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Changes in Physico-Chemical and Microbiological Parameters of Pangas (*Pangasius pangasius*) Muscle During Ice Storage

F. H. Shikha, M. I. Hossain* and S. Yeasmin
Department of Fisheries Technology,
Bangladesh Agricultural University, Mymensingh-2202
*Corresponding email: ihossain.ft@bau.edu.bd

Abstract
Temperature has great influence on the changes in physico-chemical and microbiological parameters of fish muscle. The present study was conducted to observe the changes in physico-chemical and microbiological parameters of pangas (*Pangasius pangasius*) muscle during ice storage. At the initial stage of storage TVBN-value was found 1.37mg/100g, peroxide value 1.1m.eq/kg of oil, breaking force 1005.67 (± 3.93g), protein solubility 86.37% and microbial load 7.6×10^3 CFU/g which reached to 28.25 mg/100g, 19 meq./kg of fish oil, 480.23 (± 0.88g), 36% and 4.6 ×106 CFU/g, respectively after 16 days of storage in ice. On the basis of the obtained results the study could be concluded as- pangas (*Pangasius pangasius*) can be stored in ice up to 16 days.

Key words: Gel strength, Ice storage, Organoleptic characteristics, Pangas, Protein solubility

Introduction

There are some factors that influence the quality of fish under various storage conditions. It is suggested that an increase in acidity with rapid pH fall during post-mortem changes influence the quality of fish muscle, particularly the important characteristics of texture and water holding capacity. Such quality losses are reported to be the denaturing effect of low pH on the muscle protein (Kramer and Peters, 1981; Penny, 1967, 1969;Konagaya, 1978). There are some other parameters such as- organoleptic characteristics, TVB-N value, peroxide value, and bacterial loads etc. which determine the quality of fish during storage. Freshness test of the fishes indicate the quality of fish in terms of odor, color and appearance. The TVB-N value gradually increase with lapse of storage time and the recommended value are 25-30 mg TVB-N/100g. Peroxide value also increase with lapse of storage period. The recommended values of these are 10-20meq/kg of oil. Microorganisms are present on the external surfaces (including slime) and in the gut of fish. After death, microorganisms present on the surface and in the gut multiply rapidly and gradually invade the flesh, grow and multiply. Initially, compounds having sour, fruity or acidic notes are formed; later bitterness and sulfide or rubberizes appear; finally, in the putrid state, the character is ammonical and faecal. The bacterial population in fish greatly influence the quality of fish and fishery product. There are some recommended limits of bacterial loads by which the quality of fish and fishery products has been judged under various storage conditions.

The gel forming ability of the fish 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).

Myofibrillar proteins are the proteins that form myofibrils. They are soluble in concentrated saline solutions (ionic strength above 0.6) as well as extremely low ionic strength, but are water insoluble in typical physiological ionic strength in the fish muscle. Myofibrillar proteins are composed of myosin, actin, and regulatory proteins such as tropomyosin, troponin and actinin. Myofibrillar proteins make up 66–77% of total proteins in fish muscle (Tahergorabi *et al.,* 2011). Myfibrillar protein solubility indicates the denaturation rate of protein.

Pangas is one of the most popular poor men's fish species in Bangladesh. During the last few years, this species has been cultured extensively in ponds, lakes; borrow pits, ditches, ox-bow lakes and flood plains of Bangladesh. However, the market price of this fish is declining day by day compared to other cultured fishes. But this fish has a great aquaculture potential owing to its ability to grow under less nursing conditions, omnivorous feeding habit and common disease resistance capacity. A sustainable aquaculture of pangas catfish can be achieved by increasing its utilization through addition of value. Considering above mentioned points the present study was undertaken to observe the changes in physic-chemical and bacteriological parameters of pangas muscle during ice storage.

Materials and Methods

Fish sample collection

Freshwater-pangas (*Pangasius pangasius*) used in this experiment were collected live from the K R market, Bangladesh Agricultural University campus and were brought to the laboratory of Department Fisheries Technology, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh and stored in ice in insulated box (fish: ice ratio 1:1). Ice used for storage purpose in insulated box was changed regularly each 5-6 hours interval.

