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Post-mortem Biochemical, Microbiological and Sensory Quality Changes in the Grass Carp (*Ctenopharyngodon idella*) during Ice Storage

Md. Golam Rasul[1], Md. Omor Faruk[1], Md. Shirajul Islam Sarkar[1], Chunhong Yuan[2], Taslima Akter[3], A. K. M. Azad Shah[1,*]

[1]Department of Fisheries Technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

[2]Department of Food Production and Environmental Management, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Japan

[3]Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

[*]**Corresponding Author: azad@bsmrau.edu.bd**

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ABSTRACT

In this study, biochemical, bacteriological, and sensory changes of ice stored (0 ˚C) grass carp (*Ctenopharyngodon idella)* were investigated. Whole, ungutted fish were stored in insulated ice boxes maintaining a 1:2 fish ice ratio and analyzed periodically at every 3-day interval. On a fresh matter basis, moisture, crude protein, crude lipid, and ash content of grass carp during ice storage varied significantly ($P < 0.05$). The initial pH value of 6.76 increased to 7.05 on the 12th day of ice storage. Throughout the storage period, total volatile basic nitrogen and thiobarbituric acid reactive substances values were increased. K-value also increased from 2.32% to 68.74% within 12 days. Aerobic plate count and total psychrotrophic count suggested that the fish was safe for human consumption until the 9th day of storage; a similar acceptance period was also revealed by the result of sensory evaluation. From the evidence, it can be concluded that the shelf life of ice stored *C. idella* was 9 days in an insulated ice box with the above-mentioned fish ice ratio.

INTRODUCTION

Bangladesh is one of the major fish producing country in the world, having 735 freshwater and marine fish species **(Shamsuzzaman *et al.*, 2017)**. According to **FAO (2020)**, Bangladesh obtained 2nd position in aquaculture sector and exported 73,171 metric tons of fisheries products worth of BDT 42.50 billion **(DoF, 2020)**. Due to easy cultivation practice, rapid growth rate, and good nutritional value, grass carp (*Ctenopharyngodon idella*) is cultured and traded all over the world (**Cudmore and Mandrak, 2004)**. In 2018, globally 5,704 thousand tons grass carp was produced and reported as the largest production among all aquaculture finfish species **(FAO, 2020)**. Grass carp contributed 1.58% of total fish production in Bangladesh **(DoF, 2017)**. Such

higher production arises the question of preservation after harvesting as fish is a highly perishable commodity.

Fish begins to spoil immediately after harvesting as a result of the series of complex, irreversible physical and biochemical changes that become evident by sensory manifestations e.g. objectionable odor, off flavor, loss of muscle texture, dull appearance etc. **(Gram and Huss, 1996; Sallam, 2007)**. Bacterial action, oxidation of fat and endogenous enzymatic decomposition are the major cause of fish spoilage. Lipid oxidation is a common phenomenon in fish because it contains higher amount of polyunsaturated fatty acids. Even after death of fish, enzymatic activity progresses, which causes breakdown of tissues that affects freshness, flavor, texture and appearance as well as accelerate bacterial decomposition **(Liu *et al.*, 2013)**. In post-rigor stage, bacterial metabolic activity produces volatile basic nitrogenous compounds that alter the muscle pH from acidic to alkaline range. Such post-mortem physical and biochemical changes in fishes varied upon species, size, physical condition, harvesting method, place of fishing ground, season etc. **(Nowsad, 2007)**. Moreover, higher atmospheric temperature of summer season (30-35 °C) in tropical regions accelerates the quality deterioration of fish. Therefore, it is suggested to preserved fish at low temperature **(FAO, 1993)**.

