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Changes in muscle gel-forming ability, protein solubility and pH of three marine fish species of Bangladesh during ice storage

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Abstract. Studies were conducted to evaluate the changes in muscle gel-forming ability, protein solubility and pH of three marine fish species of Bangladesh namely, Silver jewfish (*Johnius angentatus*), Ribbon fish (*Trichienus lepturus*) and Bombay duck (*Harpodon nehereus*) during storage in ice. The fish samples were collected from the fish landing center of Cox's Bazar and frozen immediately for transportation. The fish samples were stored in ice for 10 days and the gel-forming ability, protein solubility and pH of fish muscle samples were determined following the standard procedures. The results showed that in one-step heating at 50°C, the highest breaking strength was found for all three species of fishes on "0" day then the strength decreased with lapse of storage time. In case of two-step heating, a similar trend was observed for the fishes. In case of Bombay duck, breaking strength could not be measured after 3rd day of storage both in one-step and two-step heating as the samples were too weak to measure. The protein solubility of all three species of fishes was also found highest at the "0" day of ice storage which decreased with progress of storage time. The pH values of the fishes also increased with lapse of storage time in ice. These results suggest that- Silver jewfish and Ribbon fish could be utilized to produce various fish gel products like kamaboko and surimi and different types of value-added products.
Keywords: Marine fishes, Gel-forming ability, Protein solubility, pH

Introduction

The gel forming ability of the fish muscle varies from species to species and within the species depending on the biological conditions of fish. The variation within the species is due to age, season, sex, death condition, -freshness, fishing place, etc. (Shimizu *et al.* 1981, Kurokawa 1982, Shimizu and Kaguri 1986, Roussel and Cheftel 1988). There are number of other factors which influence the gel forming ability of the mince, for example, high fat content, instability of muscle proteins, large amount of sarcoplasmic protein and high proportion of dark to ordinary muscle. High fat content in the muscle weakens the gel forming ability and it is impossible to make mince products from the fishes that are not fresh even if the effective processing technique is applied (Suzuki and Watabe 1987).

In Bangladesh, present annual landing of marine underutilized species is around 0.1 million mectric tons, 50% of which is the shrimp by-catch species. Thousands of metric tons of undesirable species are discarded from the government operated and private owned shrimp trawlers annually and the by-catch that are landed are generally used in fishmeal production. As the population continues to increase faster than fish production, it is necessary to utilize these

underutilized marine fishes as a potential source of animal protein. These fishes may be presented to the people in the form of surimi based products. In order to utilize this available low priced fish species through production of various value added products it is necessary to study their gel forming ability and other related characteristics. Therefore, the present study was undertaken to study the gel forming ability, protein solubility and pH of three marine fish species during ice storage for 10 to finding out the suitable condition and duration of ice storage of these fishes to proceed to produce different mince or surimi based products.

Materials and Methods

Collection of fishes: Three commercially important marine fish species such as Silver jewfish (*Johnius argentatus*), Ribbon fish (*Trichiurus lepturus*) and Bombay duck (*Harpodon nehereus*) caught from the Bay of Bengal were collected from BFDC landing station of Cox's Bazar (Plate I). The average body length of Silver jewfish, Ribbon fish and, Bombay duck were 19 cm, 32.28 cm and, 18.50 cm, respectively and average body weight were 530 g, 565 g and, 255 g, respectively.

The collected fish were kept in an insulated ice box at landing center. The ratio of ice and fish was maintained 1:1. The iced fish were transported to the laboratory of the Marine Station, Bangladesh Fisheries Research Institute (BFRI), Cox's Bazar for freezing using a deep freezer. The frozen fish were packed in airtight polythene bags and set in an insulated box with enough flack ice. and transported to the Fish Processing Laboratory of the Bangladesh Agricultural University, Mymensingh, which required about 15 hours bus journey from Cox's bazar.

