



SENSORY AND BIOCHEMICAL QUALITY CHANGES OF CATLA (*CATLA CATLA*) IN IMMEDIATE AND DELAYED ICING AND AT AMBIENT TEMPERATURE

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KUS-08/12-100408

Manuscript received: April 10, 2008; Accepted: November 30, 2008

Abstract: Changes in the quality of Catla (*Catla catla*) stored immediately (0 h) in ice, after six (6) hours in ice and at ambient temperature soon after catch was investigated. The samples were examined by sensory (organoleptic and rigor mortis progress) and biochemical parameters (total volatile base nitrogen, trimethylamine nitrogen and pH). The rigor mortis proceeded faster in fish stored at ambient temperature (28°C-30°C) than those stored in ice (0°C). The duration of full rigor stage and total rigor period were 8h and 13h for fish stored in ambient temperature and 77h and 127h for fish stored in ice (both immediate and delayed icing) respectively. Organoleptically, the acceptability of fish varied between 16-20 days in both the iced storage conditions and 12-13.5h at ambient temperature. The fishes were organoleptically just acceptable on the 16th day of storage and just unacceptable on the 20th day. The values of total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) and pH were 18.9 mg% (0h), 16.74 mg% (6 h), 5.40 mg% (0h), 4.86 mg% (6h) and 6.93 (0h), 6.65 (6h) respectively at the time of rejection for fish stored in ice. Those values for fish stored at ambient temperature were 28.44 mg%, 6.22 mg% and 7.18 respectively at the time of rejection.

Key words: Rigor mortis, biochemical quality, icing, TVB-N, TMA-N, *Catla catla*.

Introduction

Biochemical spoilage of fish includes oxidation of fat by atmospheric oxygen, resulting in rancidity and hydrolytic changes in fats and protein. Determination of trimethyl amine-nitrogen (TMA-N), total volatile base nitrogen (TVB-N) and pH are useful for the assessment of the freshness of fishes; however, they are time-consuming and serve only to confirm the organoleptic rating. Spoilage in fish occurs due to microbial activity, autolytic activity of enzymes and rancidity of fat (Clucas and Ward, 1996). It is very important that fish should be healthy and fresh for human consumption. But it is not an easy task to preserve fish scientifically as well as to maintain nutritional quality and flavour like the fresh one. Besides temperature is one of the main hindrances of the scientific preservation of fish and fish products because temperature enhance the enzyme activity even after death Reay and Shewan (1949). Icing is a very effective way of reducing fish spoilage and it is particularly effective for the tropical fish (Hattula *et al.*, 1993). The International Code of Practice for fresh fish prepared by the Codex Alimentarius Commission (Anon, 1977) recommends that fish should be chilled to the temperature of melting ice (0°C) as soon as possible after capture and should be maintained at this temperature until it reaches the consumer.

Catla (*Catla catla*) is one of the fresh water major carps in Bangladesh and very popular for its big size and glamorous look. Of the total fish harvested in Bangladesh, major carps and exotic

carps contribute about 31.83% (Anon, 2001). In the open water fisheries (beels) Catla comprised 48.97%, which included 18.21% of the total catch from pond fisheries (Anon, 1996). Fish provides the chief source of animal protein to human. In Bangladesh 63% of animal protein is supplied from fish. Fresh fish always contains higher amount of nutrients and human beings always desire to buy and consume fresh fish.

Considerable volume of information is available on the post harvest quality changes in fish from temperate and cold waters (Adebona, 1982; Howgate, 1996; Siang and Kim, 1992; Surendran *et al.*, 1985; Shewan and Ehrenberg, 1977; Connel, 1975) but a few exist on the spoilage patterns of tropical fish, particularly Indian major carps which are commercially very important in this region (Castell and Triggs, 1955; Alam, 2002; Clucas and Ward, 1996). Considering this, the study was carried out in order to investigate the effect of immediate icing (0 h), delayed icing (after 6 h) and exposure to ambient temperature on the overall quality of Catla.

