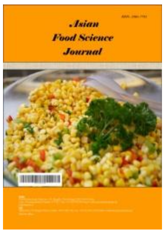

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Biochemical and Organoleptic Assessment of Asian Stinging Catfish (*Heteropnuestes fossilis)* in Two Storage Conditions

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Authors' contributions

This work was carried out in collaboration among all the authors. Authors US and MH designed the study and wrote protocol, authors US and LI conducted analysis and wrote the first draft, authors MH and SS performed statistical analyses, corrected the draft and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The proximate composition, rigor-index, organoleptic quality, and pH of Asian stinging catfish stored at iced condition (SIC) and stored at ambient temperature (SAT) were studied. Rigor mortis for both ice and ambient temperature started immediately after death and the rigor-index was maximum 82.35 % and 63.42% at 4 hrs and 8 hrs storage in SIC and SAT, respectively. Fish SIC showed a remarkably extended maximum rigor-index period of 14 hrs and 2 hrs in fish SIC and SAT, respectively. Fish SIC showed post-rigor period for upto 30 hrs, it was shorter in fish SAT for 18 hrs, though in both storage the rigor-index of the fish did not ceased completely. The moisture, protein, lipid, and ash content of fresh, SIC and SAT fish were 77.30%, 75.57%, and 78.81%, 15.04%, 15.34%, and 14.42%, 6.10%, 6.30%, and 3.47%, 1.99%, 2.67%, and 2.95%, respectively. The initial pH of fresh fish muscle was 6.89 which decreased to 6.32 and 6.12 after 18 hrs in fish

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SIC and SAT, respectively. The organoleptic quality assessment was in acceptable condition up to 12 days in fish SIC whereas a very short period of 10 hrs at fish SAT. Considering the findings of present study, it is highly recommended to store Asian stinging catfish at iced condition for maintaining shelf-life and quality.

Keywords: Asian stinging catfish; ice storage; ambient temperature; rigor-index; proximate composition; pH.

1. INTRODUCTION

Asian stinging catfish (*Heteropnuestes fossilis),* is a freshwater fish of Heteropneustidae family of Siluridae order and found in Southeast Asian countries including Bangladesh, India Pakistan, Thailand, and Sri Lanka. It is a high valued and very popular fish in Bangladesh due to be considered as a highly palatable, nourishing, and tasty [1]. It is well preferred by consumers because of its less fat, less spine, and high digestibility [2]. This species of fish is rich in iron (226 mg/ 100g muscle) and calcium and so why it is recommended in the diet of sick and convalescents [1]. As it is a lean fish, suitable for the people who are not interested to take animal fats [3]. In previous times, this species of fish were captured from natural water bodies but due to over exploitation and diseases out break the natural stock is ceased and insufficient enough to fulfill the consumers demand. Recently, the induced breeding of this fish is commercially started and the culture practice of this species is highly expanded. Though it is a fish species with accessory respiratory organ and can survive long periods after captures, sometimes it is crucial to transport this species in live at distance place. Transporting fish to a long distance with insufficient amount of ice for short-term storage hamper shelf-life of fish [4]. So, it requires proper preservation to maintain shelf-life of the fish during transportation and marketing to ensure safe food to the consumers. As fish are highly perishable, it is essential to determine the changes, especially the rigor mortis and post mortem changes to keep it safe for the consumers.

Rigor mortis is a phenomena which causes stiffness in the muscle after death which results in the rigidity. Rigor-mortis is one of the most prominent post-mortem events in muscle. The progress of rigor mortis depends on many factors such as species, size, age, season, handling pressure, temperature, etc. [5]. Physical and biochemical changes are responsible for occurring rigor mortis of the body after death. Physical changes include general appearance, texture, and stiffening of the body. Different biochemical changes occur in the muscles after several hours of death. When fish dies, the post mortem changes take place due to breakdown of cellular structure by autolysis or microbial enzymes and growth of microorganisms [6]. However, post-harvest handling is highly responsible for post mortem biochemical changes in fish. These biochemical changes include ATP depletion, lactic acid development, and decrease in pH level. Rigor mortis consist of three stages: pre-rigor, in rigor, and post-rigor. The process of rigor mortis is very important for post-harvest preservation and processing of fish. Quality of fish depends largely on the process of rigor mortis and usually confirmed on the pre-rigor period. If the progress of the rigor-mortis period takes long time, the shelf life of the fish remains longer. By increasing the pre-rigor period, the shelf-life of fish can be increased. This helps in keeping freshness of the fish flesh.