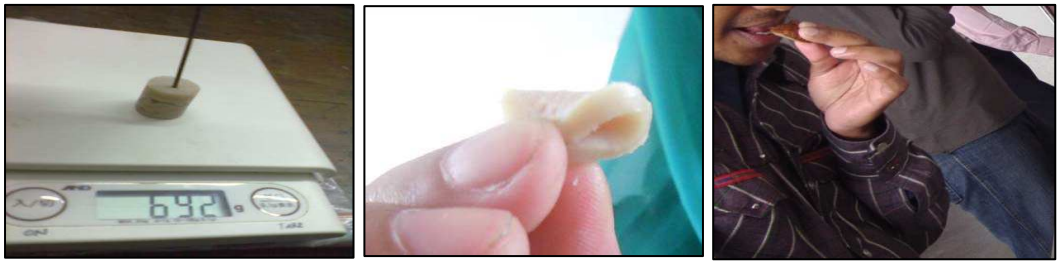
***Storage of experimental fish*:** The melting ice of the box was replaced with fresh flack ice and stored for 10 days in ice to carry out further studies. During storage flack ice was replaced at least twice in a day for maintaining the cool temperature.

Changes in Gel-forming Ability

***Preparation of meat paste*:** Subsamples of the fishes were washed in chilled freshwater before they were beheaded and gutted. Dorsal and lateral muscles were excised from whole fish as fillet form. Kidney tissues, skin and belly fats were carefully removed. To prepare mince from fillets a manually operated meat mincer was used which was cooled at 4°C before every operation. After deboning procedure the mince was grinded with 3% salt for 20 minutes in a ceramic grinder. At the time of excision of fillet and preparation of mince and paste a cooled condition was maintained.

***Preparation of gel*:** For one step heating, the paste in cylinders was heated to produce gel in water baths at 40°C, 50°C, 60°C, 70°C and, 80°C for 120 min. On the other hand, in the case of two step heating process, the same temperatures and time were used and mentioned as pre heating where the gels were further cooked at 90°C for 30 minutes. After heat treatments, the samples were taken out from the water bath, kept in ice water for 1 hour and then left for 20 minutes at room temperature before gel-strength measurement. The gels left at room temperature were then subjected to the following tests:

***Measurement of gel-strength*:** The gel strength of products was assessed by organoleptic methods. A five person panel as described by Poon *et al*. (1981) was provided for the organoleptic assessments. The gels were removed from the cylinder and subjected to puncture test, folding test and teeth cutting test for physical measurement of the gel (Plate I).



Puncture Test Folding Test Teeth Cutting Test

Plate I. Measurement of Gel Strength.

Puncture test: The gels were removed from the tube and cut into equal pieces of 2 cm. The puncture test was done by measuring breaking force of the gel against the penetration of a ball type plunger. The cut gel was placed on the pan of an electric balance and a spherical plunger was penetrated onto it. The force in gram required to break the gel by the plunger was recorded from the balance.

Folding test: For folding test, a spherical disc of 1 mm thick gel was cut off and placed on the index and middle finger of the right hand, the disc was folded first into halves and then quarters by the help of thumb and index finger. The gel was graded using the scores presented in Table I.

Teeth cutting test: For teeth cutting test, disc gel of the same size were used in folded test and supplied to the panelists to recognize the taste by cutting it through incisors and the gel strength was evaluated by the following numeral scores presented in Table II as suggested by Shimizu *et al*. (1981).

Table I. Grades used in the Folding Test of the Gel

Grade	Characteristics of Gel for Folding Test
AA	No crack visible when disc is folded into quarter
A	No crack visible when disc is folded into half, but one or more cracks or breaks are visible when folded into quarter.
B	One or more cracks or breaks are visible when folded into half.
C	Breaks, but does not split into halves.
D	Split into halves when folded into half.
0	Sample too soft to evaluate.

Table II. Scores used in the Teeth Cutting Test of the Gel

Score	Characteristics of Gel for Teeth Cutting Test
0-1	Paste or mud like gel
2-3	Very frail gel
4-5	Frail gel
6	Medium gel strength
7-8	Strong gel
9-10	Very strong gel

Changes in the protein solubility: As myofibrils are a part of muscle proteins so myofibrillar protein solubility indicates the denaturation rate of the proteins. To study the changes in protein solubility, the following procedure was followed in the laboratory:

Preparation of Myofibrils: Myofibrils were prepared from ordinary muscles immediately after excision according to Perry and Grey (1956) with slight modification and the procedure is shown in Flowchart I.