Materials and Methods

Collection of samples: Fish samples were collected from Chuknagor, Khulna, Bangladesh at around 8 A.M. when the temperature of pond water was between 26°-27°C and air temperature was 28°C. For the second time, the fish were collected from a commercially poly-cultured pond adjacent to Khulna University around 6 AM. The water temperature was between 28°C to 30°C and average air temperature was 32°C at the time of sample collection. After collection, the experiments were carried out in the Nutrition and Quality Control Laboratory, Fisheries and Marine Resources Technology Discipline, Khulna University, Khulna.

Experimental design: The fish obtained at the first time was divided into two lots (25 fish lot⁻¹). One lot was iced (1:1 ice to fish ratio) and stored in an insulation box at the site (0 h) while the other was transported in a bamboo basket without ice. The uniced batch was iced after 6h of harvesting. Storage conditions were maintained by draining the insulated fish boxes of melted ice intermittently and more ice added to keep the temperature at 0°C throughout the storage period. Firstly, the samples from both containers were taken out at intervals of 3-4 days to determine the overall quality by organoleptic and biochemical analyses. Secondly, the fish samples were collected same way as the first and samples were also divided into two lots (28 fish lot⁻¹) and both batches were transported in the laboratory to determine the overall quality by organoleptic and biochemical analyses.

Visual assessment of rigor mortis: Five fish sample from each treatment was carried out hourly up to 16 hours and then at 24, 48, 60, 72, 84, 96, 120 and 144 h following death in case of iced storage fish (Azam *et al.* 1990). A simple method modified from that described by Curran *et al.* (1986) was used.

Organoleptic assessment: The organoleptic assessment of fish for this study involved a score sheet, which is mainly based on the score sheets of Shewan and Ehrenberg (1977). Eleven trained panels of three members were used to evaluate the organoleptic condition of the fish samples. They were made familiar with the objective of the study and the method of scoring each of the criteria used.

Biochemical assessment

pH measurements: Fish flesh suspension was prepared using 1 gm fish flesh in 20 ml of distilled water. The pH was measured with a pH meter (HANNA 8424).

Determination of Total Volatile Basic Nitrogen (TVB-N) and Trimethyl Amine Nitrogen (TMA-N): TVB-N and TMA-N were determined according to Conway micro-diffusion technique (Conway, 1977) with a small modification. This technique was carried out in a special glass dish called Conway dish with an outer annular space and inner circular space. The whole dish was made airtight by closing it with a glass plate coated with sealing agent. The principle consists of simple diffusion of the volatile substances from the outer chamber into the inner one.

Data Analysis: For all the variables mean, SD, ANOVA, correlation coefficient were calculated and graphically presented by using SPSS and Microsoft Excel 2003.

Results

Effect of immediate (0 h) and delayed icing (after 6 h) on the quality of *C. catla*

Visual assessment on the progress of rigor mortis: Visual assessments of Catla stored at room temperature (28°C-30°C) and in ice (0°C) are shown in Table 1 and Table 2. The rigor mortis proceeded faster in fish stored at room temperature.

Table 1. Visual assessment of the progress of rigor in *C. catla* stored at ambient temperature (28°C-30°C).

Sample Id.	Onset of rigor (Hours)	Onset of full rigor (Hours)	Duration of rigor (Hours)	Resolution of rigor (Hours)	Duration of total rigor (Hours)
F1	1	3	7	14	13
F2	2	4	8	14	12
F3	2	3	9	13	11
F4	1	2	10	16	15
F5	2	3	8	15	13
Average	1.6	3	8.4	14.4	12.8

Table 2. Visual assessment of the progress of rigor in *C. catla* stored in ice (0°C).

Sample Id.	Onset of rigor (Hours)	Onset of full rigor (Hours)	Duration of rigor (Hours)	Resolution of rigor (Hours)	Duration of total rigor (Hours)
F1	1	2	72	120	119
F2	-	1	84	136	135
F3	1	2	72	120	119
F4	-	1	72	144	143
F5	-	1	84	120	119
Aver. time		1.4	76.8	128	127

Organoleptic Changes: The results (Fig. 1) show that *C. catla* iced immediately and after 6 hours were acceptable, according to taste panel, up to 16 and 20 days. The initial total score was 97.67 and it decreased to 62.67 and 52.33 respectively on the 16th and 20th days for immediate storage. While in case of delayed icing, the initial total score was 93.33 and decreased to 62.33 and 52.00 respectively for 16th and 20th days.