Review of the literature showed that post mortem and biochemical changes of Asian stinging catfish are not available. It is a tropical fish and the spoilage rate of tropical and sub-tropical species in storage considerably differs from those of cold water species. Spoilage pattern and loss of freshness in fish is markedly varies from species to species [7]. Though the fish is mostly marketed lively, it also needs to be ice stored for transported to remote area where the fish is not available. Ice storage is a very cheap, easy, efficient and adapted process for post-harvest short term fish preservation. For this reason, it is important to know the status of rigor mortis at ice storage and at room temperature. Nowadays, the freshness and quality of fish are evaluated by measuring post mortem changes associated with organoleptic, physical and chemical changes, and microbial growth [8]. Additionally, as far we know, there is no report on the changes of organoleptic condition at storage of the Asian stinging catfish at ice storage and room temperature. Therefore, the objectives of the present study were: i) To evaluate the rigor-index and organoleptic assessment of Asian stinging catfish stored both at iced condition and ambient temperature ii) To measure the proximate composition and pH of fresh fish, and fish store at iced condition and at ambient temperature.

2. MATERIALS AND METHODS

2.1 Sample Collection

Asian stinging catfish, *Heteropneustes fossilis* was collected from the Borobazar market, Doratana, Jashore, Bangladesh and brought to the laboratory in live condition. A 50 Lit size plastic drum containing water was used for transporting 90 fishes in laboratory within 30 mins of harvest. Total body length and weight of the fish were 18.0±1.5 cm and 35.0±2.25 g, respectively. Ten (10) fishes were separated randomly to analyze proximate composition of fresh fish. The fishes were then grouped in two categories where a portion (40 fishes) was ice stored (2±1°C) (fish: ice ratio=1:2) in an ice box and the rest (40 fishes) were stored at ambient temperature (28±3°C) in an ice box without ice. The storage period was not pre-determined. The storage period was depended on the organoleptic characteristics of the stored fish and it was 18 hrs for fish SAT and 14 days for fish SIC.

2.2 Proximate Composition Analysis

Proximate composition such as moisture, crude protein, lipid, and ash content of the muscle of fresh fish, fish SIC, and fish SAT were analyzed by following the standard procedures described by AOAC [9].

2.3 Determination of Rigor-index

Rigor-index was measured following the method of Bito et al. [10]. Immediately after death, the fish was put on a horizontal table in such a way that half of the body (tail part) kept out of the table. At every 2 h interval, the rigor-index of both categories of fish SIC and SAT was measured. The rigor-index of fish was measured following the equation:

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

Where, Do 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.

2.4 Organoleptic Assessment

A large number of schemes have been introduced for the sensory assessment of various types of fish. The guidelines and process adopted here using score on the organoleptic characteristics of fish as narrated by EC freshness grade for fish and fishery products [11] which is noted in (Table 1 and Table 2).

Table 1. Grade points table used to determine the freshness of Asian stinging catfish

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

Table 2. Defect characteristics and defect points used to determine the freshness of Asian stinging catfish

Defect Parameters	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) Sunken dye covered with yellow slime	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 slightly opaque or milky	1	Acceptable
	B) Turbid opaque and milky slime, with markedly increase in amount.	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

2.5 Measurement of Muscle pH

The pH of fish muscle was measured following the method of Haq et al. [12]. A pH meter (Model 250 pH /ISE, Denver Instrument Company, Colorado, USA) was used for measuring pH of fish muscle. Homogenate mixture was prepared by blending 5 g of fish muscles with 20 mL distilled water in a blender.

2.6 Statistical Analysis

All the analyses were done in triplicates. One-way analysis of variance (ANOVA) was used to analyze obtained data by SPSS software (SPSS version 20, Chicago, IL). Graphs were prepared using MS Office, Excel 2010.