Organoleptic evaluation

A large number of schemes have been proposed for sensory evaluation of various types of fish. The evaluation methods used in the study are based on the one currently in used in various institutes and industries of the world. The following set of guidelines has been prepared to get maximum value from them by being able to compare the results. The guidelines and methods given here using score on the organoleptic characteristics of fish as described by EC freshness grade for fishery products (Howgate *et al.,* 1992) which is shown in Table1 and 2.

Table1. Grading of fresh fish

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

Table 2. Determination of defect points

Sl.No.	Characteristics of whole fish	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) Reddish lateral line; dull; no bloom	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 be slightly opaque or milky	1	Acceptable
		b) Becoming turbid opaque and milky, with marked increase in amount of slime present in skin	1	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		
		d) Limp and floppy	3	Acceptable
			5	Reject

Determination of TBV-N value

Total Volatile Base Nitrogen (TVB-N) was determined according to the methods given in AOAC (1984) with certain modification using the following formula-

$$\text{Amount of TVB-N (mg/100 g) sample} = \frac{\text{ml titrant} \times 0.014 \times \text{normality of acid}}{\text{Sample weight}} \times 100$$

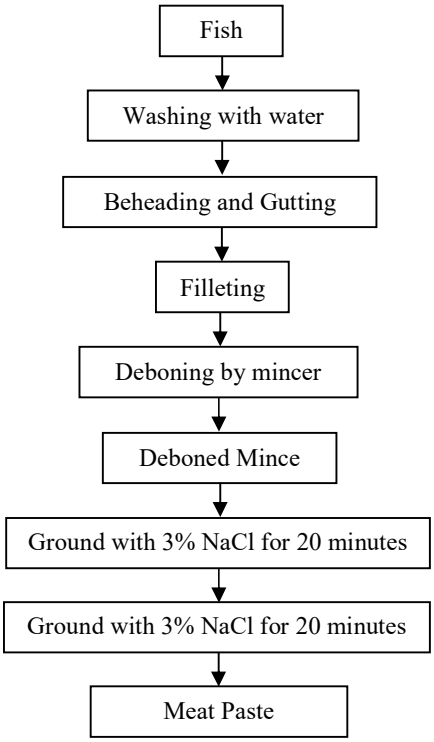
Peroxide value
Peroxide values of the samples weredetermined as the method described by Egan et al. 1981 and adopted from (Wood and Aurand, 1977) using the following formula.

The peroxide value was calculated as follows:
Peroxide value = 2 (S-B)/W, meq. /kg of oil

Where,
'S' is sample titre
'B' is blank titre
'W' is weight of sample oil in gm.

Microbiological study
Aerobic plate count (APC) expressed as colony forming units per gram (CFU/g) of fish sample on different days of ice storage were determined by consecutive decimal dilution techniques using spread plates as described by Seely and Vandemark (1972).

Changes in gel forming ability
Preparation of meat paste
On arrival at the laboratory, the fishes were washed in chilled freshwater before they were headed and gutted. Dorsal and lateral muscles were excised as fillet form. Attention was paid to remove kidney tissues as they form globular masses which affect both texture and appearance of the product. The steps of preparation of meat paste is presented in flow chart 1.



Flow chart 1. Preparation procedure of meat paste

Preparation of gel
The past in cylinders was heated to produce gel. Some samples were heated once only in well stirred water bath, whilst the rest were heated twice. For convenience, the former method of heating is called one-step heating and the later two-step heating. All heating treatments were triplicate.In one-step heating samples were heated for 120 min in water of 40ºC. In two-step heating, the first heating was for 120 min in water of 40ºC, which heating will be conveniently called pre-heating. After this preheating treatment, they were immediately heated for another 30 min in water of 85ºC. After heat treatments, the samples were taken out from the water bath, kept in iced water for 1 hour and subjected to the following tests.