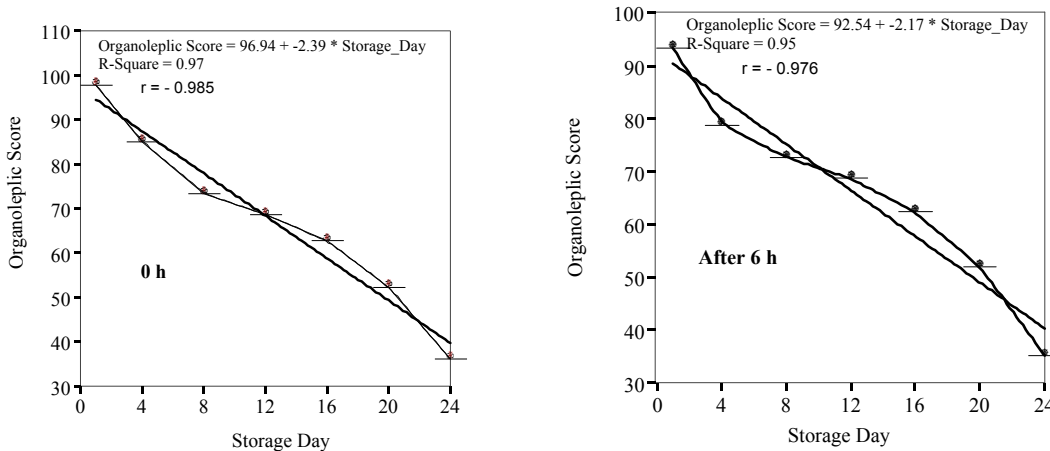


Fig. 1. Organoleptic evaluation of *C. catla* stored in ice (0 h and after 6 h).

Table 3. Changes in organoleptic attributes of *C. catla* stored in ice (0 h) in an insulation box.

Days in storage	Appearance	Odour	Texture	Acceptability
1	Shiny, good looking, bright eyes and clear dark with clear margin, gills are bright red, transparent slime.	Fresh fishy odour.	Firm, compact, elastic flesh. Whitish, transparent. Belly wall and visceral organs are intact	9.77 Highly Acceptable (HA)
4	Thin transparent slime present on the skin, slightly loss of characteristic colour, eyes opaque, gills become red brownish, thin slime, fishy odour, tight filaments.	Loss of Characteristics odour	Muscle firm and in rigor stage, fish flash transparent but slightly soft.	8.50 Acceptable (A)
8	Skins are slightly dull, thin slime, eyes become dull; red brownish gills.	Neutral, slightly spoilage and metallic odour	Rigor stage slightly resolved, fish flesh transparent, slightly soft and loose	7.33 Moderately Acceptable (MA)
12	Slime slightly thick and dull with slight discolouration; sunken eyes dull grayish; gills become pale brownish, thick slime.	Slightly metallic acid odour/moderately spoilage odour.	Rigor almost resolved, fish flesh white, dull, slightly soft and loose.	6.87 Just Acceptable (JA)
16	Slime slightly thick and dull skin, slightly sunken eyes with slight discolouration; gills become brownish/ whitish, thick slime and off odour.	Very fishy, strong metallic acid odour.	Rigor resolved completely, fish flesh soft and loose. Meat layers easily separated.	6.27 Just Acceptable (JA)
20	Thick slime, sticky, dull brownish colour in appearance; sunken eyes dull, gills become dark brown, thick slime, off odour and dull	Off odour	Moderately soft flesh	5.23 Just Unacceptable (JUA)
24	Scales become reddish with thick rough slimes; eyes pupils become digested	Strong off odour	Flesh condition soft and loose from the bone	3.60 Unacceptable (UA)

Table 4. Changes in organoleptic attributes of *C. catla* stored in ice (after 6 h) in an insulation box.

