.81	6.85	6.87
4	6.71	6.75	6.84	6.86
6	6.64	6.68	6.84	6.85
8	6.57	6.63	6.82	6.84
10	6.52	6.59	6.8	6.83
12	6.45	6.54	6.78	6.81
14	6.33	6.49	6.76	6.79
16	6.28	6.43	6.73	6.76
18	6.23	6.37	6.7	6.73
20	6.15	6.30	6.67	6.7

Organoleptic quality assessment

Organoleptic quality assessment results of freshwater snake headed fishes, Taki and Shol during storage in ice are presented in Table 5. The grades were defined as the total number of defects points (Table-2). The points less than 2were considered as excellent. The points from 2 to less than 5 were considered as good or acceptable and while the score point was 5 and above was considered as rejected. The scores based on sensory assessments, the freshwater snake headed fishes, Taki and Shol were found in acceptable condition until 32 days of storage in ice. During this period, changes in quality can roughly be divided into 8 steps corresponding from 0 to 4, 5 to 8, 9 to 12, 13 to 16, 17 to 20, 21 to 24, 25 to 28 and 29 to 32 days in iced condition. In step 1 (0 to 4 days) both fishes were fresh initially with bright appearance, soft-firm texture and characteristic natural fishy odor though at the later phage brightness and natural odor started to reduce. Slight soft texture; loss in natural fishy odor, some slime on surface could be found at the advance stage of step-3 (8 to 12 days). In step 7 (25 to 28 days) texture begun to show definite signs of spoilage with dull appearance, ununiformed distribution of blood and slime on surface. The fishes were found spoiled in all consideration by losing their all characteristic odor, texture, general appearance at the la t stage of step 8 (28 to 32days).

Table 5. Changes in organoleptic characteristics of *Channa punctatus* and *Channa striatus* during storage in ice in an insulated box.

Days of storage	Organoleptic qualities	Mean defect points		Grade		Overall qualities	
		Channa punctatus	*Channa striatus*	*Channa punctatus*	*Channa striatus*	*Channa punctatus*	*Channa striatus*
0	Fresh, bright appearance, soft and firm texture with characteristics of natural fishy odor.	1.25	1.2	A	A	Excellent	Excellent
4	A decrease in the brightness; slightly softer texture, natural fishy odor.	1.6	1.5	A	A	Excellent	Excellent
8	Some loss in brightness; slight loss of the natural fishy odor.	2.2	2.1	B	B	Acceptable	Acceptable
12	Some slime on surface and slight soft texture; some loss of natural fishy odor.	2.5	2.4	B	B	Acceptable	Acceptable
16	Slimy surface and moderately soft texture; some loss of natural fishy odor.	2.9	2.8	B	B	Acceptable	Acceptable
20	Soft texture and slime on surface; loss of natural fishy odor.	3.45	3.4	B	B	Acceptable	Acceptable
24	Soft texture; bloody appearance; loss of natural fishy odor.	3.85	3.8	B	B	Acceptable	Acceptable
28	Definite signs of spoilage with dull appearance, ununiformed distribution of blood and slime on surface.	4.6	4.5	B	B	Acceptable (in a limit)	Acceptable (in a certain limit)
32	The fishes are putrid in all consideration by losing all characteristic odor, texture, general appearance.	5.0	5.0	C	C	Rejected	Rejected

Some available information on Indian major carps and other commercial fish species have showed that these fishes can be stored in ice as edible condition for about 2-3 weeks. Kamal *et al*. (1994) have reported that hilsa which was transported in an insulated box in ice immediately after catch, remained acceptable until 18 days; while the fish obtained from Mymensingh wholesale fish market and stored in ice in a wooden box were organoleptically acceptable for 8 days. Faruk (1995) assessed the organoleptic quality of rohu during storage in ice in an insulated box and found the fish in acceptable condition till 20 days. The shelf life of *Catla catla* and*Labeo fimbriatus* was reported to be 18 days while stored in ice (Bandyopadhyay *et al*, 1986). Rubbi *et al*., (1985) studied the shelf life of six freshwater fish species in different storage temperature by subjective and objective parameters. The spoilage rates were found to increase with the increase of storage temperature for all the six varieties. Bamboo baskets and wooden boxes, insulated with hogla mat (the spongy leaves of a local plant), were reported to be most efficient for maintaining the quality of fish in ice for transportation. Muslehuddin *et a1*. (1986) observed that soaking of fish in 15% salt concentration contribute to extend the shelf life of Mola fish (*A. mola*) up to 134 h at low temperature (2°C). On the other hand Hye *et a1*., (1990) suggested that fish-ice ratio of 4:1, 2:1 and 1:1 could preserve the shelf life of fish up to 2 days, 5 days and 6 days, respectively in the insulating box consisting of polyethylene material. However, information on the shelf life of the