Measurement of gel-strength
The gel strength of the products was assessed by objective and organolaptic methods. A five person panel as described Poon *et al*. (1981) was provided for the organoleptic assessments.The gel were removed from the cylinder and subjected to puncture test, folding test (Plate1) and teeth cutting test for physical measurements of the gel. Puncture test measured the breaking strength of the gel against insertion of a ball type plunger. The folding test measured the resistance against breaking along with the folds when samples discs of 1 mm thickness were folded into halves and then quarters and the teeth cutting test was a measure of the resistance of the disc cut by the incisors of members of the panel.

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 penetrating onto it. The force in gram required to break the gel by the plunger was recorded from the balance.

(a) **(b)**

Plate 1. Gel forming test; (a) puncture test; (b) folding test

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 quarter by the help of thumb and index finger. The gel was graded using the scores presented in Table3.

Table 3. Grade used in the folding test of the gel

Grade	Results on folding
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.

Teeth cutting test

For teeth cutting test the disc gel of same size are used in folded test was 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 Table4 as suggested by Shimizu *et al*. (1981).

Table 4. Score used in the teeth cutting test of the gel

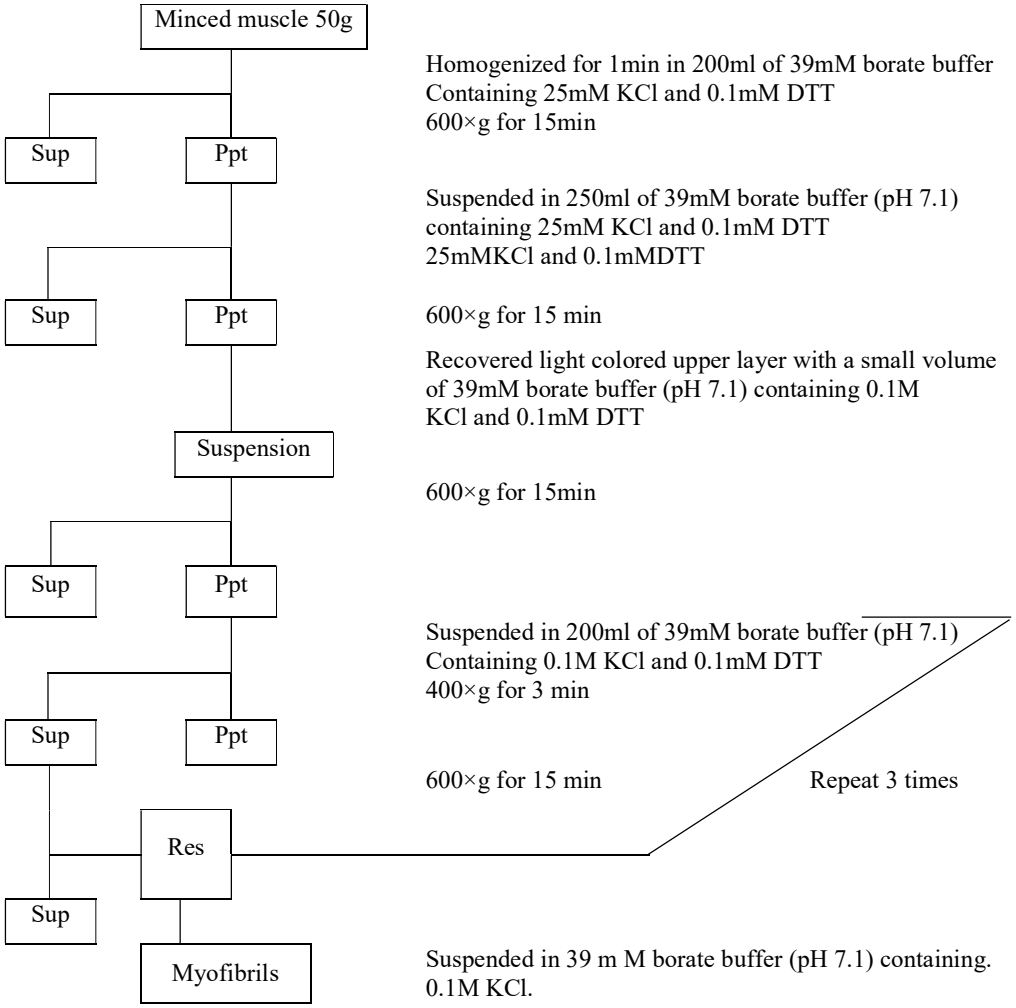
Score	Characteristics of the gel
0-1	Paste or mud like gel
2-3	Very frail gel
4-5	Frail gel
6	Medium gel strength
7-8	Strong gel
9-10	Very strong gel