3. RESULTS AND DISCUSSION

3.1Proximate Composition Analysis

The proximate composition of fresh fish, fish SIC, and fish SAT is shown in Fig. 1. The moisture content of fresh fish was 77.30%, which was decreased in fish SIC to 75.57% and increased in fish SAT to 78.81%. The moisture content was slightly decreased in ice storage; this might be due to evaporation of moisture from fish surface in ice storage because of various factors like relative humidity, chemical changes, and storage temperature. Dhanapal et al. [13] evaluated the proximate composition during ice storage of *Labeo rohita* for 18 days and reported that there was reduction in moisture content in ice storage. There was increase of moisture in fish SAT due to reduction of lipid content by oxidation and protein degradation. Obemeata and Christopher [14] conducted study on the cold storage of *Tilapia guineensis* and reported that the moisture content of fresh fish was 55.12% which decreased to 46.32% after 4 weeks storage. Gandotra et al. [15] reported that there was reduction of moisture content in *Labeo rohita* fillet stored at low temperature for 21 days. The moisture content of present study is in good agreement with the reported values.

Fish protein is considered as a unique source of animal protein because of containing high amount of myofibrillar protein and less stroma protein [16]. The protein content is fresh fish, fish SIC, and fish SAT was found to be 15.04%, 15.34%, and 14.42%, respectively. The protein content in ice stored fish did not varied significantly (P≤0.05) from the fresh fish whereas it was reduced significantly in fish SAT (Fig. 01). Gandotra et al. [14] reported that the protein content decreased to 13.06% from 15.93% due to cold storage at -12°C for 21 days. Obemeata and Christopher [14] reported the protein content of *Tilapia guineensis* was 15.13% in fresh fish and was decreased to 11.11% and 9.98% in -18°C and 4°C storage, respectively. The reduction of crude protein content of stored fish might be due to the degradation of protein and formation of various volatile compounds such as total volatile bases, trimethyl amine, ammonia, and hydrogen sulfide [17]. Chomnawang et al. [18] stated that the reasons of protein content reduction is due to the reduction of water soluble or salt soluble protein whether Hultman and Rustard [19] stated that autolytic deterioration process facilitated by microbial or autolytic enzymes caused protein content reduction. The protein content in fish SAT might also be decreased due to the destruction and spoilage of protein stored at ambient temperature. Protein content is reduced by amino acid destruction, formation of protein lipid complex, and peptide scission when exposed to oxidizing environment [20-22].

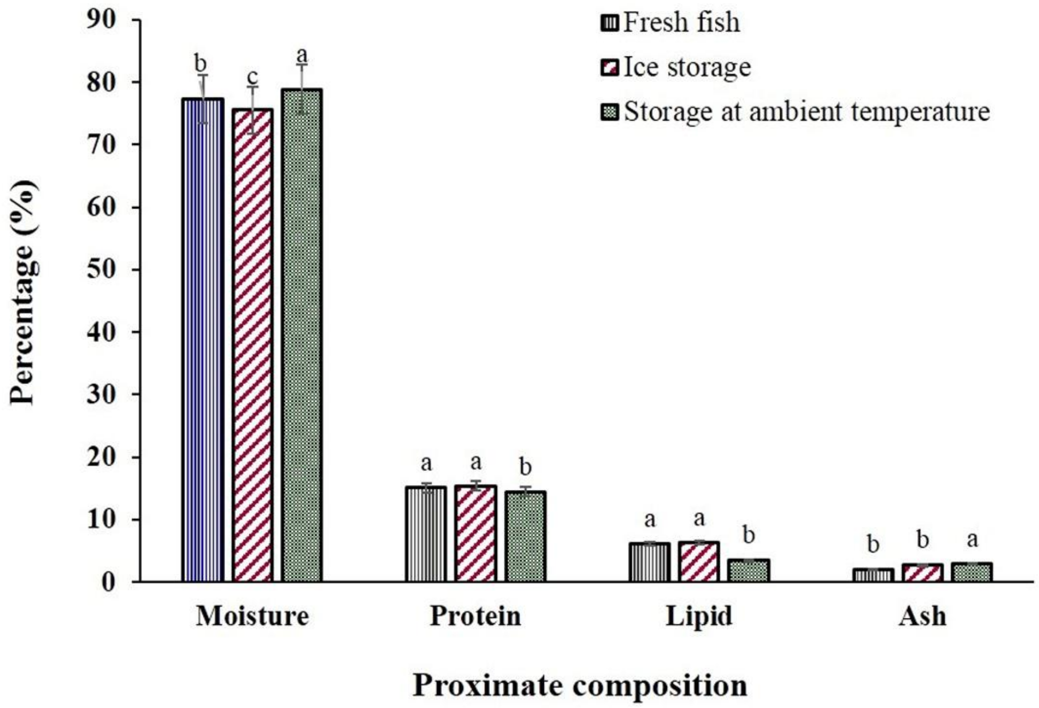


Fig.1. Proximate composition of fresh, ice stored, and ambient temperature stored fish
Different small letters on each bar indicates significant differences (P<0.05)

