Bangladesh J. Fish. Res., 13(1), 2009: 65-76

Organoleptic and microbiological quality changes of catla (*Catla catla*) in immediate and delayed ice storage and at ambient temperature

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Abstract
An investigation was carried out on the quality changes of Catla (*Catla catla*) stored immediately (0 h) in ice, after six (6) hours in ice and at ambient temperature. The samples were examined for organoleptic and microbiological parameters in summer. Organoleptically, the acceptability of fish varied between 16-20 days in both the iced storage conditions and 12-13.5 hours at ambient temperature (28^{0}C). When fish were organoleptically just acceptable on the 16th day of storage, bacterial load were 6.23 and 6.17 log10 cfu/g, respectively for 0 hour and after 6 hours iced fish. But on the 20th day of storage, when fish were just unacceptable SPC were 6.51 and 6.62 log10 cfu/g. In case of ambient temperature storage condition standard plate count was 8.36 log10 cfu/g on 13.5 hours, when fish were organoleptically just unacceptable. At the time of rejection for fish stored in ice (0 hour and after 6 hours) on 20th day, gram negative and gram positive values were 55.45%, 44.55% and 44.52%, 55.48% respectively. While fish were rejected after 13.5 hours at ambient temperature gram negative and gram positive bacteria were found as 43.02% and 56.98%. The differences in SPC, gram positive and gram negative bacteria between the storage times were statistically significant ($p<0.05$).

Key words: Delayed ice storage, *Catla catla*

Introduction

Catla (*Catla catla*), a major carp is one of the most popular fish in Bangladesh for its big size, glamorous look and marvelous taste. After catch, several strains of terrestrial bacteria find their way on to the fish from several sources like boat decks, fish boxes, ice including air with which they come in contact (Govindan 1985). These may at times even include organisms of public health importance like *Escherichia coli, Strepotococcus, Staphylococcus, Salmonella* and *Vibrio* presence of which in the fish muscle beyond certain limits renders it unfit and dangerous for human consumption.

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 Alimentarious Commission (FAO/WHO 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. The practice of icing immediately after capture and throughout the distribution chain retards spoilage and the shelf life of fresh fish can be extended quite considerably. A considerable 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), but few studies exist on the spoilage pattern of tropical fish, particularly Indian major carps which are commercially very important fish in this region. Only limited information is available on the spoilage pattern of tropical fish stored in ice immediately, delayed icing and at ambient temperature. Considering this, the study was carried out to investigate the effect of immediate icing (0 h), delayed icing (after 6 h) and exposure to ambient temperature on the overall quality of Indian major carp, Catla (*Catla catla*).

Materials and methods

Collection and preparation of samples: Fish samples (S_1) were brought from Chucknagor, Khulna, Bangladesh around 8 am when the water temperature of the water pond was between 26^0-27^0C and air temperature 28^0C. Another lot of fish samples (S_2) were collected from a commercially poly-cultured pond adjacent to Khulna University around 6 am. Water temperature was then between 28°C-30^0C and average air temperature was 32°C. Ice was collected from Gollamary Fish Market.

Experimental design: The S_1 were divided into two lots (25fish/lot). One lot was iced (1:1 ice to fish ratio) and stored in an insulation box at the site (0 h) while the other lot was transported uniced in a bamboo basket to the Quality Control Laboratory, FMRT Discipline, Khulna University, Khulna. 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 entire storage period. The samples from both containers were withdrawn at intervals of 3-4 days to determine the overall quality by organoleptic and microbiological analyses. The S_2 fishes were collected as the same way of S_1 fishes and S_2 fishes were also divided into two lots (28fish/lot) and both batches were transported to the Quality Control Laboratory to determine the overall quality by organoleptic and microbiological analyses.

Organoleptic assessment: The organoleptic assessment of fish for this study involved a score sheet, which was mainly based on the score sheets of Shewan and Ehrenberg (1977). In the sensory score technique, the fish were judged by following quality factors: General appearance (eye, pupil, gill, body surface, flesh, belly wall and viscera), odor, texture. Each of the factor was given 10-0 scores and the highest being

the best. In addition overall acceptability was measured by the taste panel. Eleven trained panels of three members evaluated the organoleptic quality of the fish samples. They were made familiar with the objective of the study and the method of scoring each of the criteria used.