freshwater snake headed fishes, Taki and Shol are scare. Therefore, the results obtained in the present study suggest that-the shelf life of the freshwater snake headed fishes, Taki and Shol are not similar organoleptically to that of reported for carps and other freshwater fish species, because the biochemical changes after death is slower in snake headed fishes. The available studies also reveal that the shelf life of the fishes varies from the species to species due to some factors like- the chemical composition of the fish, the temperature at which the fishes are stored etc.. Fatty fish are more susceptible to spoilage even in ice. Herring with a fat content of 50% or more can become inedible after only one to two days and mackerel become inedible after 4 to 5 days in ice (FAO, 1975).

Proximate composition

The proximate composition of Taki and Shol muscles are presented in Table 6. Moisture, protein, lipid and ash contents in muscles immediately after death were 76.83, 20.73, 1.08 and 1.36% in Taki and 77.47, 20.44, 0.94 and 1.15% in Shol, respectively. Percent moisture increased in the samples with the progress of storage period and at end of 32 days of storage the values increased from 76.83 to 80.15 and 77.47 to 80.61; protein content decreased from 20.73 to 17.63, and 20.44 to 17.48 in Taki and Shol, respectively. Percent lipid values decreased from 1.08 to 0.84 and 0.94 to 0.73 in Taki and Shol, respectively. Ash consent did not show any significant change in the fishes.

Table 6. Changes of proximate composition of *Channa punctatus* and *Channa striatus* during ice storage in an insulated box.

Days of storage	Moisture (%) (Mean ± SE)		Protein (%) (Mean ± SE)		Lipid (%) (Mean ± SE)		Ash (%) (Mean ± SE)	
	Channa punctatus	*Channa striatus*	*Channa punctatus*	*Channa striatus*	*Channa punctatus*	*Channa striatus*	*Channa punctatus*	*Channa striatus*
0	76.83±1.10	77.47±2.00	20.73±1.10	20.44±0.98	1.08±0.046	0.94±0.012	1.36±0.006	1.15±0.029
4	76.86±2.03	77.51±0.99	20.68±2.60	20.38±1.02	1.07±0.012	0.93±0.012	1.39±0.006	1.18±0.012
8	76.93±1.00	77.91±0.99	20.63±0.94	20.01±2.00	1.06±0.017	0.92±0.012	1.38±0.006	1.16±0.012
12	77.65±0.80	78.26±1.90	19.82±0.90	19.56±0.97	1.04±0.012	0.90±0.012	1.49±0.006	1.28±0.012
16	78.16±0.90	78.72±1.98	19.41±1.04	19.21±0.21	1.01±0.006	0.87±0.012	1.42±0.012	1.20±0.006
20	78.89±1.01	79.51±0.90	18.76±1.12	18.49±0.49	0.97±0.025	0.84±0.006	1.38±0.012	1.16±0.006
24	79.35±1.00	79.89±1.01	18.33±0.84	18.12±0.12	0.93±0.006	0.81±0.006	1.39±0.006	1.18±0.023
28	79.69±0.80	80.33±1.80	18.00±0.05	17.69±1.99	0.89±0.006	0.77±0.017	1.42±0.006	1.21±0.006
32	80.15±1.85	80.61±0.90	17.63±1.10	17.48±0.98	0.84±0.006	0.73±0.012	1.38±0.012	1.18±0.012