Various methods such as chilling, freezing, salting, frying, pickling etc. are employed to extend shelf life of fish. Chilling by means of ice known as icing, is one of the easiest and low cost method among these preservation methods. It is also more practical and economical for short term preservation as ice storage do not require electrical supply **(Mehta *et al*., 2014)**. Ice melts at 0 ºC, which does not freeze the fish, however, controls the temperature at ideal chilled level **(Clucas and Ward, 1996)**. Rate of biochemical reactions become half and shelf life doubles if storage temperature is decreased by 5-6 ºC **(Mjelda and Urdahl, 1974)**. Initial microbial load of the fish and storage temperature greatly influence the shelf life of ice stored fish **(Church, 1998)**. Moreover, melted ice water wash away surface bacteria and contaminants, keeps fish surface wet that prevents dehydration and retains the glossy appearance. The biochemical, microbiological and organoleptic characteristics during ice storage have been determined in many fishes such as *Gibelion catla, Scomber scombrus* **(Bennour *et al.*, 1991)**. As grass carp is a herbivorous fish, therefore, it is assumed that physical and biochemical changes and duration of shelf life during ice storage might be different from other fish species. In Bangladesh most consumers highly prefer this species in fresh condition. So, fish mongers generally sell this fish at iced condition. Until now, about shelf life extension of grass carp using icing has been scarcely studied. Therefore, this study was aimed to determine the biochemical, bacteriological, sensory characteristics, and shelf life of ice stored grass carp.

MATERIALS AND METHODS

Sample collection and storage

Live grass carp (*Ctenopharyngodon idella*) (n=24) (average weight 1100 ± 100 g) were obtained from a fish farm located at Kapasia, Gazipur, Bangladesh and transported to laboratory in an ice box. Fish were prepared by ikejime method **(Wikipedia, 2022)** through inserting a sharp needle in the hindbrain. Whole *C. idella* were immediately iced (0 ˚C) in ice boxes at 1:2 fish ice ratio. Everyday melted ice water was drained out and ice was added at 9.00 am and 9.00 pm. Boneless, skinless edible anterior-dorsal muscle part was separated from randomly selected fishes, homogenized, and analyzed at 3 day's interval until apparently unacceptable for human consumption.

Chemical analysis

Crude protein, crude lipid, moisture, and ash content were measured by **AOAC (2002)** method. The pH value was directly measured using a pH meter following the method of **Rasul *et al.* (2021)**. Thiobarbituric acid reactive substances (TBARS) value was determined colorimetrically according to **Kirk and Sawyer (1991)** and the value was expressed as mg malondialdehyde (MDA)/kg of lipid. According to **AOAC (2002)**, the total volatile basic-nitrogen (TVB-N) was assessed and expressed as mg N/100 g muscle. According to **Ryder (1985)**, ATP, ADP, AMP, IMP, inosine and hypoxanthine were determined by HPLC. Standards were purchased from Sigma-Aldrich, India.

Microbial analysis

According to the Chinese National Standard (GB4789.2-2010) aerobic plate count (APC) was measured. To enumerate the psychrotrophs, bacteria culture plates were incubated at 5 °C for 72 hours **(Sharifian *et al.*, 2011)** and in both cases, the values were expressed as log CFU (colony forming units)/g of flesh.

Sensory evaluation

Method described by **Howgate *et al.* (1992)** was used to determine organoleptic acceptability of fish samples. In individual sensory booths, fish samples were served to 12 panelists (age 24-37 years) from the Department of Fisheries Technology.

Statistical analysis

The data were expressed as mean ± SD (standard deviation). One-way analysis of variance (ANOVA) was performed and Duncan's multiple range test was done to compare the means ($P < 0.05$).

RESULTS AND DISCUSSION

Changes in proximate composition of *C. idella* during ice storage

The proximate composition of *C. idella* muscle during 12 days of ice storage are shown in Table 1. Sensory characteristics and microbial growth are influenced by proximate composition of fish, which strongly contribute in acceptability of that fish for human consumption **(Ibrahim *et al.*, 2007)**. The proximate composition of fresh *C. idella* muscle was 76.48% moisture, 19.07% crude protein, 1.93% crude lipid and 1.12% ash before storage in ice.

Table 1. Changes in proximate composition of *C. idella* muscle during ice storage[1]

Storage Time (Days)	Moisture %	Crude protein %	Crude lipid %	Ash %
0	76.48 ± 1.12^{c}	19.07 ± 0.91^{a}	1.93 ± 0.11^{a}	1.12 ± 0.11^{b}
3	77.05 ± 1.00^{bc}	18.77 ± 0.69^{a}	1.72 ± 0.12^{b}	1.51 ± 0.15^{a}
6	78.01 ± 1.05^{abc}	17.96 ± 0.72^{ab}	1.65 ± 0.11^{bc}	1.36 ± 0.14^{ab}
9	78.97 ± 1.14^{ab}	17.40 ± 0.55^{b}	1.49 ± 0.11^{cd}	1.30 ± 0.26^{ab}
12	79.37 ± 1.20^{a}	16.93 ± 0.66^{b}	1.31 ± 0.11^{d}	1.27 ± 0.19^{ab}