Microbiological analysis: Microbiological analyses were done according to the Bacteriological Analytical Manual of the United States Food and Drug Administration (FDA 1998). All the tests were done in triplicate.

Data analysis: All the statistical analyses (mean, SD, correlation co efficient, and ANOVA) among different variables were calculated and graphically presented by using the SPSS and Microsoft Excel.

Results and discussion

Organoleptic changes: The results show that Catla iced immediately (0 h) and after 6 hours were acceptable according to taste panel between 16th and 20th days (Table 1). The initial total score was 97.67 and it decreased to 62.67 and 52.33 respectively on the 16th h and 20th days during 0 h-iced storage. While in case of delayed icing (6 h), the initial total score was 93.33 and decreased to 62.33 and 52.00 respectively on 16th and 20th days. For fish stored in ice after 6 h, the initial score was lower than that of immediate iced fish. This was due to the exposure of the batch to the room temperature (28 ^{0}C-30 °C) for 6 hours before icing. At the end of the 24 days of storage, the score decreased to 36 and 35.33 for fish stored in ice immediately and after 6 h respectively. During storage trials, taste panel acceptability is used to determine the quality changes of fish. Taste panel, visual and olfactory assessments are subjective in nature although they are essentially the most important methods for determining quality since they are the basis on which a consumer accepts or rejects the fish.

Table 1. Organoleptic changes of *Catla catla* stored in ice (0 h and after 6 h)

Storage day	Organoleptic score	
	0h	6h
0	97.67±2.08	93.33±0.58
4	85.00±2.00	78.67±1.53
8	73.33±1.53	72.67±1.53
12	68.67±1.53	68.67±2.08
16	62.67±1.53	62.33±2.08
20	52.33±1.53	52.00±1.00
24	36.00±1.00	35.33±1.53

The results obtained from the taste panel clearly indicated that fish stored immediately in ice showed a gradual deterioration during the storage period and almost similar result was obtained in case of delayed icing. But delay of icing up to 6 hours did not have adverse effect on quality of fish. Nair *et al.* (1974) had reported that delay up to 7 h before icing did not affect storage life. Dawood *et al.* (1986) also had reported similar finding about rainbow trout. The changes occurred in the organoleptic quality during this period can roughly be divided into four phases corresponding to periods of 0 to 4, 4 to 12, 12 to 16 and over to 16 days in ice depending on acceptability level. In phase 1, the fish were very fresh with a species-specific taste and natural flavour and odour. At this stage, fish had the characteristics of excellent quality indicating highly acceptable. In phase 2, there was a little deterioration apart from some slight loss of natural flavour. At this stage there was little loss of the characteristic odour and the flesh was neutral but had on off odour; fishes at this stage remained at both the acceptable and moderately acceptable level. In phase 3, there were signs of early spoilage with off-flavour. In the beginning of this phase, the off-flavour was slightly sour, sickly sweet, fruity or like dried fish but the fish were judged as just acceptable quality. In phase 4, the fish began to taste stale, its appearance and texture began to show obvious signs of spoilage and the gills and belly cavity had an unpleasant smell clearly indicating unacceptability. At the beginning of this stage, the fish samples were at just unacceptable range and later on at unacceptable range.

The gill condition and odour would appear to be the most useful of the visual and olfactory parameters. The odour to be detected gives an indication of the quality of the *Catla catla*. Gills colour changed gradually from bright red to dark pale colour. The condition of the flesh and walls were also useful since these soften gradually and gave an indication of quality. The skin showed a noticeable change especially with the presence and colour of the slime, which developed. The eyes changed from clear, protruding to opaque, and sunken, but not bloody. All of these parameters were considered at the same time with the aid of the taste panel and when considered together, they give a good indication of the qualities of the fish samples.

Regression analysis between total organoleptic score and storage time for the batches proved to be linear with negative correlation (r values -0.985 and -0.975 for 0 h and 6 h delayed icing respectively) (Fig. 1) and statistically highly significant ($p<0.05$).