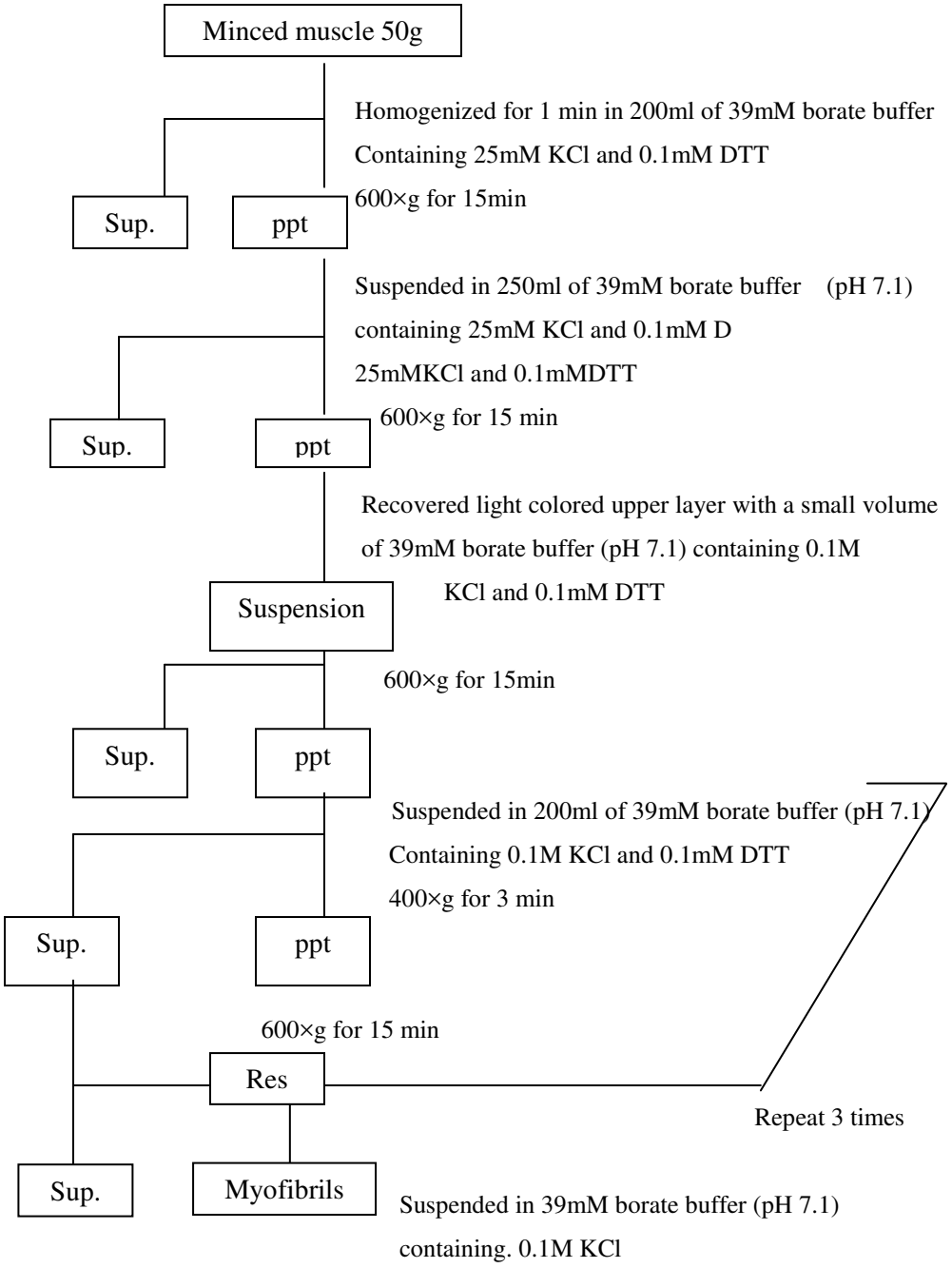
Myofibrillar protein solubility: From the suspension of myofibril 2ml (5mg/ml) of myfibrillar protein was taken, heated from 15-50°c for 30minutes, then stored at 0°c for overnight. Next day the suspension was omogenized with 2ml of 1M KCl and 100mM Phosphate then centrifuged. After centrifugation the supernatant was used to measure protein solubility.by Biuret method (Gornall *et al*. 1949).