Changes in the protein solubility

Proteins are polymer of amino acids. Myofibrils are a part of muscle protein. Myfibrillar protein solubility indicates the denaturation rate of protein.

Preparation of myofibrils

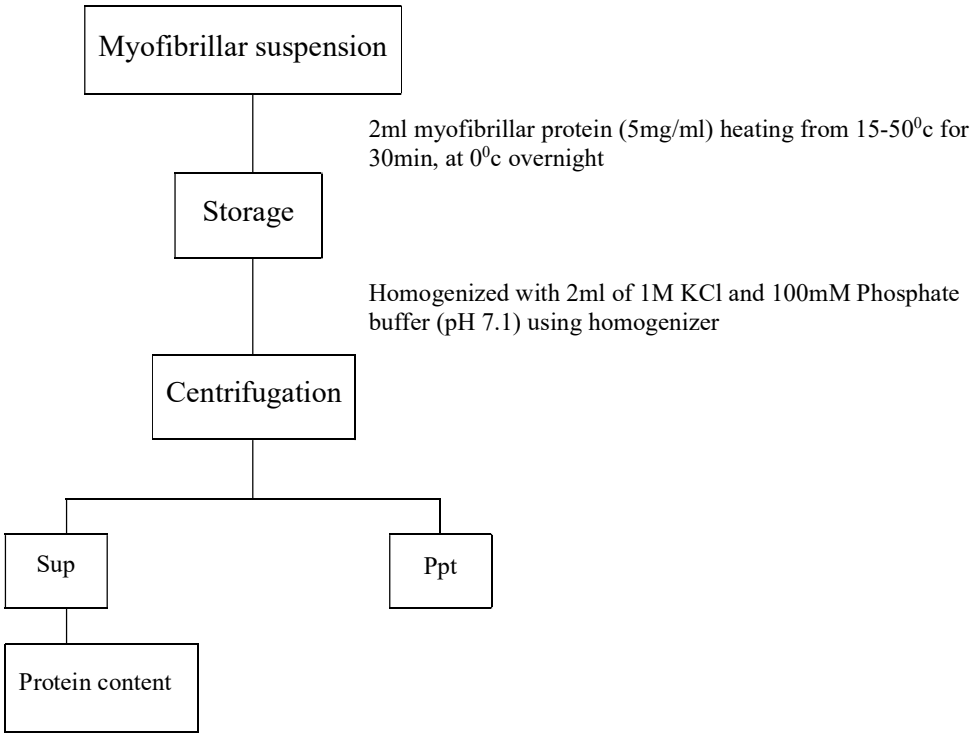
Myofibrils were prepared from ordinary muscles immediately after excision according to Perry and Grey (1956) with slight modification and the procedure is shown in flow chart2.



Flow chart 2. Preparation of fish muscle myofibrils

Myofibrllar protein solubility

Two ml of myofibrillar suspensions (5mg/ml) were homogenized with 2ml of 1M KCl plus 100mM phosphate buffer (pH 7.0) using a homogenizer. The homogenate was allowed to stand at refrigerated temperature (4^0c) overnight. The suspension was centrifuged for 30min at 900×g in cold condition. The protein in supernatant was determined by Biuret method (Gornall *et al*., 1949). The procedure for myofibrillar protein solubility test is shown in Flow chart 3.