There is lots of information on the shelf life and spoilage patterns of the fish particularly from the temperate and cold waters but very little are known on the fish of Bangladesh. In the present study, organoleptic quality of 0 h and 6 h (delayed) iced storage Catla fish was acceptable between 16th and 20th days. This change in the organoleptic characteristic pattern was almost similar to that reported by some other researchers (FAO 1975, Kamal *et al.* 1994).

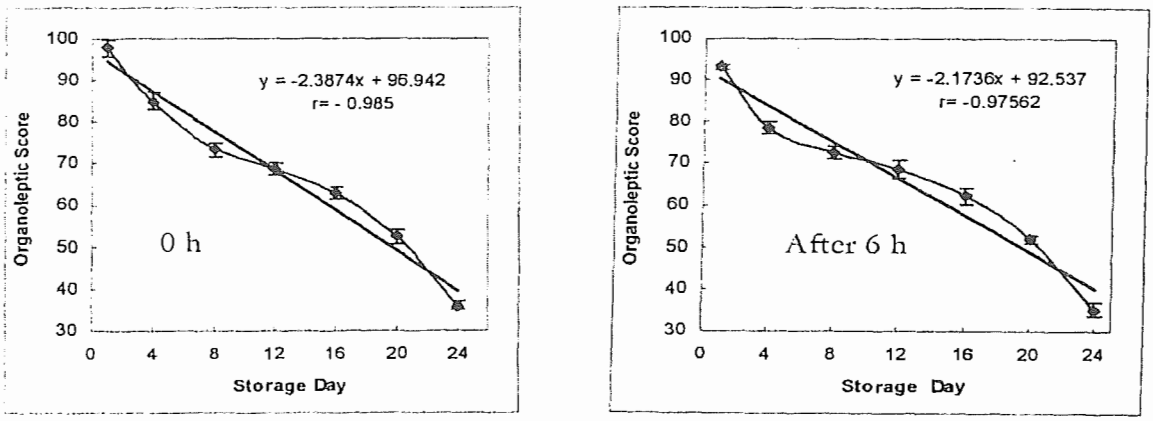

Fig. 1. Organoleptic evaluation of *Catla catla* stored in ice (0 h and after 6 h).

Microbiological changes at immediate and delayed icing

Standard plate count: The results of the standard plate count (SPC) of the samples stored in ice (0 h and after 6 h) are presented in Table 2 and Fig. 2. The results are expressed in $\log_{10}$ cfu/g fish muscle.

Table 2. Standard plate count (Log_{10} cfu/g) of *Catla catla* stored in ice (0 h and after 6 h).

Storage period (Day)	SPC			
	0 h		6 h	
	Log_{10} cfu/g	cfu/g	Log_{10} cfu/g	cfu/g
0	5.27±0.43	2.42 x10^5	5.58±0.23	2.42 x10^5
4	5.05±0.52	1.6 x10^5	5.40±0.02	2.53 x10^5
8	4.33±0.07	2.14X10^4	4.44±0.10	2.82X10^4
12	5.80±0.47	8.4 x10^5	5.88±0.14	7.8 X10^5
16	6.23±0.19	1.8 x10^6	6.17±0.38	1.97 x10^6
20	6.51±0.15	3.3 x10^6	6.62±0.12	4.25 x10^6
24	7.61±0.16	4.2 x10^7	7.63±0.27	4.8 x10^7

The initial log number of bacteria in ice (0 h and after 6 h) were 5.27cfu/g and 5.58 cfu/g respectively. The bacterial load in the 4th day of storage when the fish were started to relax from the rigor state were Log_{10} 5.05 and 5.4 cfu/g respectively for both samples. However, these populations decrease over a period of 8 days and then a gradual increased to 7.61 cfu/g and 7.63 cfu/g on 24th day. After death of fish quality of fish gets reduced because of enzyme (autolysis) and microbial activity. Icing is an effective means

of reducing microbial activity and rate of autolysis but autolysis continues slowly even after icing. After several days of icing slower rate of autolysis produce various volatile and polluting substance and provides opportunity to the bacterial population to increase.

