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Biochemical and Microbial Changes during Ice Storage of *Mystus gulio* and *Puntius sophore*

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Abstract

Biochemical, microbial and organoleptic changes in two commercially important fishes (*Mystus gulio* and *Puntius sophore*) during ice storage have been studied. Ash content, lipid content, total nitrogen and non-protein nitrogen showed a decreasing trend whereas pH and salt soluble nitrogen showed reverse trend during the ice storage period. TVBN increased to 29.87 mg% on 10^{th} day in *P. sophore* whereas, it crossed the border line of spoilage on 13^{th} day in *M. gulio*. Aerobic plate count reached to $10^6 g^{-1}$ on day 13 and day 10 for *M . gulio* and *P. sophore* respectively. Results obtained in the sensory analysis correlated well with the observed biochemical changes. The study indicated that the edible quality of *M. gulio* and *P. sophore* was maintained upto 13 and 10 days respectively when stored with ice.

Keywords: *Mystus gulio, Puntius sophore,* ice storage, biochemical changes, shelf life

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Introduction

It is known that loss of freshness and spoilage pattern in fish markedly varies from species to species. Post-mortem biochemical changes in fish muscle are strongly influenced by the post-catch handling practices. Fish decomposition is a progressive proteolysis of the muscle tissue brought about mainly by the action of microorganisms and, to a lesser extent, by autolytic enzymes. Because the changes during the decomposition are known to be very complex, a single chemical index may not be a reliable indicator for a particular sample of fish (Castillo-Yanez et al., 2007).

The rate of post mortem biochemical changes determines the shelf-life of fish. Number of works has been reported on biochemical changes of medium to big sized freshwater fish during ice storage, whereas, such reports on small variety fishes are very limited. *Mystus gulio* and *Puntius sophore* are two small sized food fish of high commercial importance in Northeastern region of India. This study reports post mortem biochemical and microbial changes of *P. sophore* and *M. gulio so* as to establish their shelf-life under ice storage conditions.

Materials and Methods

Fresh *M . gulio* and *P. sophore* used for the study were procured from the local market in Lembucherra and brought to laboratory in iced condition. Average size and weight of the fish were 13.3 ± 0.3 cm, 26.6 ± 1.6 g and 9 ± 0.5 cm, 12.26 ± 0.99 g respectively for *M. gulio* and *P. sophore*. In the laboratory, fishes were washed thoroughly and stored in insulated boxes (each species in two boxes) in alternating layer of ice-fish-ice. Flake ice was used at the rate of 1:1. The boxes were kept in ambient temperature (23.5 – 34.6^{0}C). Sampling for sensory and biochemical analysis was done every two days during period of storage. During sampling, partial replenishment of ice was done as per requirement. Sensory and biochemical analysis was also done for fishes before icing.

During sampling eight to ten fish in the case of *P. sophore* and four to five fish in the case of *M. gulio* were collected from different locations of each box. Since *P. sophore* is consumed as whole (with bone), the fishes were descaled and mixed in a grinder to consider as representative sample. But for *M. gulio*, flesh with skin was taken from each fish and mixed to make a representative sample. Entrails and gills were removed in the case of both the fishes.

Moisture, pH, ash, total lipid, total nitrogen and non-protein nitrogen analysis of both the fish was carried out using standard procedures of AOAC (2000). Salt soluble nitrogen (SSN) was estimated following the method of Dyer et al.(1950). Total volatile basic nitrogen (TVBN) was determined by using Conway's micro-diffusion method (Conway, 1947). Peroxide value (PV) was determined on chloroform extracts of tissues according to the methods suggested by Jacobs (1958).

Thiobarbituric acid reactive substances (TBARS) were determined as described by Benjakul & Bauer (2001). The flesh (0.5 g) was dispersed in 2.5 ml of a solution containing 0.0375% thiobarbituric acid, 15% trichloroacetic acid and 0.25N HCl. The mixture was heated in boiling water for 10 min, followed by cooling in running tap water. The mixture was centrifuged at 8000 rpm for 20 min at room temperature. The absorbance of supernatant was measured at 532 nm using a spectrophotometer (SHIMADZU, UV 2550). Aerobic plate count was estimated as per APHA (2001).