[1]The values are expressed as mean ± standard deviation (n=5). Means with different superscripts within a column are significantly different ($P < 0.05$)

Moisture content increased from 76.48% to 79.37% during ice storage. Ice melt water absorbed by fish flesh increases the moisture content. Similarly, **Sravani (2011)** found that, in rohu (*Labeo rohita*) moisture content increased from the initial value of 78.55% to 80.16% at 10th day of ice storage. At the end of the experiment, no significant difference was observed among 0, 3rd and 6th day's sample but significant difference ($P < 0.05$) was observed between 0, 9th, 12th day's samples, and 3rd and 12th day's sample. Crude protein content decreased gradually with the increase of storage period. Significantly ($P < 0.05$) the highest crude protein (19.07%) content of *C. idella* muscle was observed on fresh matter basis at day 0 that ultimately reduced to 18.77%, 17.96%, 17.40% and 16.93% at 3rd, 6th, 9th and 12th day of ice storage, respectively. Drain out of water soluble proteins and dilution effect due to entry of ice melted water through osmosis process are the reasons behind decrease in crude protein content **(Dileep *et al.*, 2005)**. Similar result was also observed in pink perch during ice storage **(Reddy and Srikar, 1991)**, and in minced channel cat fish when stored at 4˚C **(Suvanich, *et al.*, 2000)**. Total crude lipid content also shown a significant ($P < 0.05$) decrease throughout the entire study period. The crude lipid content was reduced to 1.72%, 1.65%, 1.49%, and 1.31% at 3rd, 6th, 9th and 12th day's sample, respectively. Similar results were reported in *Cyprinus carpio* **(Meenakshi *et al.*, 2010)**, and in rohu (*Labeo rohita*) **(Sravani, 2011)** during ice storage. The loss of crude lipids might be due to oxidative rancidity **(Afrin *et al.*, 2021)**. Like crude protein and crude lipid content, the ash content was also decreased

significantly ($P < 0.05$) during the entire period of ice storage. Ash content was 1.12%, 1.51%, 1.36%, 1.30% and 1.27% at 0, 3rd, 6th, 9th, 12th days of ice storage, respectively. Ash content at the 3rd day was increased due to relative loss of proteins. **Garcia-Arias *et al.* (2003)** suggested that ice-melted water leaches out ash content. **Zhao *et al.* (2019)** found that ash content in *Sebastes schlegelii* and *Hexagrammos otakii* decreased with storage time.

Changes in pH values

Changes in pH values of *C. idella* muscle during ice storage are shown in Fig. 1. The initial pH of fresh *C. idella* muscle was 6.76. The pH value of fresh fish muscle usually range from 6.0 to 7.0 **(Li *et al.*, 2012)**. At day 3, the pH value decreased slightly. The fish remained in stress and struggled during harvesting which causes more accumulation of lactic acid and octopine in fish muscle derived from glycogen metabolism **(Cai *et al.*, 2014)**. Then the pH value were increased to 6.67 and 6.93 at 6th, and 9th day, respectively and finally reached to 7.05 at 12th day. No significant difference in pH value was observed between 0 and 3rd day, whereas significant difference was observed among the pH values when analyzed at 3rd, 6th, 9th and 12th day. The subsequent increase of pH values in this study might be due to the formation of volatile basic nitrogenous compounds, such as trimethylamine and ammonia that resulted from either microbial or endogenous enzymatic activities **(Duman and Ozpolat, 2015)**. Similar results were found by **Dilip *et al.* (2007)** in *Labeo rohita*.

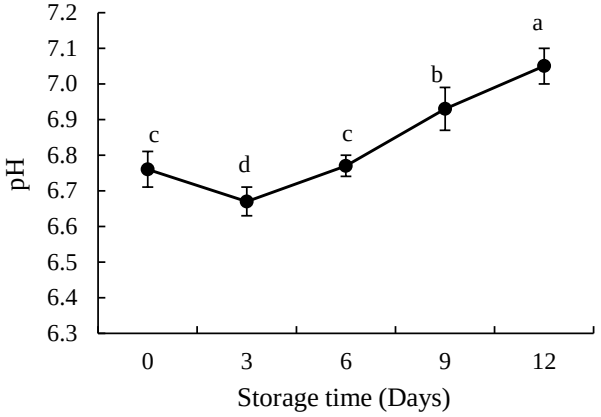

Fig. 1. Changes in pH values of *C. idella* muscle during ice storage. The error bars represent means ± SD (n=5). Means with different superscripts with error bars are significantly different ($P < 0.05$).