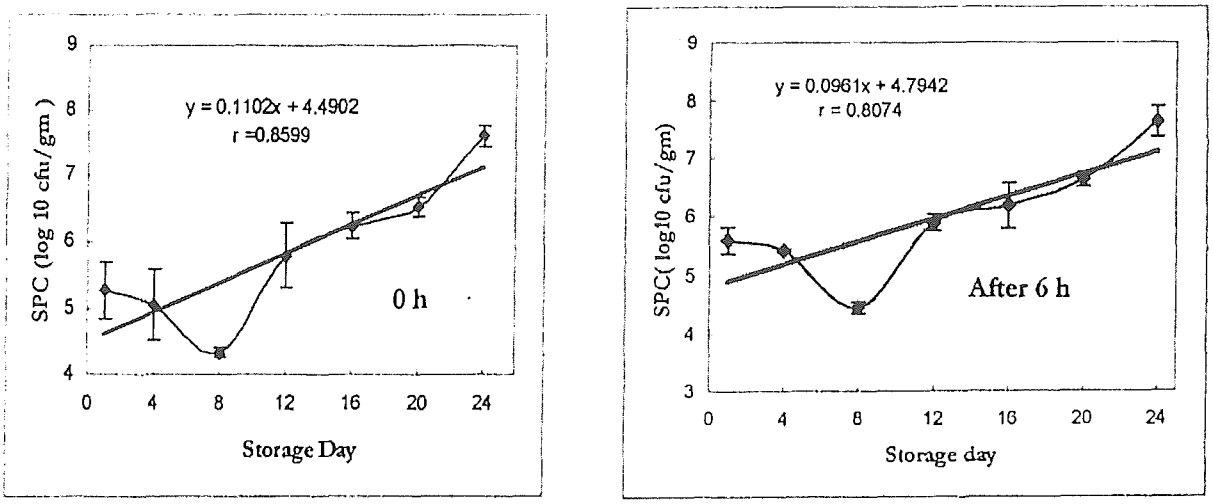

Fig. 2. Standard plate count (Log_{10} cfu/g) of *Catla catla* stored in ice (0 h and after 6h).

The standard plate count of the muscle of fish stored in delayed icing (6 h) showed an initial higher number of bacteria than those fish stored immediately (0 h). The bacterial population of both the batches between 16th and 20th days stood at 1.8 x 10^6 and 3.3x10^6 cfu/g of fish stored immediately in ice (0 h). While the number was 1.9X10^6 and 4.25t10^6 for fish stored in ice after 6 h (Table 2). These values are nearly similar as laid down by the (ICMSF 1986 and IFST 1997) which stipulate that the SPC should be less than 10^6 cfu/g and not exceed 10^7 cfu/g. Statistical presentations also support the results because r values of both the batches were 0.8599 and 0.807 respectively for 0 h and 6 h iced storage fish; clearly indicating a moderately strong positive correlation of bacterial load with the passage of storage period (Fig. 2). The differences of the results were also statistically significant ($p<0.05$). The results highlight the fact that there is a little difference between the two batches that are in agreement with the paper on the preservation of some Indian freshwater fish (Durairaj and Krishnamurthi 1986).

Gram negative bacteria and gram positive bacteria: Table 3 and 4 shows gram negative and gram positive bacteria in log number and their percentage present in *Catla catla* stored in ice immediately (0 h) and after 6 hours. The initial percentage of gram negative and gram positive bacteria were 55.92% and 44.08% respectively in immediately iced fishes. Gram negative bacteria present were higher when fish were stored in ice immediately while the initial percentage of gram positive bacteria was

higher than the gram negative bacteria (43.97% and 56.03%), when stored in ice after 6 h.

Table 3. Load of gram negative bacteria of *Catla catla* stored in ice (0 h and after 6 h)

Storage period (Day)	Gram Negative Bacteria			
	0 h		6 h	
	Log_{10} (cfu/g)	Percentage (%)	Log_{10} cfu/g)	Percentage (%)
0	5.02±0.41	55.92	5.22±0.21	43.97
4	4.77±0.53	52.39	5.06±0.06	45.69
8	4.06±0.06	54.47	4.12±0.08	47.83
12	5.56±0.46	58.16	5.57±0.14	48.37
16	5.95±0.17	53.55	5.85±0.37	47.47
20	6.26±0.11	55.45	6.26±0.14	44.52
24	7.35±0.15	54.45	7.29±0.26	46.27