Flow chat 3. Solubility determination of muscle myofibrils

Results and Discussion

Organoleptic evaluation

The results of the organoleptic quality assessment of pangas during ice storage in insulated box are presented in Table-5. The quality of fishes was graded in using the score from 1-5. The grades were defined in terms of the total number of defects points. The points less than 2 were considered as excellent. The points from 2 to less than 5 were judged as good or acceptable conditions. While 5 and above considered as rejected. On the basis of the scores among the freshwater pangas, (*Pangasius pangasius*) was found in acceptable condition up to 16 days in ice storage. During this period, changes in quality can roughly be divided into 4 steps corresponding 0 to 3, 6 to 9 12, to 16 days in ice storage. There were very little changes occurred in step 1 without loss of natural flavor and odor but in step 2 there was a little deterioration without showing any signs of spoilage and no off-flavor. There were signs of early spoilage with slight off-flavor in step 3 and in step 4 the fish begins to taste stale, its appearance and texture begins to show signs of spoilage and moderate sour odor in gill and body cavity.

Considerable information are available on the keeping qualities of the quality of most of the species from temperate region but the scientific and practical knowledge is very limited on commercially important tropical fish species of the tropical regions. Some available information on Indian major carps and other commercial fish species have indicated that the fish can be kept in ice in edible condition for up to 2-3 weeks. Kamal *et al.* (1994) have reported that, hilsa immediately after catch transported in the insulated box in ice remained in acceptable condition for 18 days of storage, while the fish obtained from Mymensingh wholesale fish market and stored in wooden box were organoleptically acceptable for about 8 days in ice storage. Faruk (1995) has reported the organoleptic quality assessment of rohu fish during ice storage in an insulated box, and the fish were found in acceptable condition for 20 days of storagebeforeitbecomes inedible. The shelf life of *Catla catla* and *Labeo fimbriatus* was reported to be 18 days in ice storage (Bandyopadhyay *et al.*, 1986). Rubbi *et al.* (1985) studied the shelf life of six freshwater fish species in different storage temperature by subjective and objective parameters. The spoilage rates were found to increase with the increase of storage temperature for all the six varieties. Bamboo baskets and wooden boxes, insulated with hoglamat (the spongy leaves of a local plant), were reported to be

most efficient for maintaining the quality of fish in ice for transportation. Muslehuddin *et a1.* (1986) reported that the shelf life of Mola fish (*A. mola*) could be increased up to 134 h at low temperature (2°C) when fish soaked in 15% salt concentration. On the other hand Hye e*t a1.* (1990) suggested that fish-ice ratio of 4:1, 2:1 and 1:1 could preserve the shelf life of fish up to 72h, 120h and 144hrs respectively in the insulating box consisting of polvurethene material. White sardines were reported to be acceptable for human consumption up to 9 days in ice storage (Jeyasekaran and Saralaya, 1971).The available studies also reveal that the shelf life of the fish varies from species to species, their chemical composition and ambient temperature at which the fish are stored. Fatty fish are susceptible to spoilage very rapidly in ice. For an example, herring with a fat content of 50% or more can become inedible after only one to two days and mackerel become inedible after 4 to 5 days in ice (FAO, 1975). Information arelacking on the shelf life of the freshwater pangas species. The results obtained in the present study suggest that organoleptically the freshwater pangs species is almost similar to that of reported for carps and other commercially fresh water fish species.

Table 5. Changes in organoleptic qualities of fresh water pangas (*Pangasius pangasius*) during ice storage

Days of storage	Organoleptic qualities	Defect points	Grade	Overall qualities
0	Fresh, bright appearance, soft and firm texture with characteristics of natural fishy odor.	1.25	A	Excellent
3	A decrease in the brightness; slightly softer texture, natural fishy odor.	1.9	A	Excellent
6	Some loss in brightness; slight loss of the natural flavor; some slime in the surface.	2.2	B	Acceptable
9	Slimy surface and slight soft texture; considerable loss of flavor and odor.	2.6	B	Acceptable
12	Moderately soft texture and slime on surface; loss of bloom.	3.2	B	Acceptable
16	Soft texture and slime on surface; moderate loss of flavor and odor.	4.2	B	In the limit Acceptable
18	Fish has dull appearance with blood and slime on surface not uniformly distributed; texture begin to show obvious signs of spoilage.	5.0	C	Rejected