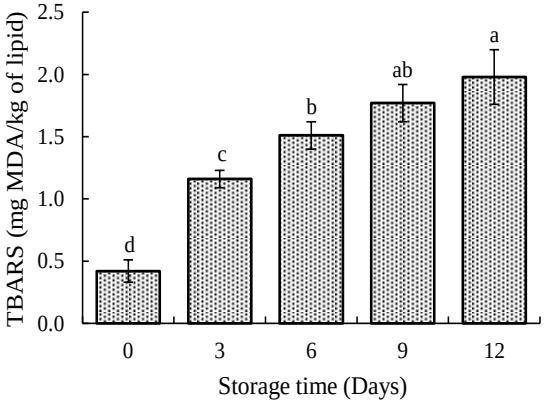

Fig. 2. Changes in TBARS values of *C. idella* muscle during ice storage. The error bars represent means ± SD (n=5). Means with different superscripts with error bars are significantly different ($P < 0.05$).

Changes in thiobarbituric acid reactive substances

The TBARS value indicates secondary lipid oxidation products as malonaldehyde. The lipid oxidation of fish during ice storage causes malondialdehyde (MDA) formation, which leads to undesirable off-flavors and toxic products formation **(Wenjiao *et al.*,**

2013). The initial TBARS value of *C. idella* muscle was 0.42 mg MDA/kg of lipid indicating that the fish was fresh before icing. The acceptable limit of TBARS value is 1-2 mg MDA/kg of lipid for fresh fish **(Connell, 1990)**. The TBARS value was increased significantly during the storage period and increased to 1.16, 1.51, 1.77 and 1.98 mg MDA/kg of lipid at the 3rd, 6th, 9th and 12th day's sample, respectively (Fig. 2). **Kyrana *et al.* (1997)** reported that icing slow down the production of malonaldehyde in whole, ungutted ground fish. The TBARS values increased very slowly and were in acceptable range in sutchi catfish during 18th days of ice storage **(Viji *et al.*, 2015)**. In tilapia fillet, TBARS value remained at a very low level (0.11- 1.35 mg MDA/kg of lipid) throughout ice storage **(Liu *et al.*, 2010)**. The results are similar to the findings of **Taliadourou *et al.* (2003)** in sea bass and **Chytiri *et al.* (2004)** in cultured rainbow trout during ice storage.

Changes in total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) is a group of compounds composed of primary, secondary and tertiary amines and degraded products of protein and non-protein nitrogenous compounds caused by microbial activity and endogenous enzymatic actions **(Li *et al.*, 2013)**. TVB-N is often used as an indicator of fish meat deterioration. Fish products are considered as spoiled and unacceptable for human consumption when TVB-N value become higher than 30 mg N/100 g of fish muscle **(Huss, 1995)**. In this study, TVB-N content increased significantly ($P < 0.05$) during ice storage up to 12 days due to combined effects of microbiological and autolytic deamination of amino acids **(Truelstrup *et al.*, 1995)**. Whole and ungutted fish resulted the higher hydrolysis of nitrogenous compound by visceral and gill microorganisms **(Benjakul *et al.*, 2003)**. The values of TVB-N content of *C. idella* muscle was 12.69, 24.45, 27.25, 29.58 and 37.66 mg/100 g at 0, 3rd, 6th, 9th, and 12th day of storage, respectively; which indicates that the fish was acceptable for consumption up to 9th day of ice storage (Fig. 3). Similarly, it is reported that, TVB-N value increased throughout ice storage in farmed seabass (*Dicentrarchus labrax*) and reached 26.77 mg N per 100 g muscle at 13th day **(Taliadourou *et al.*, 2003)**. Again, TVBN values of *Mugil cephalus* and *Otolithes ruber* also increased throughout 15 days ice storage period **(Ninan and Zynudheen, 2014)**.