The lipid content was 6.10%, 6.30%, and 2.95% in fresh fish, fish SIC, and fish SAT, respectively (Fig. 1). The lipid content was sharply reduced in fish SAT due to high tendency of lipid oxidation at ambient temperature. The lipid content in fish SIC was similar to fresh fish because the lipid oxidation at cold condition was very slow. Kyrana and Lougovois [23] reported that the lipid auto-oxidation process was insignificant during ice storage and in whole iced sea bass lipid autoxidation appears to be a minor spoilage process. The lipid content in fresh *Tilapia guineensis* was 14.50% which decreased to 8.50% in cold stored fish after four weeks [14]. Similar result of lipid content reduction in storage of fish was reported by some other researchers [15,24-25]. The reduction of lipid content in storage is attributed due to oxidation and formation of undesired and obnoxious chemical compounds. The lipid content in stored fish might be associated with the oxidation of polyunsaturated fatty acids available in fish tissues to other products such as free fatty acids, aldehydes, ketones, and peroxides [26]. The ash content in fresh fish and fish SIC did not vary significantly whereas the value was increased in fish SAT. Actually, the ash content is not to be fluctuated in dry wet basis but the obtained value was different due to fluctuating the values of other components. Obemeata and Christopher [14] obtained similar result of increasing ash content at 4ºC stored *Tilapia guineensis*.

3.2 Changes in Rigor-index

The rigor-index of Asian stinging catfish SIC and SAT is shown in Fig. 2. Immediately after death, the rigor process started in both storage conditions and the rigor-index reached to the maximum of 82.35 % and 63.42% at 4 hrs and 8 hrs storage in SIC and SAT, respectively. Fish SIC showed a remarkably extended maximum rigor-index period of 14 hrs whereas the fish SAT showed maximum rigor-index period of 2 hrs. Fish SIC showed post-rigor period for up to 30 h whereas it was shorter in fish SAT for 18 hrs, though in both storage the rigor-index of the fish did not ceased completely. The present study showed that the rigor mortis begins much faster in fish SIC and the total rigor period was longer compared to fish SAT. On the other hand, rigor started later in the fish SAT compared to the fish SIC. Similar pattern of rigor index was reported by some researchers and stated that lower temperature slows down the rate of progress of rigor mortis showing an extended in-rigor period [27-29].