Changes in gel strength

One step heating

Changes in gel forming ability of pangas during ice storage is presented in Table-6. The result showed that- the breaking force of one-step heating gel was 669.33 (± 0.67g) after heating at 40ºC for 120 minutes, which decreased to 205 (± 0.88g) after 16 days of ice storage. The initial folding test (FT) of one-step heating gel was found 'AA' which decreased to'A' and the score of teeth cutting test (TCT) was 8 which decreased to 5 after 16 days of ice storage. The results obtained from the study clearly indicated that the gel forming ability decreased with the progress of storage period which might be resulted due to denaturation of myofibrillar protein.

Two step heating

The changes in the gel forming ability oftwo-step heading gels of pangas during ice storage is also presented in Table-6. The initial breaking force was obtained 1005.67 (± 3.93g) which decreased to 480 .23 (± 0.88g), at theend of 16 days of ice storage. The initial folding test (FT) of two-step heating gel was 'AA' which decreased to'A' and the score of teeth cutting test (TCT) obtained 8 which decreased to 5 after 16 days of ice storage. The results obtained from the study clearly indicated that the gel forming ability decreased with the lapseof storage period.

Table 6. Changes in gel-strength of pangas (*Pangasius pangasius)* meat paste during one-step and two-step heating

Type of Heating	Heating temperature (°C)	Duration (min)	Storage time in ice (days)	Breaking force (g)	Teeth cutting test	Folding test
One-step heating	40	120	0	669.33±(0.67)	AA	8
			3	535.67±(2.33)	AA	8
			6	446.00±(2.08)	AA	7
			9	346.33±(0.88)	A	6
			12	266.33±(0.88)	A	5
			16	205.00 ±(0.88)	A	5
Two-step heating	40 (1st step) + 85(2nd step)	120 + 30	0	1005.67±(3.93)	AA	8
			3	875.00±(1.53)	AA	8
			6	776.67 ±(0.88)	AA	7
			9	666.33±(0.88)	A	6
			12	555.67±(0.88)	A	6
			16	480.23±(0.88)	A	5

BF = Breaking force (Mean± SE)

The comparison of breaking force between one and two step heating gels is shown in Figure-1, where the sample fishes for gel preparation were stored in ice. The study showed that- the breaking force was higher in two steps heating gels than that of one step heating gels but in case of both gels a similar decrease in breaking force was observed. The result obtained from the present study is more or less similar to the findings of Ishikawa (1978) and Ishikawa *et al.* (1979), who reported, the gel-strength of freshly caught sardine fishes was higher than the gel made from sardine stored few days in crushed ice. Shikha*et al*. (2012) studied the gel strength of silver carp in fresh and iced condition of fish. They found the breaking force 690.34±2.26g at the initial stage of ice storage which declined to 498.22 ±2.95 at the end of 15 days storage. For one step heating gels also similar trend was followed. Finding of Shikha*et al.* (2012) is in agreement with the results of present study. With the lapse of time even in ice storage the decrease in breaking force occur and this is might be due to denaturation of myofibrillar proteins (Shimizu and Kaguri, 1986).

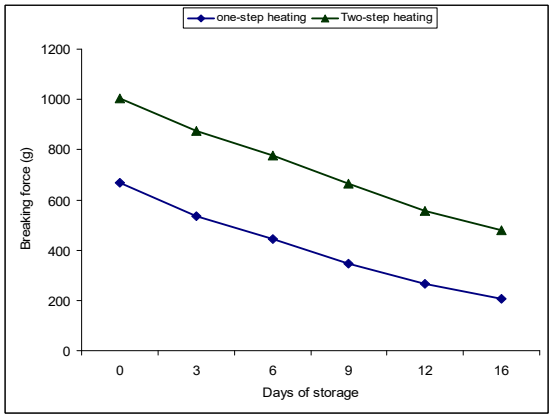


Fig. 1. Comparison of changes in breaking force (g) of one and two step heating gels during storage of fish in ice