Changes in K-value

Post-mortem ATP to IMP conversion usually completed within a day. Subsequent accumulation of inosine or hypoxanthine is attributed to both autolytic and microbial activities. Such nucleotide products calculated by K-value is generally considered as freshness indicator of seafood **(Nowsad, 2007)**. K-value increased from 2.32% to 68.74% within 12 days (Fig. 4). K-value of fresh fish remains as low as zero, 10-20 in moderate quality fish and acceptable up to 60% which can go up to 90 in spoilt fish **(Gopakumar, 2002)**. Acceptable level of K-value varies with species. **Ninan and Zynudheen (2014)** reported that, acceptable level of K-value is above 60% in case of *Mugil cephalus* and

Otolithes ruber in ice storage. So, in regard to K-value, grass carp in ice storage is acceptable for 9 days. Besides, ice stored Greenling showed a quick increase in the K-value throughout the study period due to production of a strong protease by spoilage bacteria that degraded muscle protein **(Yoshioka *et al.*, 2019)**.

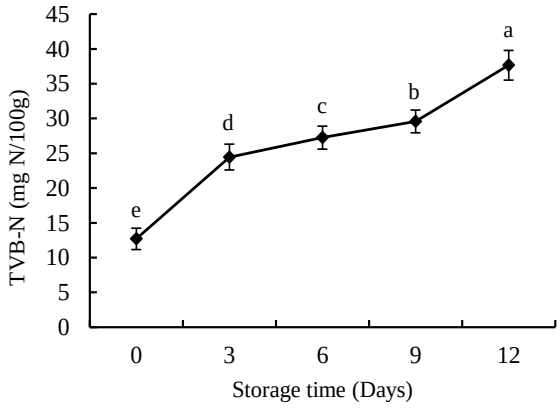

Fig. 3. Changes in TVB-N values of *C. idella* muscle during ice storage. The error bars represent means ± SD (n=5). Means with different superscripts with error bars are significantly different (P < 0.05).

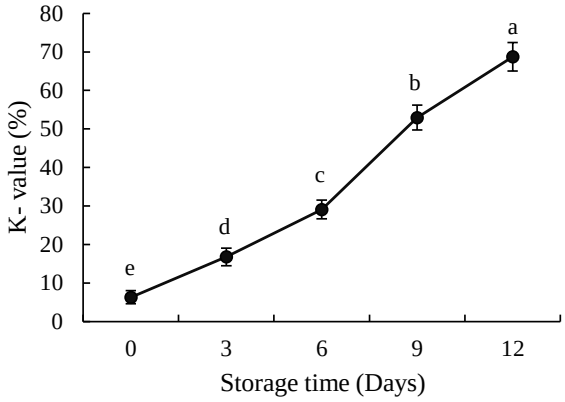

Fig. 4. Changes in K-value of *C. idella* muscle during ice storage. The error bars represent means ± SD (n=5). Means with different superscripts with error bars are significantly different (P < 0.05).

Changes in microbial count

Microbial activity determines the quality of the fish. In case of acceptability of food, aerobic plate count (APC) has often been used as an indicator **(Soares *et al.*, 2013)**. The APC of *C. idella* varied from 3.19 to 8.19 log CFU/g during the study period (Fig. 5). The initial APC 3.19 log CFU/g increased up to 8.19 log CFU/g at the 12^{th} day of storage. APC was significantly (P < 0.05) increased over the storage period. Similar result was found in mearge fish and *Heteropneustes fossilis* stored in ice **(Hernández *et al.*, 2009; Yeasmin *et al.*, 2010)**. The APC of some marine fishes like silver jewfish, bombay duck and ribbon fish species were in acceptable level upto 10 days during ice storage period (**Reza *et al.*, 2009**). Microbial quality of the waters from which the fish caught determines the initial bacterial load of fresh caught fish **(Huss, 1988)**. The eventual increase observed in mean APC could be attributed to multiplication of microbes due to favorable conditions found in ice storage **(Ibrahim and El-Sherif, 2008)**.

Psychrotrophic count of *C. idella* during ice storage has been shown in Fig. 6. Like APC, psychrotrophic count also increased over storage period. Within 12 days, psychrotrophic count increased from 0 to 8.03 log CFU/g. **Sharifian *et al.* (2011)** found that during ice storage psychrotrophs increased with progression of storage time and ultimately taken over the initially dominant mesophiles. To be considered safe, however, bacterial count of fish should never exceeds 7 log CFU/g wet weight **(Ojagh *et al.*,**

2010). In this study, both APC and psychrotrophic count exceeded 8 log CFU/g at 12th day. So, grass carp is safe for human consumption till 9 days stored in ice.