Days in storage	Appearance	Odour	Texture	Acceptability
1 (Just before icing)	Shiny, good looking, bright eyes and clear dark with clear margin, gills are bright red, transparent slime.	Fresh fishy odour.	Firm, compact, elastic flesh. Whitish, transparent. Belly wall and visceral organs are intact.	9.33(HA)
4	Thin transparent slime present on the skin, slight loss of brightness. Eyes slightly sunken, Slightly dull, Gills become slightly dull red, thin slime, fresh fishy odour, and tight filaments.	Sweetly odour,	In rigor stage, Solid, compact, fish flash transparent but slightly soft.	7.87(A)
8	Skins are slightly dull, thin slime, eyes become dull; Red slightly pale.	Neutral slightly fishy and metallic odour	Compact, fish flesh transparent, slightly soft.	7.27(MA)
12	Slime slightly thick and dull with slight discolouration; sunken eyes dull grayish; gills become pale brownish, thick slime.	Slightly metallic acid odour/moderately spoilage odour.	Compact but less elastic. Fish flesh white, dull, and soft	6.87(JA)
16	Slime slightly thick and dull skin, slightly sunken eyes with slight discolouration; gills become brownish/ whitish, thick slime and off odour.	Very fishy, strong metallic acid odour.	Post rigor stage, fish flesh soft and dull.	6.23(JA)
20	Thick slime, sticky, dull	Off odour	Dull and red fish flesh.	5.20(JUA)

	brownish colour in appearance; sunken eyes dull, gills become dark brown, thick slime, off odour and dull			
24	Scales become reddish with thick rough slimes; eyes pupils become digested	Strong off odour	Flesh condition becomes reddish loose, slight waxy and very soft.	3.53(UA)

The changes in organoleptic characteristics of fish stored in ice immediately and after 6 h are given in Table 3 and 4.

Biochemical Quality Changes

Total Volatile Basic Nitrogen: TVB-N values of fish stored in ice have been presented in Fig. 2. TVB-N levels for fish stored in ice immediately (0 h) increased over storage day 8.07 mg % initially and 22.99 mg % on day 24. In case of delayed ice storage condition, TVB-N levels were 8.41 mg% on the 1st day and highest level was 27.07 mg% on 24th day.

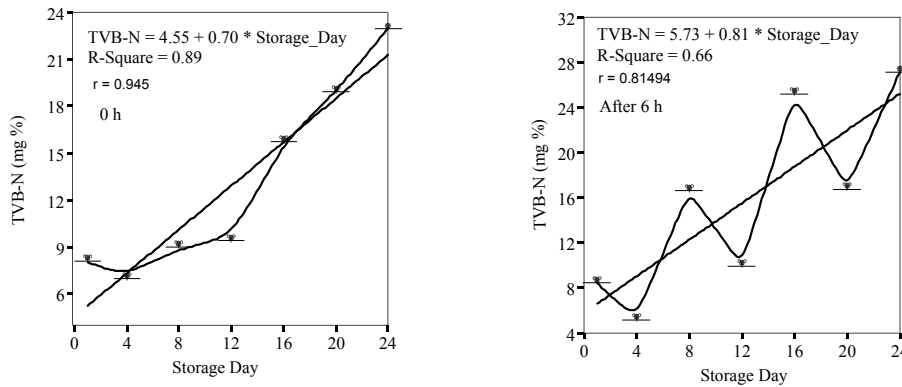


Fig. 2. Total Volatile Basic-Nitrogen content (mg %) of *C. catla* stored in ice (0 h and after 6 h).

Trimethyl Amine-Nitrogen: The changes in TMA-N value of the fish stored in ice (0 h and 6 hours) are shown in Fig. 3. On the 1st day of the experiment, TMA values of both the samples were 0.99 and 1.16 mg % and raised to 7.48 and 7.58 mg % respectively at the end of storage trial. TMA values were found to fluctuate for fish stored in ice after 6 hours. But TMA-N value reached constant up to 8th storage day of storage and of than accelerated for fish stored in ice immediately (0 h).