Sensory characteristics and overall acceptability were assessed by a panel of six members belonging to the College of Fisheries, Lembucherra, Tripura on the basis of ten point scale on each sampling as suggested by Sukumar et al. (2007). Sensory characteristics study included general appearance, odour and texture of fish. The scores were given in the decreasing order scale with 10 – 9 for excellent, 8 – 7 for good, 6 – 5 for fair and acceptable, 4 – 3 for poor and 2 – 1 for very poor. The mean of the scores given by the panel represented the overall sensory quality. A score of 3 to 4 was considered unacceptable. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 11.0 for Windows).

Results and Discussion

Proximate composition of *M. gulio* and *P. sophore* is given in Table 1. The percentage of protein, salt soluble protein and non-protein nitrogen is almost similar in both the fish and is comparable to carps. With respect to fat content, the fishes are in the fatty fish group. Since *Puntius* like small variety fishes are consumed whole, the estimated high value of ash content was due to ashing of whole fish.

Table 1. Proximate analysis of *Mystus gulio* and *Puntius sophore*

Composition	*Mystus gulio*	*Puntius sophore*
Moisture	74.76 ± 1.75	72.55 ± 1.79
Crude protein (%, muscle)	16.06 ± 0.12	16.19 ± 0.25
Salt soluble protein (%, muscle)	10.56 ± 0.19	10.5 ± 0.12
NPN (%, muscle)	0.35 ± 0.02	0.37 ± 0.03
Lipid (%, muscle)	5.69 ± 0.05	4.49 ± 0.34
Ash (%, muscle)	1.58 ± 0.05	5.72 ± 0.25

Changes of biochemical parameters during ice storage for *M. gulio* and *P. sophore* respectively are presented in Table 2 and 3. The moisture content registered a steady increase for both samples during storage *viz.*, increase was 6.18% in the case of *M. gulio* and it was significantly different ($p<0.05$).In the case of *P. sophore*, the increase (3.6%) was statistically not significant ($p>0.05$). The correlation (r) between the period of storage and change in moisture content was found to be 0.82 and 0.87 in the case of *M. gulio* and *P. sophore* respectively. Joseph et al. (1988) reported a similar trend during iced storage of cultured *Labeo rohita*. The uptake of moisture due to exposure of fish to ice melt water was found to be more in the case of *M. gulio* than that of *P. sophore*. This difference could have occurred due to presence of scales in the case of *P. sophore* whose barrier effect is more than the scale-less *M. gulio*.

A significant ($p<0.05$) increase of pH in both the fishes during storage was observed *viz.*,6.65 and 6.63 in the case of *M. gulio* and *P. sophore* respectively at the end of the storage. The correlation coefficient (r) between the period of storage and change in pH were found to be 0.99 and 0.89 in the case of *M. gulio* and *P. sophore* respectively. Ababouch et al. (1996) reported increase in pH in sardine while Nunes et al. (1992) and Ruiz-Capillas & Moral (2001) noted similar results in other fish species stored in flake ice. Variations among the initial pH values may be due to the species, season, and diet, level of activity or stress during the catch as well as type of muscle.

In both species, ash content remained almost constant with non significant changes through the storage period. Total nitrogen (TN) content in both the fishes showed a gradual decrease and reached to 2.27% (88.3% initial value) and 2.45% (94.6%

Table 2. Biochemical changes during ice storage of *Mystus gulio** (n=3)

Composition	Storage period (Days)				
	1	4	7	10	13
Moisture	74.76[a]	78.25[b]	79.2[b]	79.16[b]	79.38[b]
pH	6.32[a]	6.38[b]	6.46[c]	6.59[d]	6.65[e]
Ash	1.58[c]	1.51[b]	1.54[a]	1.48[a]	1.5[a]
Total nitrogen	2.57[d]	2.43[c]	2.37[b]	2.28[a]	2.27[a]
NPN	0.35[a]	0.32[a]	0.31[a]	0.32[a]	0.31[a]
SSN	1.69[a]	1.72[a]	1.75[a]	1.74[a]	1.75[a]
Lipid	5.69[d]	5.26[c]	4.93[b]	4.78[b]	4.21[a]
TVB-N	14.0[a]	20.53[b]	24.73[c]	29.87[d]	38.27[e]
Peroxide value	3.55[a]	6.22[b]	9.44[c]	14.11[d]	18.22[e]
TBA	0.68[a]	0.87[b]	1.15[c]	1.39[d]	1.74[e]
Sensory	9.05[d]	8.78[d]	7.76[c]	6.82[b]	6.11[a]