Absorption of moisture from surrounding ice and losses of other components (protein, lipid) might be the reason of increase in percent moisture. Total crude protein in fish flesh decreased with the lapse of storage period. Some loss of organic nitrogenous constituent's largely sarcoplasmic proteins and inorganic salts with free drip are probably the contributing factors for such loss as Tarr (1965) reported for chilled fish. A decrease in lipid content during storage period could be explained by their individual variation and oxidation since lipid content varies greatly even within the same species. Devadasan *et al.* (1978) examined the proximate composition of *Labeo rohita, Catla catla, Cirrhinus mrigala, Labeo calbasu, Mystus seenghala* and *Wallago attu.* The moisture content in the above mentioned raw fishes were 77.71, 76.28, 77.47, 76.26, 80.83 and 77.11%, respectively. The protein values for the same fishes were 19.60, 17.74, 18.72, 16.66 and 15.76%, respectively; the lipid values were 1.83, 1.32, 2.89, 1.19 and 3.16% and ash values were 1.31, 0.93, 1.40, 1.03, 0.91 and 0.72%, respectively. Gheyasuddin *et al.* (1979) determined the proximate composition of some important fishes of the Bay of Bengal such as silver pomfret *(Stromateus cinereus),* ribbon fish *Trichiurus haumela),* Indian salmon *(Polynemus indicus)* and Bombay duck *(Harpodon nehareus).* The moisture contents of the above mentioned fish species were 78.70, 77.34, 77.24 and 82.21%, respectively. The protein values were 16.70, 16.60, 17.67 and 15.10%; lipid values were was 2.10, 2.52, 2.61 and 1.53% whereas the values for ash content were 1.95, 2.60, 2.40 and 1.20%, respectively. Hossain *et al.* (1999) obtained the crude protein and lipid in *Mystusvittatus*17.59 and 2.76%, respectively. The proximate composition analysis of fresh chapila showed percent moisture value 77.51, protein 15.59% , fat 3.58 and ash 2.75 whereas in tengra the percent moisture value was 78.54, protein 17.01, fat 2.1 and ash 2.06 in edible portion (Chakraborty *et al.*, 2003). The proximate composition of different fishes varied might be due to species variation. The results of present study coincides with the above mentioned finding.

Changes in TVB-N

The changes in TVB-N (mg/100g) value of experimental fishes are presented in Figure 1. Initially the TVB-N values were found 1.76 and 1.37 mg/100g in Taki and Shol, respectively, which gradually increased with lapse of storage time. After 24 days of storage, TVB-N values increased up to 23.87 and 22.98 mg/100 in Taki and Shol, which was within the range of recommended values of 25 to 30 mg/100g for fresh fish. On 28th day of storage, the values increased up to 28.98 and 27.65 mg/100g, respectively in Taki and Shol which were still within the recommended value for fresh fish. However, the TVB-N Values were 36.45 and 33.79 at the end of 32 days of ice storage that exceeded the recommended values.

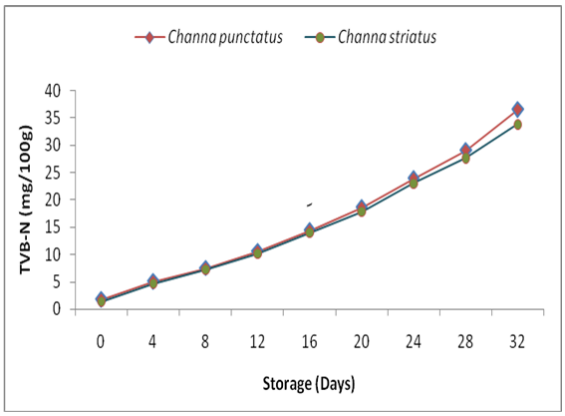

Figure 1. Changes in Total Volatile Base Nitrogen (TVB-N) mg/100g of freshwater snake headed fishes (*Channa punctatus* and *Channa striatus*) during ice storage in an insulated box.

The available reports suggest that- the upper limit of TVB-N/ 100g is considered 30 mg for finfish acceptability (Connell, 1975). The increase in TVB-N with the lapse of storage period may be attributed to bacterial spoilage. However, the information indicate that, TVB-N mainly accumulated in the fish flesh during the later phase of spoilage while the bacterial population has grown. Thus the TVB-N is low during the edible storage period and relies when the fish is near rejection. Also there is a large variation between species in the development of TVB-N values.