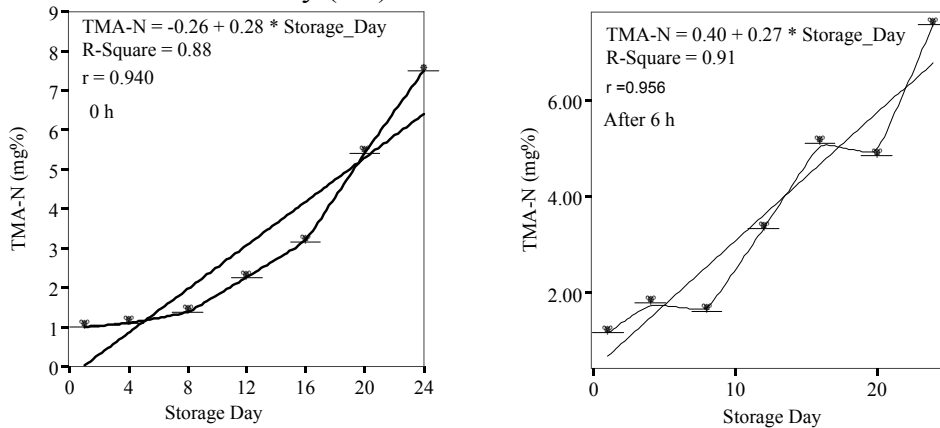


Fig. 3. Trimethyl Amine-Nitrogen (TMA-N) content (mg %) of *C. catla* stored in ice (0 h and after 6 h).

pH: The pH changes in *C. catla* stored in ice (0 h and after 6 h) are shown in Fig. 4. The pH of *C. catla* varied between 6.95 to 7.09 if iced immediately after catch, while for fish iced 6 h after catch, it ranged from 6.84 to 7.17. The initial pH values of Catla were 6.95 and 6.84 respectively, which tend to decrease with storage period up to 12th day and increased to 7.09 and 7.17 at the end of experiment.

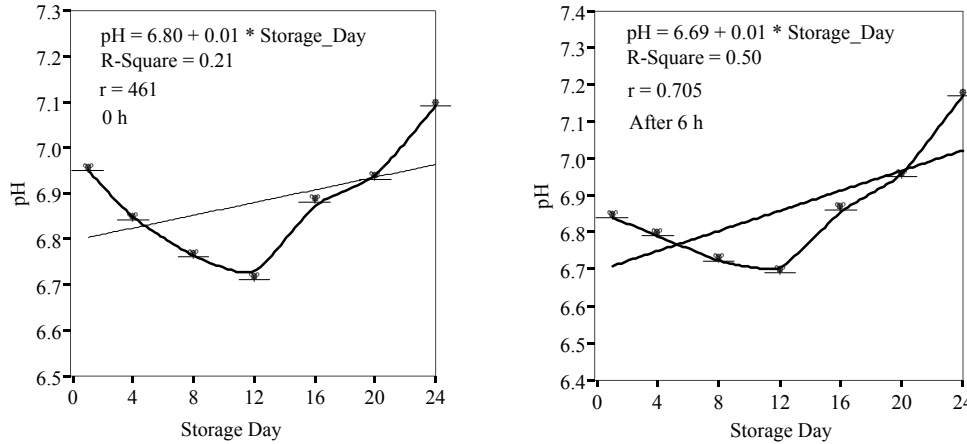


Fig. 4. Changes in pH of *C. catla* stored in ice (0 h and after 6 h).

Quality changes in *Catla catla* stored at ambient temperature

Organoleptic changes: The keeping time of Catla varied between 12 to 13.5 hours for fish stored at ambient temperature as judged organoleptically (Fig. 5). Total score decreased from 100 to 28.33 in 16.5 hours of storage time.

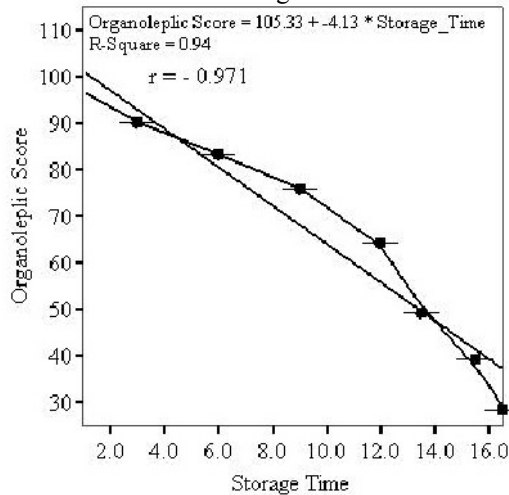
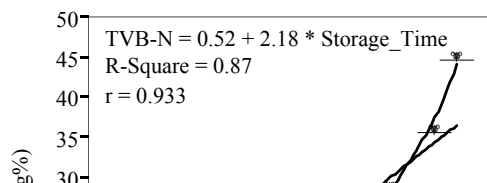


Fig. 5. Organoleptic changes in *C. catla* stored at ambient temperature.