Changes in TVB-N value

The result of the TVB-N (mg/100) is presented in the Figure 2. The initial TVB-N value was 1.37mg/100g, which gradually increased with lapse of storage period. At the end of 16 days of ice storage TVB-N value reached to 28.25mg/100g, which is within the range of recommended value of 25to30mg TVB-N/100g for fresh fish. However, at the end of 18 days of ice storage the TVB-N value increased to 35.1mg/100g which exceeded the recommended value. The available report suggests that the upper limit of TVB-N value should be 30g/100g for finfish acceptability (Connell, 1975). The increase in TVB-N value with the lapse of storage may be attributed due to bacterial spoilage. However, the available information indicates that TVB-N mainly accumulated in fresh fish during the later phase of spoilage after the bacterial population has grown. Thus the TVB-N is low during the edible storage period and only when the fish is near rejection level increasing amount of TVB-N are found.

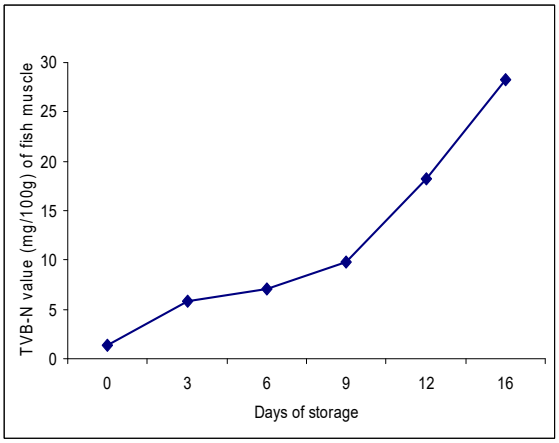


Fig. 2. Changes of TVBN-value (mg/100g) of pangas (*Pangasius pangasius*) muscle during ice storage

The result obtained for this study is more or less in agreement with the findings of Ahamed (2000) who reported the TVB-N value of tilapia (*Oreochromis*

niloticus) increased from 5.3mg/100g to 28.4mg/100g at the end of 16 days in ice storage.

Changes in peroxide value

The result of the changes in peroxide value of pangas (*Pangasius pangasius*) muscle during ice storage is shown in Figure 3. The initial value was below 5meq/kg of oil, which increased gradually with the lapse of storage period. At the end of 16 days of storage in ice, the peroxide value reached to 19.00meq/kg of oil, which was within recommended value of 10 to 20 meq/kg of oil. At the end of the 20 days of storage, the peroxide value found 28.18meq/kg of oil that exceeded the recommended value. According to Connell (1980) the recommended value of peroxide for fresh finfish is 10-20meq/kg of oil. The value above 20 meq/kg of fish, found to be emitting smell and rancid taste. The peroxide were presumed to be eventually further oxidized to aldehydes and ketones which had a very disagreeable "fishy" odor and taste. However, depending on the fish species and storage condition a good correlation between peroxide value and organoleptic quality was found.The results obtained from the present study indicate that there was an oxidation of fat during storage condition. The results of the present study, more or less in agreement with the findings of Ahamed (2000). In his study, the peroxide value of tilapia (*Oreochromis niloticus*) increased from 5meq/kg to19.2meq/kg at the end of 16 days of ice storage.

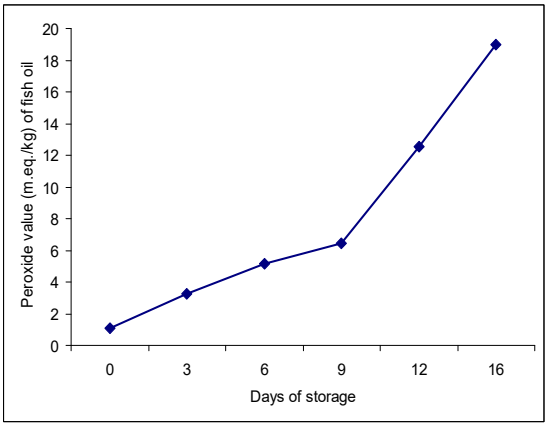


Fig. 3. Changes in peroxide-value (meq/kg) of fish oil of pangas (*Pangasius pangasius*) muscle during ice storage