Changes in Non-Protein Nitrogen (NPN)

Figure 2 shows the changes in NPN values of fresh water snake headed fishes (*Channa punctatus* and *Channa striatus*) stored in ice. The initial NPN values were 0.06 of total nitrogen in both fishes. A clear trend of increase in NPN content with the progress of storage period was observed. At the end of 32 days of storage the NPN values increased to 0.23 and 0.21 of total nitrogen in Taki and Shol, respectively. However, organoleptically Taki (*Channa punctatus*) and Shol (*Channa striatus*) were in acceptable condition up to 28 days of ice storage which corresponds with NPN values of 0.20 and 0.18 of total nitrogen, respectively.

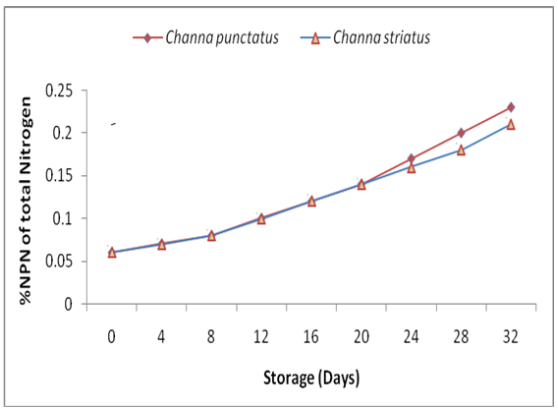

Figure 2. Changes in % Non Protein Nitrogen (% NPN) of freshwater snake headed fishes (*Channa punctatus* and *Channa striatus*) during ice storage in an insulated box

NPN usually accounts from 9.2to 18.3% of total nitrogen in teleost and from 33 to 38.6% in elasmobranch fishes (Tarr, 1958). However, Indian marine fishes are very low in NPN content (Velankar and Govindan, 1958), the reason is not yet explained. For fresh water fishes also Stansby (1963) has given a similar opinion. Kamal (1977) found NPN content in frozen ribbon fish *(Trichiurus haumela);* Bombay duck *(Harpodon neherius)* and Indian salmon *(Polvnemus indicus)* 0.38, 0.36 and 0.39g/100g. Lower NPN value confirms comparatively better quality product. The results obtained in the present study are in agreement with the above mentioned findings.

Changes in peroxide values

The changes in peroxide values of fresh water snake headed fishes (*Channa punctatus*, Taki *and Channa striatus*, Shol) during ice storage are shown in Figure 3. The initial peroxide values were 1.3 and 1.1 m.eq/kg of oil in Taki and Shol, respectively which increased gradually with the progress in storage period. On 24th day of storage, peroxide values were found 15.79 and 14.06 m.eq/kg of oil in Taki and Shol, respectively later on which values reached to 18.45 and 17.56 m.eq/kg of oil on 28th day of storage. At the end of 32 days of storage in ice, the values were 23.89 and 21.78 m.eq/kg of oil.

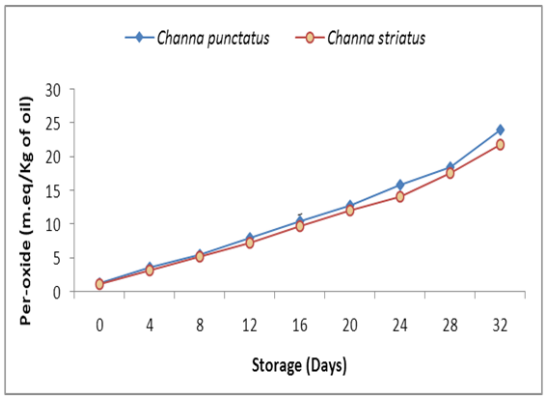

Figure 3. Changes in peroxide value (m.eq. /kg of oil) of freshwater snake headed fishes (*Channa punctatus* and *Channa striatus*) during ice storage in an insulated box.

The recommended value of peroxide for fresh fish is 10-20 m.eq/kg of oil (Connell; 1975). The value above 20, the fishes emit bad smell and rancid taste. The peroxides are further oxidized to aldehydes and ketones which have a very disagreeable fishy or rancid odor and taste. However depending on the fish species and storage condition a good correlation between peroxide value and organoleptic quality may be found. In the present study the peroxide value for both fishes exceeded the recommended value after 28 days of storage indicating the inedible condition of the fishes.

Changes in protein solubility

Myofibrillar protein solubility were 86.32% and 88.24% in Taki and Shol, respectively just after catch of fish which decreased to 32.48% and 33.69% at the end of 32 days of ice storage (Figure 4). The solubility decreased continuously with the increase of storage period.