Biochemical Quality Changes

Total Volatile Basic-Nitrogen (TVB-N): Fig. 6 shows the changes in the TVB-N content of fish muscle stored at ambient temperature. It can be seen that the TVB-N content increased with storage time. The initial value of sample was 6.69mg-N 100g⁻¹ which increased to 44.55mg-N 100g⁻¹ at the end of the storage trial (16.5 hours).



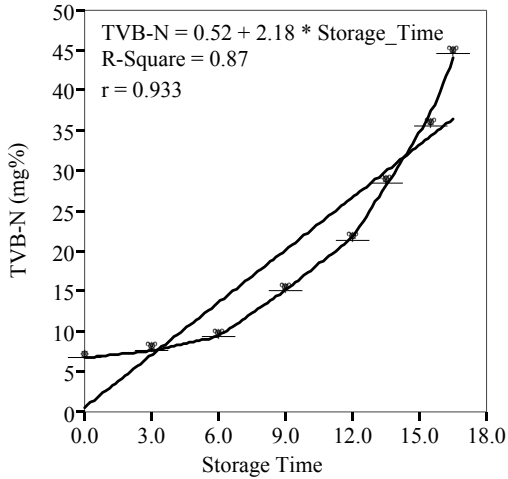


Fig. 6. Changes in TVB-N concentration of *C. catla* stored at ambient temperature.

Trimethyl Amine-Nitrogen (TMA-N): TMA-N content of fish muscles of *C. catla* increased with storage time and ranged from 0.89 to 8.46 mg-N 100g⁻¹ of muscle. At the initial period of the investigation, the TMA-N content was 0.89 mg-N 100 g⁻¹. TMA-N showed positive correlation (Fig. 7).

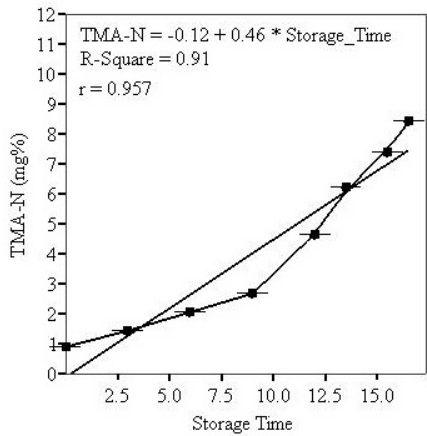


Fig. 7. Changes in TMA-N content in *C. catla* stored at ambient temperature.

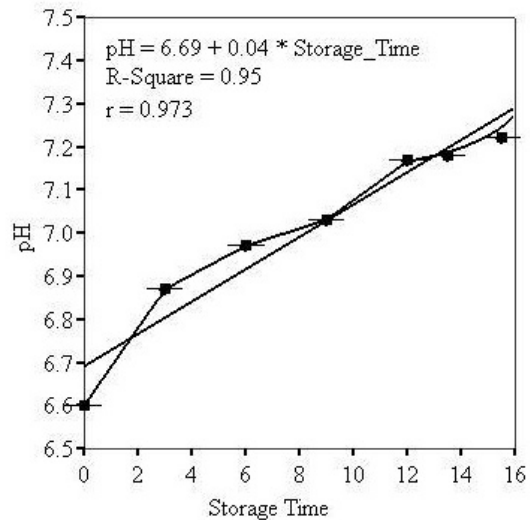


Fig. 8. Changes in pH of *C. catla* stored at ambient temperature.

pH: Initially, the average muscle pH was 6.60 in *C. catla* stored at ambient temperature. It increased slightly during storage, reaching an average of 7.32 at the end of the storage trial.