***pH measurement*:** The pH was measured at room temperature following the method described by AOAC (2005).

Results and Discussion

Changes in Gel-forming Ability of Marine Fishes

One Step Heating: Studies were conducted to evaluate the gel forming ability of three marine fish species of fishes named as Silver jewfish, Ribbon fish and Bombay duck. In one-step heating, samples were heated for 120 min in water bath at 40°C, 50°C., 60°C, 70°C and, 80°C. Here gels obtained after heating at 50°C was used for measurement of the gel strength as at this temperature the gel strength was found to be highest for all the fish samples. At the initial stage the breaking force of gel prepared with Silver jewfish was found 757.42±(0.76)g which decreased to 434.67±(1.66)g at the end of 10 days of ice storage (Table III). Similarly for Ribbon fish (Table IV) the breaking strength decreased from 803.12 ±(1.35)g to 470.64 ±(2.21)g. On the other hand for Bombay duck, the breaking strength could be measured until 3rd day of ice storage and the value ranged from 204.31±(2.06)g to 160.75±(0.76)g then for rest of days the obtained gels were too soft to measure (Table V). The initial folding test (FT) grade for Silver jew fish was found 'AA' on '0' day which decreased to 'B' and teeth cutting test (TCT) score found 8 on '0' day which decreased to 4 after 10 days of ice storage. Similarly for Ribbon fish folding test (FT) grade decreased from 'AA' to 'B' and teeth cutting test (TCT) score decreased from 8 to 5 and for Bombay duck folding test (FT) grade found 'B' and teeth cutting test (TCT) score, 4 at the initial stage then after 3 days of ice storage obtained gel were too weak to carry out gel forming ability study.



Flow Chart I. Preparation of Fish Muscle Myofibrils

The results of this study clearly indicated that the gel farming ability decreased with increase in the storage period and this might be due to denaturation of myofibrillar protein. Soottawat *et al*. (2003) carried out a study on the changes in physico-chemical and gel-forming ability of lizard fish (*Saurida tumbil*) during post-mortem storage in ice. They observed that- gel-forming ability of surimi, prepared under different setting and/or heating conditions, decreased as storage time increased ($p < 0.05$). Mehta *et al*. (2014) did experiment on effect of ice storage on the functional properties of proteins from a few species of fresh water fish (Indian major carps) with special emphasis on gel forming ability. They found that- the gel forming ability of three species was significantly affected ($p < 0.05$) during 22 days of ice storage. Mousumi *et al*. (2017) studied the quality changes of Pangas catfish (*Pangasianodon hypophthalmus*) fillet during ice storage and reported that- the gel forming ability and initial breaking force was found reducing at 21st day of study period; indicate gradual decrease in quality. In their study the get strength was found highest in higher temperature. These findings are quite similar to the results of present study.

Two-step heating: At this two-step heating, breaking force of Silver Jewfish (Table III) ranged from 956.57±(4.21) to 550.46±(1.24)g, in Ribbon fish (Table IV) from 867.61±(3.53) to 537.57±(3.04)g and, in Bombay duck (Table V) ranged from 210.30 ±(0.88) to 185.68 ±(1.15). The initial folding test (FT) of Silver Jewfish was found 'AA' which decreased to 'B' and teeth cutting test (TCT) value was found 8 which decreased to 5 at the end of storage. Similarly for Ribbon fish initial FT was found 'AA' which decreased to 'A' and TCT value was found 8 which decreased to 5. In the case of Bombay duck the obtained results were somewhat different. For Bombay duck, the FT was found 'C' and the TCT value was 3 at the initial stage of storage, afterward with the lapse of storage time gel strength could not be measured as gel did not form with the ice stored Bombay duck meat paste. The result obtained from the study clearly indicated that though two step heating contributed to increase the gel strength than one step heating but the gel strength decreased with the lapse of storage period. Hossain *et al*. (2005) did experiment on the influence of ice storage on the gel forming ability, myofibrillar protein solubility, Ca^{2+} -ATPase activity of queen fish (*Chorinemus lysan*) and found maximum breaking force from both washed and unwashed mince at the incubation temperature of 50°C. The gel-strength of both unwashed and washed meat paste gradually declined with lapse of storage period. Sabina (2009) studied the gel strength and ice storage relation in case of pangas (*Pangasius hypothalmus*) and reported that, the gel forming ability of pangas decreased with increase in the storage period. In her study, the initial breaking force was found 669.33 (±0.67g) which decreased to 205 (±0.88g) in one step heating and in two step heating the breaking force was found 1005.67 (±3.93g) initially, which decreased to 480.23 (±0.88g) at the end of 16 days ice storage. Tiwo *et. al* (2018) studied on the effect of ice storage on the textural and rheological properties of proteins from freshwater fish, *Cyprinus carpio* (Common Carp). They found that- there was a significant reduction in gel strength after 15 days of ice storage. The decrease in gel strength values of the gels prepared was rapid at the end of 5-days of ice storage. The initial gel strength values of the gels were 668,28 ± 58,18 when prepared using 2.5% NaCl.