Table 4. Load of gram positive bacteria of *Catla catla* stored in ice (0 h and after 6 h)

Storage period (Day)	Gram Positive Bacteria			
	0 h		6h	
	Log_{10} (cfu/g)	Percentage (%)	Log_{10} (cfu/g)	Percentage (%)
0	4.92±0.46	44.08	5.33±0.25	56.03
4	4.73±0.50	47.61	5.14±0.01	54.31
8	3.99±0.08	45.53	4.16±0.11	52.17
12	5.42±0.49	41.84	5.59±0.14	51.63
16	5.89±0.21	46.45	5.89±0.40	52.53
20	6.16±0.20	44.55	6.36±0.12	55.48
24	7.27±0.17	45.55	7.36±0.27	53.73

Chinivasagam and Vidanapathirana (1985) isolated 59% gram negative and 41% gram positive bacteria in Trench Sardines (*Amblygaster sirm*) stored in ice immediately (0 h) and 43% gram negative and 57% gram positive bacteria after a delay of 5 hours. The lower number of gram negative bacteria in fish iced after 6 h delay at ambient temperature (43.97°C) may explain why delayed icing by 6 h had no effect on fish quality. The remaining micro-flora had been dominated by gram positive bacteria. Another possible reason for lower rate of spoilage on delayed icing of fish was explained by (Poulter *et al.* 1981). They suggested that when warm water fish are kept at high temperature, the onset of rigor is slow and of a longer duration, but when

preserved in ice immediately, it is of a shorter duration resulting in delayed bacterial growth till rigor is resolved.

The gram negative and gram positive bacteria were dropped over a period of 8 days in both the storage condition probably due to cold-shock or leaching of surface flora. After 8th day onwards, the gram negative and gram positive bacteria in both the batch gradually increased in numbers.