* Mean values followed by different letters in row denote significant differences ($p < 0.05$) as a result of the ice storage

Table 3. Biochemical changes during ice storage of *Puntius sophore** (n=3)

Composition	Storage period (Days)			
	1	4	7	10
Moisture	72.55[a]	74.36[a]	75.55[a]	75.16[a]
pH	6.27[a]	6.31[b]	6.46[c]	6.63[c]
Ash	5.72[c]	4.58[b]	4.38[a]	4.6 5[a]
Total nitrogen	2.59[b]	2.52[ab]	2.44[a]	2.45[a]
NPN	0.37[a]	0.35[a]	0.34[a]	0.34[a]
SSN	1.68[a]	1.74[b]	1.76[b]	1.77[b]
Lipid	4.49[b]	4.14[ab]	3.97[a]	4.21[ab]
TVB-N	13.07[a]	19.13[b]	26.13[c]	29.87[c]
Peroxide value	2.61[a]	6.11[b]	11.11[c]	21.22[d]
TBA	0.87[a]	1.42[b]	1.49[c]	1.5[c]
Sensory	9.12[d]	8.35[c]	7.15[b]	6.27[a]

* Mean values followed by different letters in row denote significant differences ($p<0.05$) as a result of the ice storage

initial value) at the end of 13thand 10thday respectively in respect of *M. gulio* and *P. sophore*. The correlation coefficient (*r*) between the period of storage and change in total nitrogen were found to be -0.96 and -0.92 in the case of *M. gulio* and *P. sophore* respectively. This decrease can be attributed to leaching out of soluble nitrogenous components in the ice melt water. The result shows that the loss of nitrogenous components was low in the case of *P. sophore* and may be attributed to the presence of scale. Simultaneously, the NPN content also showed a similar trend and the reduced from 88.6% to 0.31% and 91.9 to 0.34% respectively in the case of *M. gulio* and *P. sophore*. These changes are due to the breakdown of the cellular structure as well as to the growth of microorganisms that are either naturally associated with the fish or that become part of the flora because of contamination during handling (Ehira& Uchiyama, 1987).

The salt soluble nitrogen (SSN) showed a slow increasing trend throughout the period of ice storage in both the fishes although the increase was not significant (p>0.05). Increase of SSN before and after icing was significant only in case of *P. sophore*. The increase of SSN during ice storage may be due to leaching out of water extractable nitrogenous components. The correlation coefficient (*r*) between SSN and total nitrogen was -0.97 and -0.96; between SSN and NPN was -0.80 and -0.99; and between SSN and period of storage was 0.87 and 0.93 for *M. gulio* and *P. sophore* respectively. The *r*-value indicated effects of reduction of total TN and NPN nitrogen as well as the storage period on the changes of salt SSN. The same phenomenon was reported by Joseph et al. (1988) in *L. rohita*. They also reported that the initial lower value of SSN may be due to the effect of rigor on the myofibrillar proteins.

Average values of TVB-N content showed a significant increase (p<0.05) from 14.0 to 38.27 mg of nitrogen 100 g^{-1} of muscle in the case of *M. gulio* whereas it was 13.07 to 29.87 mg of nitrogen 100 g^{-1} of muscle in the case of *P. sophore*. This increase may be due to production of ammonia and other volatile amines (Mazorra-Manzano et al., 2000). TVB-N and spoilage depend on the species and the TVB-N cut-off value of 30 mg 100 g^{-1} of muscle may not coincide with organoleptic measurements of spoiled fish. The correlation coefficient (*r*) between the period of storage and change inTVB-N levels was found to be 0.99 in both fish species.