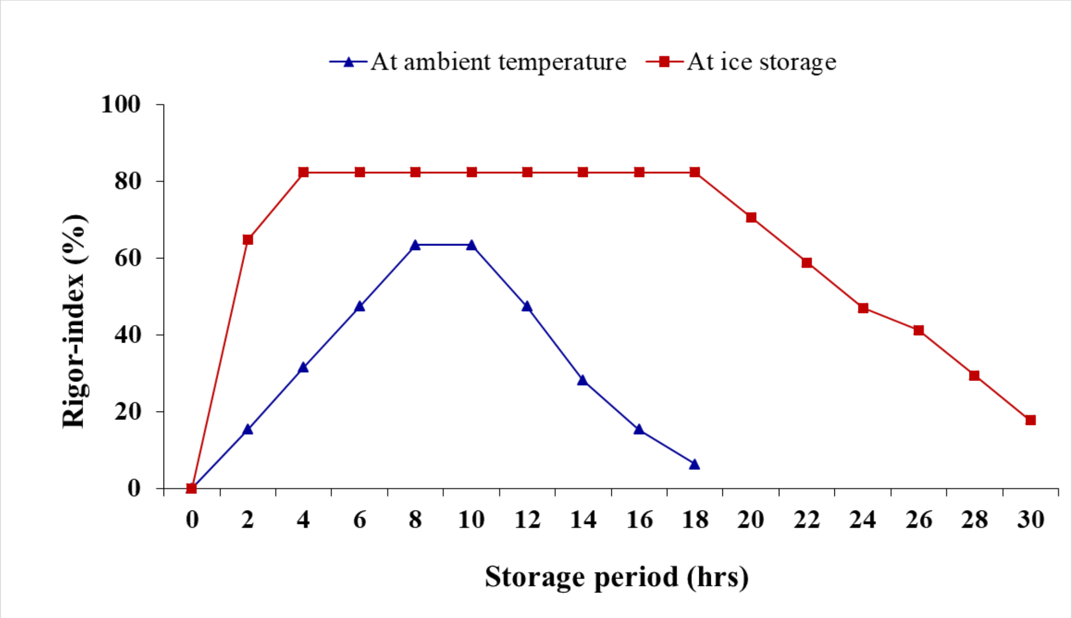


Fig. 2. Changes in rigor index of fish stored at ambient temperature and ice storage

The functions engaged in post mortem changes in fish have been illustrated by poor ability of Ca^{2+} uptake by the fish sarcoplasmic reticulum at 0°C [30]. Storage at different temperatures affected the main metabolites and parameters related to rigor mortis. Adenosine triphosphate (ATP), lactic acid, octopi, and pH were determined some of the parameters responsible to control rigor process. Seasonal variation influenced on the initial values of ATP, octopine, and pH. The seasonality did not show a significant impact on metabolite and parameter values related to rigor mortis during storage, and that 5 °C is the best storage temperature for the adductor muscles during the initial stage of rigor mortis and then reduces it to 0°C, if it is focused to the fresh market [31]. Rigor-mortis is dependent on temperature which impacts on the onset and the motion of development of rigor, among the many factors. Lower temperature delays the rigor-mortis progress and lower temperature helps in the prolongation of rigor period. Several fish species such as tilapia, red sea bream, and plaice have been indicated to minimize their pre-rigor stage at the time of storage at 0°C [27,32]. Rigor-mortis progress and storage temperature remained a polemic relationship. However, in the present study, it is clearly revealed that the onset of rigor started earlier in lower temperature by shortening the pre-rigor period. But the rate of progress of rigor-mortis was slower compared to fish preserved at high temperature.

3.3 Organoleptic Quality Assessment

3.3.1 Ice storage condition

The result of the organoleptic quality assessment of Asian stinging catfish during SIC in an insulated box is presented in Fig. 3A. The changes in quality of fish SIC were evaluated at three days interval by organoleptic examination, the fish was found in acceptable conditions for 12 days before the onset of spoilage. The changes occurred in organoleptic quality during the storage period can be divided into four phases comprising: 0 to 3 days, 3 to 6 days, 6 to 9 days and 9 to 12 days in fish SIC. In phase 1, the fishes were with a specific taste, natural flavor, and odor. At this stage the fishes had the characteristics of excellent quality. In phase 2, there was little deterioration apart from some slight loss of natural flavor. At this stage there was little loss of the characteristic odor and the flesh was neutral but had no off flavor. In phase 3, there were signs of early spoilage with sour of flavor. In the beginning of this phase the off-flavor was slightly sour, sickly sweet, fruity of like dried fish but the fishes were judged as 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. The days between 13 and 14 was kept into phase 5 when the fish grade become C, defect point 5 and it became totally rejected.

There were a lot of informations on the quality and spoilage patterns of the fish particularly from the temperate and cold waters but very little are known on the fishes of the tropical waters [33,34]. In the present study, organoleptic quality of Asian stinging catfish SIC was graded as the limit of acceptable condition up to 12 days. The available reports suggest that the quality of the fishes varies considerably depending on species and storage conditions. Pacheco-Aguilar et al. [35] indicated that quality of Pacific lions-paw scallop adductor muscle was maintained during at least 12 days of ice storage. Ocaño-Higuera et al. [7] determined that the edible quality of Cazon fish (*Mustelus lunulatus*) muscle was maintained during at least 18 days of ice storage. The Hilsa fish (*Hilsa ilisha*) transported immediately after catch in the insulated box in ice remained in acceptable condition up to 18 days in ice storage [36]. Rubbi et al. [37] studied on the shelf-life of six freshwater fish species in ice using various containers. The present study reports that Asian stinging catfish remained edible upto 12 days which was lower than *Hilsha* sp. and Cazon fish. The results obtained in the present study suggested that organoleptically the shelf life of the Asian stinging catfish was almost similar to what was reported for Pacific lions-paw scallop adductor muscle and may be similar with other commercially fresh water fish species. The available studies also revealed that the shelf life of the fish varied from species to species, their chemical composition, and ambient temperature at which the fish are stored. Fat content and storage temperature might be responsible for this. Fatty fish are susceptible to spoilage very rapidly in ice. For an example, herring with high fat content can become inedible after only one to two days and mackerel become inedible after 4 to 5 days in ice [38].