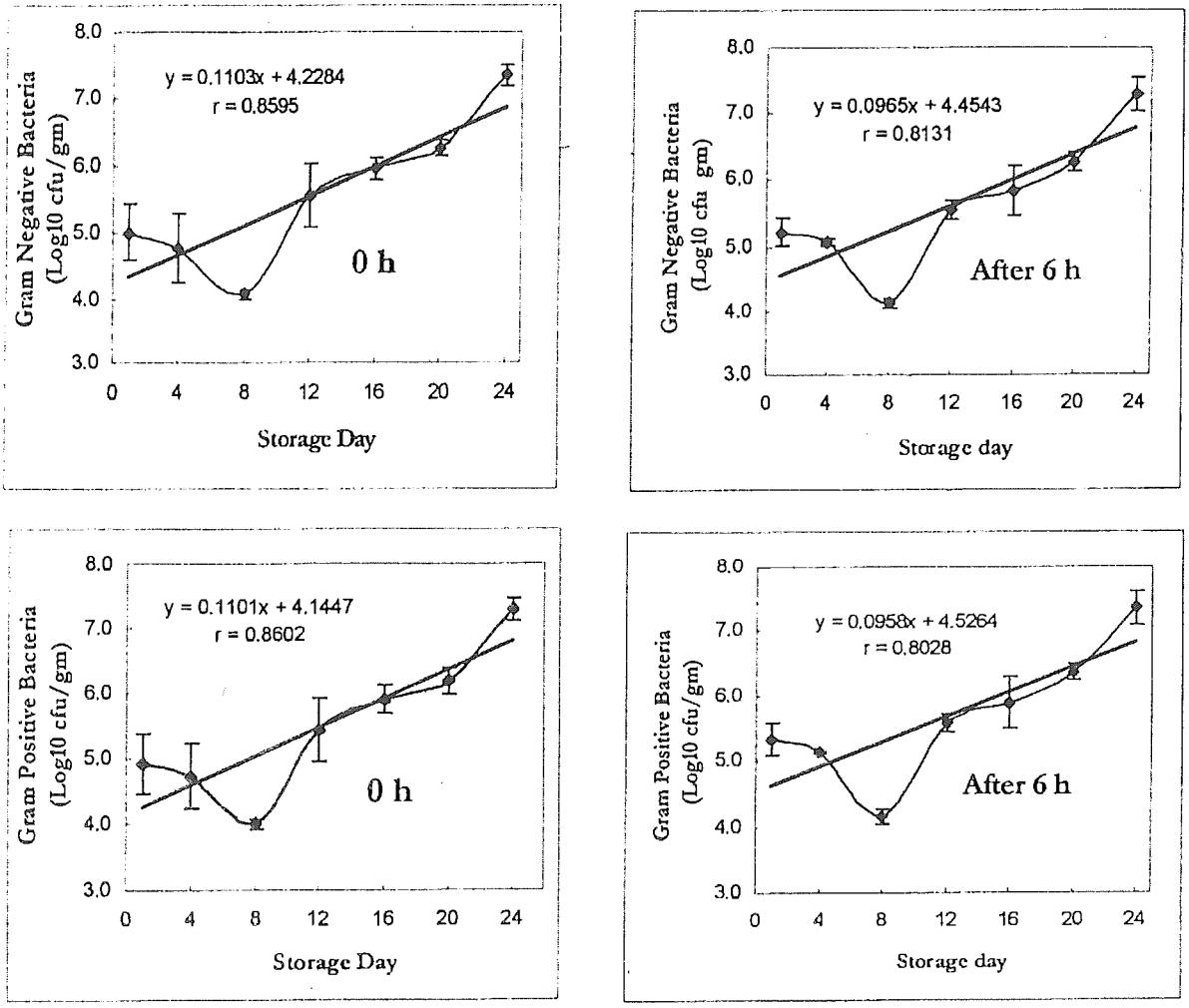

Fig. 3. Gram negative and gram positive bacteria (log_{10}cfu/g) of *Catla catla* sorted in ice (0 h and after 6 h).

Regression analysis (Fig. 3) clearly indicates a positive correlation of gram negative and gram positive bacteria with their subsequent storage period. ANOVA analysis shows a significant differences ($p<0.05$) in both the cases.

Quality changes in Catla catla stored at ambient temperature

Changes in standard plate count: Table 5 revealed that SPC count increased with storage period. At the initial stage of the experiment, the SPC value was 4.28 cfu/gm which increased rapidly to 9.62 cfu/gm after 16.5 hours of storage. A positive correlation (r=0.9812) was found between standard plate counts and storage time (Fig. 4)

Table 5. Standard plate count (Log_{10} cfu/g) of *Catla catla* stored at ambient temperature

Storage time							
0 h	3h	6h	9h	12h	13.5h	15.5 h	16.5h
4.28 ±0.83	5.25 ±0.15	6.66 ±0.66	7.74 ±0.32	7.96 ±0.58	8.34 ±0.96	8.85 ±0.46	9.62 ±0.45

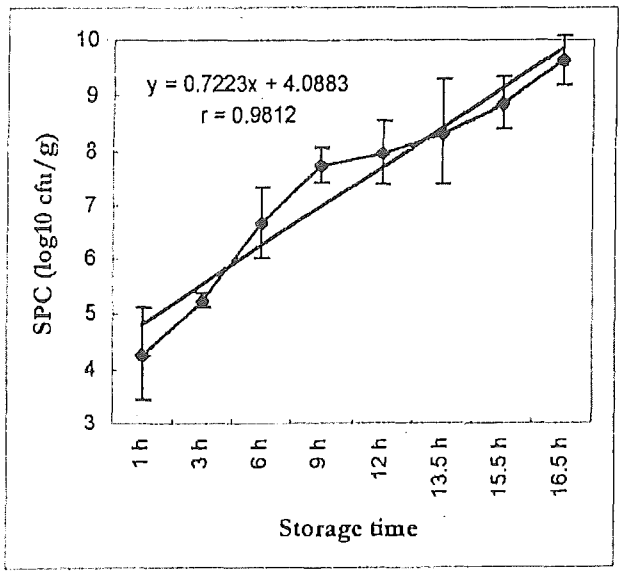

Fig. 4. Standard plate count (log_{10} cfu/g) of *Catla catla* stored at ambient temperature.

A comparison of organoleptic score and SPC data obtained in the present study indicated that acceptability for Catla showed a value of 7.96 cfu/g after 12 hours of storage when the fish was just acceptable. It was extended to 8.34 cfu/g after 13.5 hours when the fish was rejected organoleptically. Bacterial population increases with the increase of storage time but the result did not indicate any definite limit of acceptability and showed a lot of fluctuation (Rahman 1980). Present findings are confirmatory with work of many authors (Disney *et al.* 1969, Nair *et al.* 1970).