Discussion

Among the many factors, rigor mortis is known to be dependent on temperature which influence the onset and rate of progress of rigor. It is widely believed that lower temperature delays the rigor mortis progress. Several fish species such as Tilapia, Red sea bream and plaice have been reported to shorten their periods when stored at 0°C (Iwamoto *et al.*, 1985, 1987; Poulter *et al.* 1981; Imamoto and Yamanaka, 1986). The results of Azam *et al.* (1990) related to the progress of rigor in trout were similar to the findings of the present investigation.

For fish stored in ice after 6 h, the initial score was lower than that of immediately iced fish. This was due to the exposure of the batch to the room temperature (28°C-30°C) for 6 hours before icing. However, after 24 days of storage, the scores were similar both for fish stored in ice immediately and after 6 h respectively. The taste panel results indicated that fish in ice showed a gradual deterioration during the storage. Nair *et al.* (1974) reported that delay up to 7 h before icing does not have adverse effect on storage life. Dawood *et al.* (1986) also had similar finding about rainbow trout. Dawood *et al.* (1986) further reported that the changes in organoleptic quality during this period can roughly be divided into four phases like 0 to 4, 4 to 12, 12 to 16 and over 16 days in ice. Regression analysis between total organoleptic score and storage time for the batches proved to be linear with negative correlation (*r* values -0.985 and -0.976 for 0 h and 6 h delayed icing respectively) (Fig. 1) and statistically highly significant ($p < 0.05$).

TVB-N is the oldest of the chemical methods for measuring freshness. For fish stored in ice immediately (0 h), TVB-N remained nearly constant for the first 12 days and even at the end of the storage period, it was well within the usual limit of acceptability for marine fish (30 mg-N 100g⁻¹) (Nair and Lahiry, 1968).

TVB-N values for fish stored in ice after 6 hours were found to fluctuate. On the initial day, the value was 8.41 mg %, which increased to 27.07 mg % on the 24th day. In both storage conditions, the initial values were higher probably because of certain drop of microbial growth for iced cold shaking. However, regression analysis showed significant correlation between TVB-N and storage period ($r = 0.945$) for fish immediately iced but slightly lower correlation was observed ($r = 0.814$) between TVB-N levels and storage period in fish stored in ice after 6 hours (Fig. 2). ANOVA indicated significant differences ($p < 0.05$) between TVB-N value and storage time in both the cases.

Wierzychowski (1956) suggested the acceptable limit of TVB-N value as 40 mg % for estimating the freshness of fish. The level of TVB-N increases after spoilage begins both enzymatically and bacteriologically and hence, can be used as a quality index for fish (Jenson *et al.*, 1979). The TVB-N content was quite low (22.99 and 27.03 mg %) when fish were rejected organoleptically as observed in the present investigation.

It has been known that freshwater fish cannot be judged by their TMA values because they do not contain the precursor, trimethyl amine oxide (TMAO) in sufficient amounts by Bligh (1971). Furthermore, freshwater fish may spoil in a different manner than marine fish. Balakrishnan *et al.* (1971) found that, in ice freshwater fish, autolytic reactions appeared to be the dominant factor in determining quality rather than bacterial spoilage. However, regression analysis showed significant correlation between storage time and TMA values for both the batches and *r* values were 0.94 and 0.956 respectively (Fig. 3). TMA and storage periods were found statistically significant ($p < 0.05$) in both the storage condition.

Taste on the 16th day of storage, when the fish were organoleptically just acceptable, the TMA values were 3.15 and 5.12 mg % respectively for fish stored in ice (0 h and after 6 hours). The results represent relatively the higher values of TMA for fresh water fish. It may be attributed to

supplying of supplementary fishmeal of marine sources having certain amount of TMAO. Sen Gupta *et al.* (1972) found lower threshold values of TMA for species of Indian fishes. TMA values up to 1 mg % indicated fresh fish, 1-5 mg doubtful, 5 mg spoiling, and over 8 mg indicating almost spoiled. The TMA content starts to increase rapidly just as the fish start to spoil, that is when the first spoiling odours and flavours are apparent in the flesh, so TMA can distinguish between acceptable and unacceptable qualities and, later stages of spoilage, between fish which is fit and unfit for consumption (Hawgate, 1996).