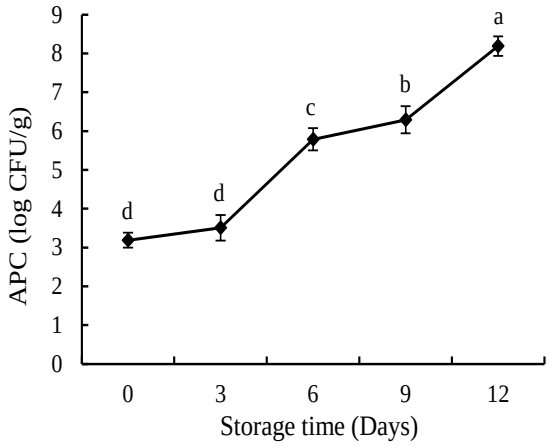

Fig. 5. Changes in APC values of *C. idella* muscle during ice storage. The error bars represent means ± SD (n=5). Means with different superscripts with error bars are significantly different (P < 0.05).

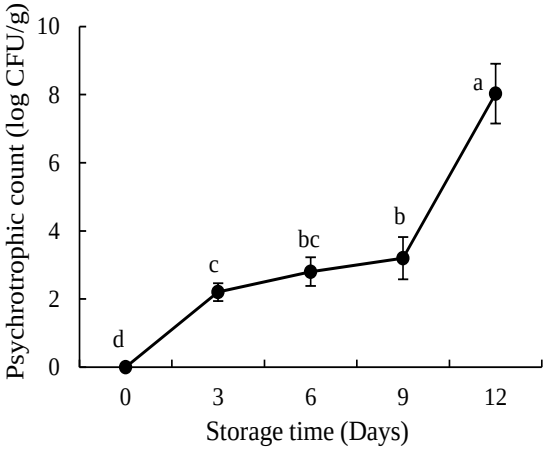

Fig. 6. Changes in psychrotrophic count in *C. idella* muscle during ice storage. The error bars represent means ± SD (n=5). Means with different superscripts with error bars are significantly different (P < 0.05).

Sensory evaluation

Sensory quality changes of *C. idella* in ice storage has shown in Table 2. Fish quality was evaluated based on scores obtained from scale 1-5. Scores <2, 2 to <5, and ≤5 were considered as Grade A (excellent), Grade B (good or acceptable) and Grade C (bad or rejected), respectively. With the increase of storage perod, the defect scores increased. Based upon such scoring, ice stored *C. idella* were acceptable for human consumption up to 9 days.

Table 2. Sensory qualities changes of *C. idella* during ice storage (n=12)

Storage period (days)	Defect scores	Grade and overall quality
0	1	A (Excellent)
3	1.80	A (Excellent)
6	3.21	B (Acceptable)
9	3.67	B (Acceptable)
12	5	C (Rejected)

At 0 day, fish had protrude and clear eyes, elastic and firm muscle, bright red gills, shiny appearance which reflected the high level freshness of fish. The sensory quality of *C. idella* declined steadily with increase of storage period. Finally, at day 12th, overall dull appearance, distinctive off-odours and flavours found in fish which made the fish unacceptable for human consumption. This phenomenon is relevant with the observed

total aerobic plate count. Similar results were reported in sea bass, common carp, and tilapia by **Taliadourou *et al.* (2003)**; **Liu *et al.* (2010)**, respectively. Raw material quality, icing method and duration determine the shelf life of fish stored in ice in a great extent. Based on the mean panel scores the fish was acceptable for 9 days during ice storage.

CONCLUSION

The values of biochemical parameters *viz*. pH, TBARS, TVB-N and K-value, and the aerobic plate count, psychrotrophic count, and sensory parameters- odor and color of gills, general appearance, eye condition and slime production of *C. idella* indicates that, even in proper ice storage, quality deterioration occur in the fish with the progress of storage time. All the parameters indicate that shelf life of grass carp was 9 days during storage in ice. After 9 days of ice storage, the fish should be considered as spoilt and unfit for human consumption. Further study could be conducted on the freshness keeping technology of fish for higher quality and longer shelf-life.

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