The results obtained from the present study indicate that denaturation of muscle protein occurred during storage and this result is more or less in agreement with Seki *et al.* (1979) who reported that solubility of carp myofibrils decreased from 95% to 20% during ice storage within 2-3 weeks. Similar results were also obtained by Hossain, (1995) for mrigal (*Cirrhina mrigala*) and Faruk, (1995) for *Labeo rohita.* According to Seki *et al.* (1979); Kramer and Peters,

(1981) the large fall in solubility during ice storage was due to lowering of pH.

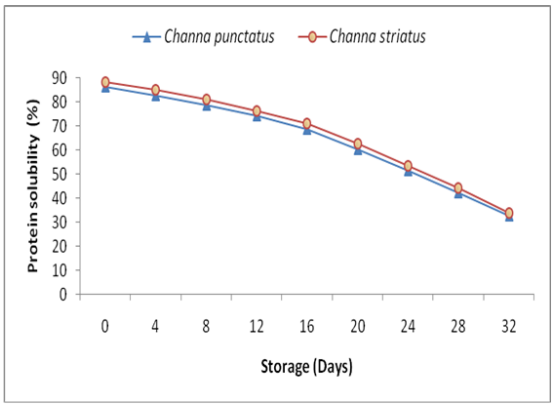

Figure 4. Changes in muscle protein solubility of freshwater snake headed fishes (*Channa punctatus* and *Channa striatus*) during ice storage in an insulated box

Bacteriological change

Very limited information are available on the microbial changes in ice stored freshwater snake headed fishes, Taki and Shol. The results of the present study on bacterial loads in muscle of fresh water snake headed fishes, Taki and Shol during ice storage are shown in Table 7. The initial bacterial loads were 7.50×10^3 and 6.2×10^3 CFU/g in Taki and Shol, respectively which gradually decreased to 6.32×10^3, and5.03×10^3 CFU/g at 4th day of storage. Then bacterial loads increased again with the lapse of storage time. On 24th day of storage in ice, bacterial load increased to 5.74×10^6 CFU/g and $4.30\text{x}10^6$ in Taki and Shol though fishes were in acceptable condition organoleptically at this stage. At the end of 32 days of storage in ice, the bacterial loads were 9.23×10^8 and 8.9×10^8 CFU/g, in Taki and Shol, respectively.

Table 7. Changes in bacterial load (CFU/g) of freshwater snake headed fishes (*Channa punctatu\s* and *Channa striatus*) during ice storage in an insulated box.

Days of storage	*Channa punctatus*		*Channa striatus*	
	CFU/g	LogCFU/g	CFU/g	Log CFU/g
0	7.50×10^3	3.88±0.012	6.20×10^3	3.79±0.006
4	6.32×10^3	3.80±0.012	5.03×10^3	3.70±0.017
8	3.45×10^4	4.54±0.006	2.14×10^4	4.33±0.012
12	7.78×10^4	4.89±0.006	6.29×10^4	4.80±0.058
16	2.85×10^5	5.45±0.023	1.73×10^5	5.23±0.012
20	5.56×10^5	5.75±0.012	4.19×10^5	5.62±0.012
24	5.74×10^6	6.76±0.012	4.3×10^6	6.63±0.001
28	8.85×10^7	7.95±0.029	7.17×10^7	7.86±0.006
32	9.23×10^8	8.97±0.012	8.9×10^8	8.95±0.012

The initial decrease in bacterial population in fish muscle found might be due to some sort of cold shock or leaching of surface flora by washing with melted ice. This is an agreement with report for some ice stored fresh water fishes (Bandyopadhyan *et al.*, 1985). Frazier and Westhoff (1990) stated that- the number of bacteria in gill, intestine and or the skin of newly caught fish vary from species to species and also depend on the microbial load of the waters in which they live.

Conclusions

The study results showed that, though rigor started in snake headed fishes1 h after spiking in both temperatures but rigor reached to it's maximum values within 8 to 9 h and 10 h at room temperature and in ice, respectively. The progress of rigor-mortis was slower in ice. Organoleptically fishes were found in acceptable condition up to 28 days then were completely rejected on 32nd day of storage in ice.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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