A gradual decreasing trend was observed in total lipid content of the fish during storage (Table 1 and 2). This could be a result of the presence of pro-oxidant enzymes (lipoxygenases, peroxidases) and chemical pro-oxidant molecules (namely, hemoproteins and metal ions) (Erickson, 1997; Sikorski & Kolakowski, 2000). The peroxide value reached to 18.22 (on day13) and 21.22 (on day10) for *M. gulio* and *P. sophore* respectively (Table 1 and 2). Although the inherent fat content of both the fish was moderate, no of rancidity was noticed till the fishes were acceptable organoleptically. This may be because of the final PV which was below the critical level of giving any rancid smell to the fish. TBARS in *M. gulio* increased by 2.5-folds, while that in *P. sophore* increased by 1.7-folds. The differences in lipid oxidation possibly result from the different fatty acid compositions (Benjakul et al., 2005). The changes of microbial growth in *M. gulio* and *P. sophore* during ice storage are shown in Fig 1. During iced storage, the bacterial count gradually increased from 10^3 g^{-1} to 10^6 g^{-1} by the 13^{th} day in *M. gulio* whereas, in *P. sophore* the same increased from 10^4 g^{-1} to 10^6 g^{-1} by the 10^{th} day. However, in both the fishes the APC did not exceed the upper acceptable limit of 10^7 cfu g^{-1} recommended by ICMSF for fresh and frozen fish (ICMSF, 2002). Fish flesh starts visibly to spoil when bacterial level rises above 10^7 cfu g^{-1} (Lakshmanan, 2005). Chytiri et al. (2004) reported that bacterial count of whole ungutted trouts was always lower than those obtained for filleted trout sample and mesophillic count for filleted and ungutted fish exceeded the acceptable limit after 10 and 18 days of ice storage, respectively.

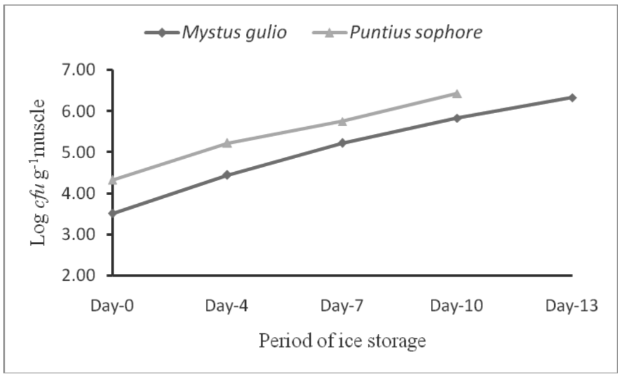

Fig. 1. Change of total aerobic count during ice storage of *Mystus gulio* and *Puntius sophore*

Overall sensory scores for *M. gulio* and *P. sophore* stored in ice are given in Fig 2. Initially the fish exhibited fresh muddy odour, shiny appearance, elastic in texture, bright red gills and perfectly fresh eyes with convex black pupil. No remarkable changes were observed till 4^{th} day for both the fishes. Gradually changes in the sensory characteristics was observed with the progress of storage period. Both the species, *M. gulio* and *P. sophore* lost their fresh odour on 16^{th} and 13^{th} day respectively and also slight decayed odour of weed was perceived. The texture became soft and scale was easily removable. Eyes were completely concave and gills exhibited bleaching with dark brown discoloration. Based on the sensory quality of the fish, the ice storage shelf life of *M. gulio* and *P. sophore* was considered to be 13 and 10 days respectively.

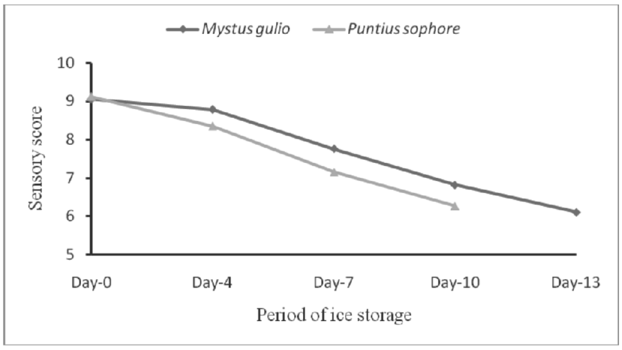

Fig. 2. Change of sensory score during ice storage of *Mystus gulio* and *Puntius sophore*

Since many small size fish are of immense commercial importance in the Northeast India, assessment of their shelf-life in ice storage condition is important. Present work on two such species, *viz., M. gulio* and *P. sophore* indicated that under proper ice storage condition, their shelf life could be approximately 13 and 10 days respectively. Further, proximate composition of these fish proved their nutritional significance.

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