Change in pH can affect proteolysis, bacterial synthesis of histamine from muscles histidine, and trimethylamine oxide (TMAO) reduction by bacteria into TMA (Ababouch *et al.*, 1991). In the storage trial, pH values initially decreased in both samples. Organoleptically the fish of both the batches were in acceptable condition between of 16th and 20th days of storage. Regression analysis showed a poor positive correlation between pH and storage time ($r= 0.461$) for fish stored in ice immediately (0 h). But in case of delay iced storage (6 h) pH values were moderately positively correlated ($r= 0.705$) with time (Fig. 4). The result was statistically significant ($p<0.05$) in both the experiments.

The initial drop of pH values causes build up of lactic acid (Eskin *et al.*, 1971). Lactic acid formation is dependent on glycogen reserves in the fish muscle tissue. Any struggle prior to death will result in depletion or loss of the endogenous substrate and the accumulation of lactic acid anaerobically by glycolysis (Eskin *et al.*, 1971).

In the present study, the total organoleptic score was reduced to 64.33 and 49.33 after 12 and 13.5 storage times respectively and a regular deterioration showed till the end of storage trial. All sensory attributes of *C. catla* gave negative correlation ($r= -0.971$) with the storage time (Fig. 5). Organoleptic score and storage time indicated statistically significant ($p<0.05$) differences. Durairaj and Krishnamurthi (1986) reported that after 10 h, *Labeo rohita* and *Tilapia mossambica* were completely spoiled when stored at ambient temperature, while *Cirrhina mrigala* became unacceptable within 11 hours.

A significant increase in the TVB-N content of the fish was observed. Such pattern of spoilage changes were also recorded by Ahamed *et al.* (1981). By 12 hours of storage time, the samples were organoleptically just acceptable. During this time, the TVB-N content was 21.35 mg %. After 13.5 hours when fish organoleptically just unacceptable, the TVB-N content increased to 28.5 mg%.

Regression analysis (Fig. 6) showed significant correlation between TVB-N and storage time ($r= 0.933$). The results indicated statistically significant differences ($p<0.05$) between the storage time and TVB-N values.

Reay and Shewan (1949) and Jenson *et al.*, (1979) also observed that during spoilage, there is an increase in the TVB-N content of fish muscle. Durairaj and Krishnamurthi (1986) reported increase in TVB-N content to 28 mg-N 100 g⁻¹ after 11 hours for *L. rohita*, 20 mg-N 100 g⁻¹ after 10 hours for *C. mrigula* and 24 mg-N 100 g⁻¹ after 10 hours for *T. mossumbica* stored at ambient temperature.

The TMA-N value slowly increased to 2.67 mg-N 100 g⁻¹ on the 9th hour. At the end of the 16.5 hours, the value was 8.46 mg-N 100 g⁻¹. TMA-N showed a positive correlation ($r= 0.957$) with the storage time (Fig. 7). The TMA-N content increased with the increase in storage time but this result did not show any significant definite limit of acceptability by De and Nazrul (1966). Results obtained in this experiment indicated statistically significant ($p<0.05$) differences between TMA-N content and storage time. Castell, (1949); Anderson and Fellers, (1952) and Somaatmadja *et al.*, (1961) have confirmed the reduced amount of TMA-N in freshwater fish.

Marrochi *et al.* (1990) proposed TMA-N levels for first grade, second grade, and intermediate freshness as <1, 1-3, and 3-5 mg-N 100 g⁻¹.

In the present study, fish was just acceptable according to the taste panel when pH was 7.17. When the fish were just unacceptable, pH was 7.18. Positive correlation ($r = 0.973$) was found between pH value and storage period (Fig. 8). Storage time and pH values of the sample were found statistically significant ($p < 0.05$). Results of present investigations were similar with the findings of Bandyopadhyay *et al.*, (1986); Poulter *et al.*, (1978) and Curran *et al.*, (1980).

Conclusion

From the investigation, it can be concluded that there is apparently no beneficial effect of using ice on fish immediately. The shelf life of fish stored in ice immediately and after 6 hours remained almost similar. Thus it would rather be wastage to use ice immediately after harvest. However, shelf life reduced drastically when stored at ambient temperature.

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