Gram negative bacteria and gram positive bacteria

Table 6 illustrates the gram negative and gram positive bacteria of *Catla catla* stored at ambient temperature during the period of 16.5 hours. Initially, the percentage of gram negative and gram-positive bacteria was 54.16% and 45.84% respectively. After 16.5 h of storage period, gram negative bacteria decreased (45.15%) while gram positive bacteria increased (54.85%).

Table 6. Load of gram negative bacteria and gram positive bacteria (Log_{10} cfu/g) of *Catla catla* stored at ambient temperature

Storage hour	Gram Negative Bacteria		Gram Positive Bacteria	
	Log_{10} cfu/g	Percentage (%)	Log_{10} cfu/g	Percentage (%)
0 h	4.01±0.81	54.16	3.94±0.86	45.84
3 h	5.02±0.13	52.82	4.86±0.17	47.18
6 h	6.35±0.65	48.79	6.37±0.66	51.21
9 h	7.42±0.32	47.84	7.45±0.33	52.16
12 h	7.37±0.58	45.57	7.80±0.59	54.43
13.5 h	7.93±0.95	43.02	8.12±0.97	56.98
15.5 h	7.75±0.43	41.92	8.78±0.48	58.08
16.5 h	8.94±0.48	45.15	9.45±0.43	54.85

On the 12 h of storage, the gram negative and gram positive bacteria showed a value of 7.37 and 7.80 (cfu/g) respectively. While on the 13.5 h of storage, the results were 7.93 and 8.12 (cfu/g). The differences in gram negative bacteria between the storage times were statistically highly significant ($p<0.05$). Gram positive were also statistically different ($p<0.05$). Fig. 5 represents the regression line with the subsequent storage time and bacterial load were highly correlated with the time.

Conclusions

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 as found in the present investigation. However, shelf life reduced drastically when stored at ambient temperature. Thus, the fishes should be preserved at ice as for the cheapest and easiest method to maintain the quality of fishes for a considerable amount of time.

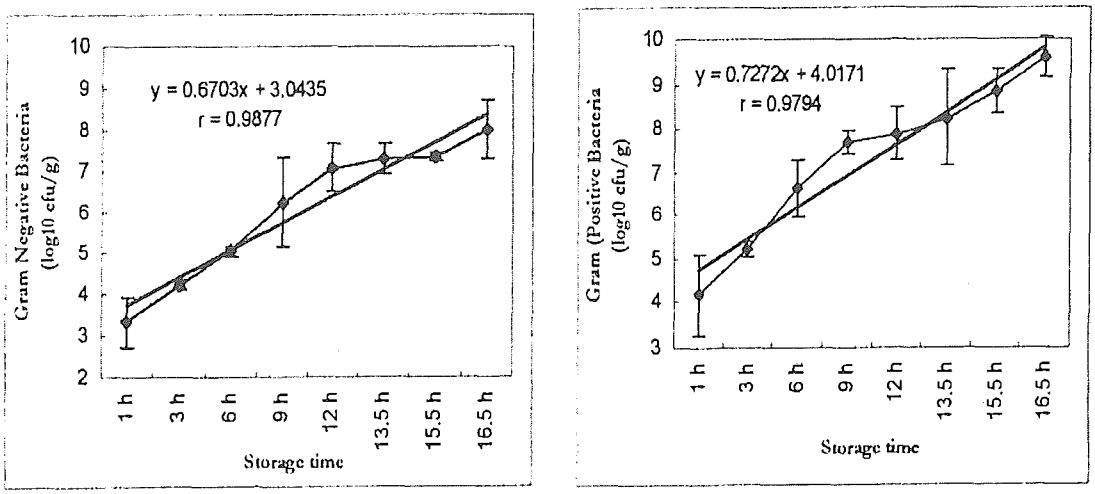

Fig. 5. Gram negative bacteria and gram positive bacteria (log_{10} cfu/g) of *Catla catla* stored at ambient temperature.

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(Manuscript received 3 November 2008)