Table III. Changes in Gel-strength of Silver jewfish (*J. argentatus*) meat paste in one-step and two-step heating at 50°C for 120 min during ice storage

Storage time in ice (days)	Breaking Force (g)		Folding Test		Teeth Cutting Test	
	One-step (heating at 50°C for 120 min)	Two-step (heating at 50°C for 120 min + cooking at 90°C for 30 min)	One-step	Two-step	One-step	Two-step
0	757.42 ±(0.76)	956.57 ±(4.21)	8	8	AA	AA
1	723.54 ±(2.53)	860.72 ±(3.39)	7	8	AA	AA
3	633.44 ±(0.66)	755.32 ±(0.81)	6	7	A	AA
5	565.71 ±(1.08)	681.83 ±(2.67)	6	6	A	A
7	503.27 ±(3.88)	624.14 ±(1.73)	5	6	A	A
10	434.67 ±(1.66)	550.46 ±(1.24)	4	5	B	B

BF = Breaking Force (Mean±SE)

Table IV. Changes in Gel-strength of Ribbon fish (*Trichiunus lepturus*) meat paste in one-step and two-step heating during ice storage

Storage time in ice (days)	Breaking Force (g)		Folding Test		Teeth Cutting Test	
	One-step (heating at 50°C for 120 min)	Two-step (heating at 50°C for 120 min + cooking at 90°C for 30 min)	One-step	Two-step	One-step	Two-step
0	803.12 ±(1.35)	867.61±(3.53)	8	8	AA	AA
1	765.43 ±(0.67)	823.76±(1.08)	8	8	AA	AA
3	674.66 ±(1.45)	737.91±(2.06)	7	7	AA	AA
5	604.52 ±(1.45)	661.66±(0.67)	7	6	A	A
7	546.63 ±(0.88)	603.73±(2.04)	6	6	A	A
10	470.64 ±(2.21)	537.57±(3.04)	5	8	B	A

BF= Breaking Force (Mean±SE)

Table V. Changes in Gel-strength of Bombay duck (*Harpodon nehereus*) meat paste in one-step and two-step heating during ice storage

Storage time in ice (days)	Breaking Force (g)		Folding Test		Teeth Cutting Test	
	One-step (heating at 50°C for 120 min)	Two-step (heating at 50°C for 120 min + cooking at 90°C for 30 min)	One-step	Two-step	One-step	Two-step
0	204.31 ±(2.06)	210.30 ±(0.88)	4	3	B	C
1	160.75 ±(0.76)	185.68 ±(1.15)	3	2	C	C
3	*	*	*	*	*	*
5	*	*	*	*	*	*
7	*	*	*	*	*	*
10	*	*	*	*	*	*

BF= Breaking Force (Mean±SE); * = Gel too soft to measure

Changes in protein solubility: Fig. I shows the changes in protein solubility (%) of three marine fish species during ice storage. At the initial stage the myofibrillar protein solubility of Silver jewfish was 74.5% which decreased to 28.5%. Similarly in Ribbon fish, the protein solubility was 77.2% at the beginning of ice storage which decreased to 38.5%, and in case of Bombay duck, the value decreased from 68.25% to 30% at the end of 10 days of ice storage. Here, the protein solubility decreased continuously with the lapse of storage period. Seki *et al.* (1979) reported that- the solubility of carp myofibrils decreased from 95% to 20% during ice storage of 2-3 weeks. Sabina (2009) found in her study with pangas fish, the protein solubility decreased continuously with the progress in storage period. She observed that- the initial protein solubility was 86.37% for pangus and decreased to 36% at the end of 16 days ice storage. Mehta *et. al*. (2014) reported- the solubility profile of proteins in high ionic strength buffer and calcium adenosine triphosphatase (ATPase) enzyme activity reduced significantly ($p < 0.05$) at the end of 22 days of ice storage. All these findings are more or less similar to the present finding.