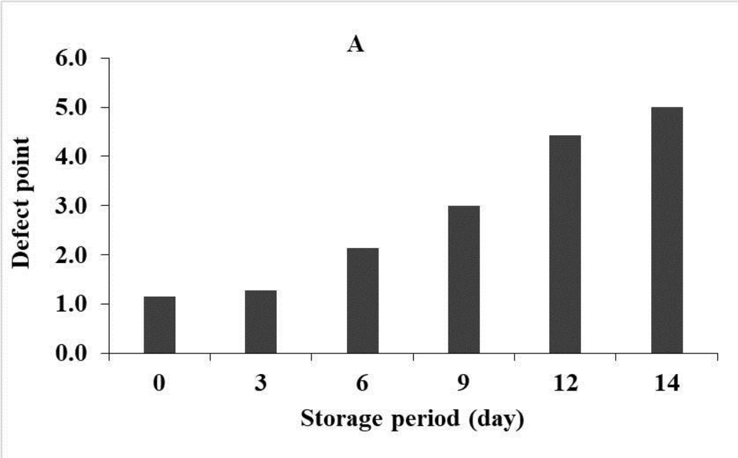
3.3.2 Storage at ambient temperature

The result of the organoleptic quality assessment of Asian stinging catfish SAT is presented in Fig. 3 B. The fish was found in acceptable condition for upto 10 hrs. The changes in quality occurred in organoleptic quality at room temperature was evaluated at 2 hrs interval. In first 2 hrs, the fish was excellent and odor of fish was natural. But the fish started to default its quality during 4 hrs storage and started to give some faint odor but was still in acceptable condition. During 6 hrs storage, there were signs of early spoilage with sour flavor and fish muscle lost elasticity. At 8 hrs storage, the fish lost organoleptic quality than previous periods. In 10 hrs, the appearance and texture began to show clear signs of spoilage, the gills, and belly cavity had an unpleasant smell. The fish was in the limit of acceptable condition upto 10 hrs and spoiled set in by all characteristics at 12 hrs. Adoga et al. [39] reported the storage life of Tilapia (*Oreochromis niloticus*) was acceptable up to 15 days in ice storage and 12 hrs at ambient temperature. Ababouch et al. [40] found the keeping times of sardines were 21–27 hrs (average 23 hrs) at ambient temperature and 8–11 days (average 9.5 days) in ice.

3.4 Changes in Muscle pH

The pH changes of Asian stinging catfish during ice storage and stored at ambient temperature is shown in Fig. 4. The pH of Asian stinging catfish muscle was 6.89 immediately after death, which was reduced to 6.43 and 6.19 in ice stored fish and fish stored at ambient temperature, respectively after 16 h storage. The reduction of muscle pH was faster in fish stored at ambient temperature than iced stored fish due to onset of rigor at ambient temperature and rapid formation of lactic acid. Reduction of pH in fish muscle after death is associated with the formation of H^+ ions due to production of lactic acid in anaerobic glycolysis of glycogen and degradation of ATP [41-42]. These two factors are related with the handling practice and conditions at which the fish is preserved after death. The results of the present study suggest that the formation of lactic acid is slower in ice stored fish compared to that stored at ambient temperature. The reduction of pH is significant for maintaining fish quality because lower pH reduces water holding capacity of the muscle [43]. The pH of fish muscle determines extent of freshness and can be used as an index of fish quality. Hossain et al. [44] reported that the Thai pangus showed pH value 7.0 immediately after death, which was reduced to 5.98 after ice storage of 14 days. Ocaño-Higuera et al. [7] reported the similar results of reducing pH level of fish fillet at ice storage.

The elementary post-mortem pH of fish muscle depends on species, catching ground and season. This change in rate is occurred due to the accelerated turnover of ATP at high temperature [30,45]. It showed that in post-mortem muscle the rate in drop of pH is a function of ATPase activity [46].