Bacteriologicalchanges

The changes in bacterial loads of muscle of pangas during ice storage is shown Table-7. The initial bacterial loads in muscle of ice stored pangas fish was7.60×10^3 CPU/g which slightly decreased to 7.10×10^3 CFU/g at 2nd day of storage and then gradually increased with the lapse of storage period. At the end of the 16 days of ice storage, bacterial load increased to 4.6×10^6 CFU/g and the fishes were in acceptable condition organoleptically. After 18 days of storage in ice, the bacterial load was found 3.8×10^8 CFU/g which exceeded the recommended limit.

The initial decrease in bacterial population in fish muscle after the first days of storage might be due to some sorts of cold shock or leaching of surface flora by washing with melted ice. This is in agreement with the report of Bandyopadhyan *et al.* (1985). It has also been reported that the number of bacteria in gill, intestinal content and or the skin of newly caught fish vary from species to species and also depend on the microbial load of the waters in which they live (Fraiger and Westhoff, 1990).In the present study, it was observed that-pangas fishes were organoleptically acceptable up to 16 days of ice stored and the bacterial population at that stage reached to 4.6×10^6 CFU/g in muscle, which was within the recommended microbial limit for fresh and frozen fish. After 18 days of ice storage, the value increased to 3.8×10^8CFU/g and the fishes become organoleptically unacceptable. This result is more or less similar to the results of Ahamed (2000) who reported an increase in bacterial loads in muscle of tilapia from 7.6×10^3 CFU/g to 4.5×10^6 CFU/g at the end of 16 days of ice storage.

Table7. Changes in aerobic plate count (APC) of pangas (*Pangasius pangasius*) muscle during ice storage

Days of storage	Bacterial load (CFU/g)
0	7.60×10^3
3	7.10×10^3
6	2.40×10^4
9	3.10×10^6
12	3.90×10^6
16	4.60×10^6

Changes in protein solubility

Figure 4 shows the changes in protein solubility of pangas muscle during ice storage. Immediately after catch myofibrillar protein solubility was 86% which decreased to 36% at the end of 16 days of ice storage. The solubility decreased continuously with the progress of storage period. The results obtained in the present study indicate that there was a denaturation of muscle protein during ice storage and this result is more or less in agreement with Seki *etal*. (1979) who reported that- solubility of carp myofibrils decreased from 95% to 20% during ice storage within 2-3 weeks. Similar results were also obtained by Hossain (1995) for mrigal (*Cirrhina mrigala*) and Faruk (1995) for *Labeo rohita.* According to Seki *et al*. (1979); Kramer and Peters (1981) the large fall in solubility during ice storage was due to lowering of pH.

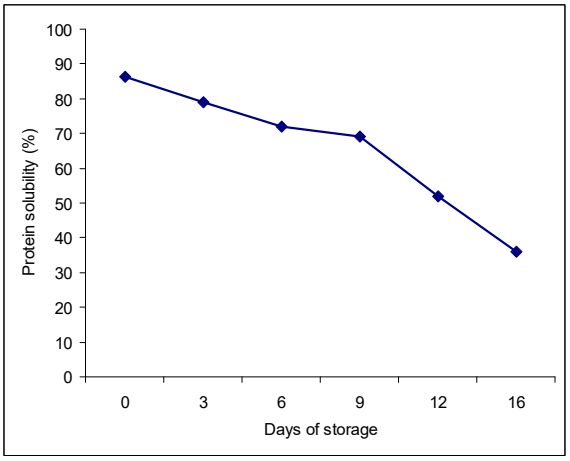


Fig. 4. Changes in protein solubility of pangas (*Pangasius pangasius*) muscle during ice storage

Conclusion

The obtained results from the present study showed that-ice storage contributed to delay the deterioration of pangas (*Pangasius pangasius*) muscle. Pangas (*Pangasius pangasius*) could be stored in ice until 16 days without emitting any objectionable odor.

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