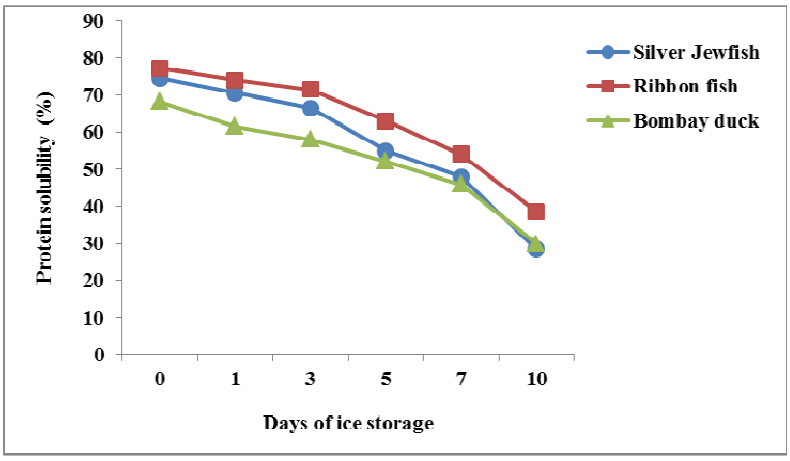


Fig. 1. Changes in protein solubility (%) of marine fishes during ice storage of 10 Days.

Changes in pH value: The pH value of the three marine species of fishes used in experiment increased gradually with the progress in storage period. In Silver jewfish, Ribbon fish and Bombay duck the pH values ranged from 6.22 to7.1, 6.30 to6.72 and 6.32 to6.67, respectively (Figure II). These results are very much similar with the result of Reza *et al*. (2009). They found that the pH value in all the samples gradually increased with the lapse of storage period and at the end of 13 days of storage the pH value increased from 7.2 to 7.98 with the lowest value for Ribbon fish and the highest value in Big-eye tuna. The initial low pH value may be because of formation of lactic acid during anaerobic glycolysis in fish muscle after the death. Mousumi *et al.* (2017) reported that- the pH of the muscle immediately after death was 7.07, fall to 5.89 after 12 days and again raised to 6.88 after 21 days of ice storage during their study on the quality changes of pangas catfish (*Pangasianodon hypophthalmus*) fillet during ice storage. The gradual increase in pH value in 13 days of ice storage indicates the accumulation of

alkaline compounds, such as ammonia compounds and TMA, mainly derived from microbial action (Hebard *et al.* 1982).

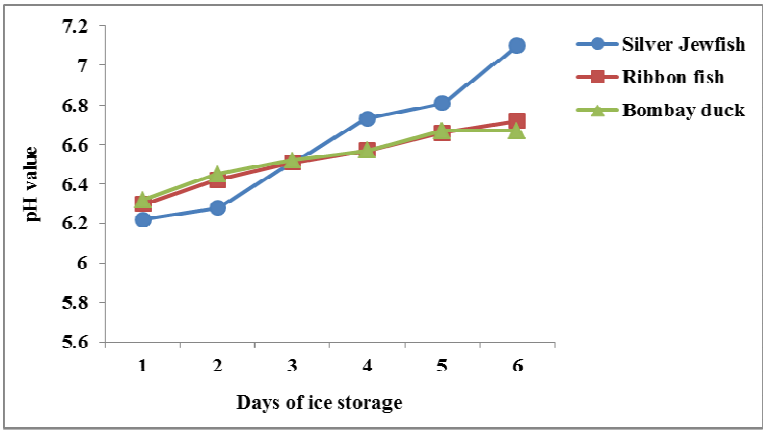


Fig. 2. Changes in pH values of marine fishes of Bangladesh during ice storage of 10 Days

The results of the present study showed that- the gel strength is highest in Silver jewfish and lowest in Bombay duck among three experimental species of fishes. The two-step heating process contributed to increase the gel strength. In both one-step and two-step heating processes the gel strength obtained highest on "o" day of 10 days storage in ice. With the lapse of storage time the gel strength decreased gradually and in case of Bombay duck, gel did not form after 3 days of storage to measure.

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