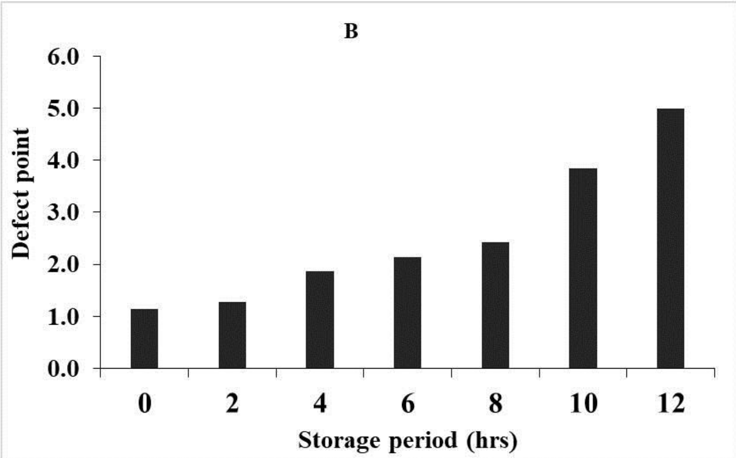


Fig. 3. Changes in organoleptic qualities of Asian stinging catfish (A) storage at iced condition and (B) storage at ambient temperature

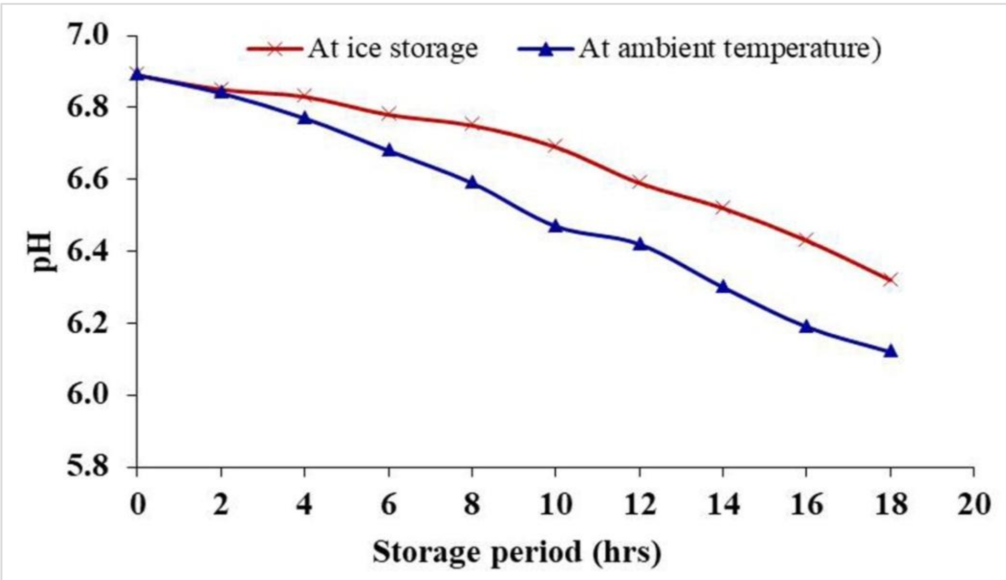


Fig. 4. Changes in pH of Asian stinging catfish store at different conditions

4. CONCLUSION

The suitable storage condition and shelf-life of Asian stinging catfish was investigated in this research. The biochemical properties and organoleptic characteristics during post mortem changes in Asian stinging catfish SIC and SAT were determined successfully. The onset of rigor-mortis was faster in fish SIC than fish SAT however, the rigor progress did not reach in full rigor state at both storage conditions. The changes in organoleptic qualities were evaluated by sensory method and fishes were acceptable up to 12 days in fish SIC and up to 10 hrs in fish SAT. In iced condition, the decrement of pH of the samples was lower than the samples stored at ambient temperature. After 18 hrs storage, the pH in fish SIC and fish SAT were 6.12 and 6.32, respectively. There was a little difference in proximate composition of fresh and iced muscle. The lipid and protein content of fish SAT was lower compared to fresh fish and fish SIC. The findings of the study instruct to store Asian stinging catfish at proper ice condition during transportation and market chaining of Asian stinging catfish keeping its quality unimpaired and the ways to extend shelf-life upto 12 days. Apart from the study of post-mortem changes in Asian stinging catfish, some other studies can be conducted such as microbial growth, determination of TVBN, and microbial activity during ice storage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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