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Changes in Fillet Quality of Pangas Catfish (*Pangasianodon hypophthalmus*) During Frozen Storage

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Abstract: Studies were conducted to determine changes in pangas catfish (*Pangasianodon hypophthalmus*) fillet quality during frozen storage by organoleptic and biochemical tests. Fillet remained acceptable for 120 days whereas after 150 days becomes inedible. Myofibrillar protein solubility for fresh samples was 88.21%; fell to 45.29% at 150 days at -20°C frozen storage. For one-step heating process gel forming ability as well as initial breaking force (IBF) was (790±4.47) g which reduced to (245±0.56) g at 50°C for 120 minutes after 21 days. IBF reduction were same at 50°C and 80°C for 120 and 30 minutes respectively for two-step heating which fall from (980±2.68 g) to (420±4.12 g). Folding test and teeth cutting test results were found same for both heating processes. Two steps heating shows higher gel strength. TVB-N values of fresh samples were 1.68 mg/100g at 150 days of storage, reached to 26.24 mg/100g that exceed the recommended range. The muscle pH immediately after death was 7.07 fell to 5.78 after 12 days, finally reached 6.43 after 150 days. Study indicated a gradual deterioration in fillet quality. This may be due to muscle protein denaturation, slight autolytic and bacterial activity.

Key words: Fillet Frozen · Organoleptic · Protein Solubility · Gel-Forming Ability · TVB-N

INTRODUCTION

Pangasianodon spp. available for culture in Bangladesh is commonly called sutchi catfish (*P. hypophthalmus*) it contributes 3.77% of the total production from culture. The total production from culture is 1065, 592 metric tons in 2011-2012 [1]. Due to low price in the market and rises in fish feed price, the profit margin has been reduced and fish farmers sometimes suffer great loss. However, 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 [2]. Demand for *Pangasianodon* remains stable throughout world. Supplies come from domestic production and through imports, both of which have been on an upward path. In Bangladesh and India, demand for *Pangasianodon* catfish in the domestic market is growing. Increasing imports from Viet Nam are complementing local harvests to satisfy this demand. In 2012 India imported close to 4000 tons of frozen fillets from Vietnam, mostly *Pangasianodon*. In the first eleven months of 2012, Malaysia imported a total of 10466 tones of frozen

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Pangasianodon fillets from Viet Nam at an average import price of USD 1.92/kg. *Pangasianodon* is now popular in the Malaysian catering and household sectors. Frozen and glazed *Pangasianodon* fillets currently sell at USD 3.6/kg in local supermarkets. This intensive, high-volume production system is very efficient, a workable commercial method providing protein to a growing world population that experts estimate could reach 9 billion by 2050. Bangladesh has as vast chance to become a supplier of pangas fish. It is anticipated that aquaculture production of *Pangasianodon hypophthalmus* will further increase in the medium term. Prices will initially remain stable, but may drop later if China solves its striped catfish production problems and increases output [2]. Value addition and diversification to satisfy the ever changing and diverse demands from the importing countries as well as urban consumers at home are most important for the world. Value addition is the most talked about word in the fish processing industry these days because of the increased realization of foreign exchange and high unit value of such products. One such value added product is fish fillet. Only lateral muscles are cut out from fish body in such a way that it removes the bone, belly fats, viscera etc. Proper storage at low temperature reduces the deterioration and improves the quality of fish. Fish fillets cut from fish body are very susceptible to different types of spoilage because fish muscles are directly exposed to air and microorganisms. Therefore, it is very essential to store at low temperature to increase the shelf life of fillet. However, some changes in fillet quality may occur during frozen storage. Physicochemical parameters of fish fillet act good indicators for determining fillet quality.

This is why the broad aim of this study is to know the preparation of fish fillet and changes in quality (organoleptic quality, protein solubility, gel forming ability, TVB-N (mg/100g) value and pH value) during frozen storage.

MATERIALS AND METHODS

Experiment Design: The fish samples were collected directly from fish farm, 8 km away from Bangladesh Agricultural University (BAU). Fish was harvested by seine net. After harvesting fishes were cleaned by super chilled water (-5°C). Within 20 minutes fishes were transferred from net to ice box to minimize the quality change. Average weight, length and width of collected catfish were 1.25kg, 43cm and 13 cm respectively. Live fish immediately after collection was sacrificed by rapid chilled shock and stored properly with crushed ice with well insulated icebox (ICEY-TEK-PET) and transported to the Fish Processing and Quality Control laboratory of BAU. Fish handling was done by following EC guidelines.

Preparation of Fish Fillet and Storage: Immediately after transportation to the laboratory, the iced fishes were washed thoroughly with chilled water (±1°C). Filleting was performed during the onset of post rigor stages to get good quality uniform fillet. Fillets were taken out by cutting the flesh from one side of the backbone the length of the fish starting just behind the head, then turning the fish over and cutting a similar strip of flesh from the other side of the backbone. Filleting was done manually with sharp knife and skinned properly followed by Mitch [3]. Immediately after filleting, these were washed with chilled water (±1°C) to remove blood, visceral part. Extra attention was paid to remove kidney tissues as they form globular masses, which affect both texture and appearance of the product. Then fillets were packed with polyethylene pouch and stored in deep fridge (Model: HCM050EC) at -20°C for 150 days and experiments were conducted at every 10 days interval. At selected time interval, the samples were obtained from freezer then thawed at refrigerated temperature condition for conducting different experiments. Handling and hygienic techniques were followed by EC guidelines and Bangladesh rules.

Organoleptic Evaluation: In sensory analysis, general appearance, odor, flavor, color and texture are evaluated using the human senses. The evaluation methods used here are based on the guidelines and methods for organoleptic quality of fish as described by EC guidelines for fish freshness and Howgate and Whittle [4], details in (Tables 1 and 2).

Protein Solubility

Preparation of Myofibrils: Myofibrils were prepared from ice stored fish fillet muscle according to Perry and Grey [5] with slight modification.

Myofibrllar Protein Solubility: Two ml of myofibrillar suspensions (5 mg/ml) were homogenized with 2ml of 0.1M KCl plus 100mM phosphate buffer (pH 7.0) using a homogenizer. The homogenate was allowed to stand at refrigerated temperature (4°C) overnight. The suspension

Table 1: Grading of fresh fish products according to [4, 16]

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 [16]

Sl. No.	Characteristics of whole fish	Defect characteristics	Defect points	Grade
1	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
2	Odor	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	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	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

was centrifuged for 15min at 1000×g in cold condition. The protein in supernatant was determined by Biuret method according to Gornall *et al*. [6] and Stoscheck [7].

Gel Forming Ability

Preparation of Meat Paste: Fillets are taken out from icebox every 3 days interval and meat mincer (NOVENAii) was used to mince the excised fillets. Operation was done at 4°C in every operation to minimize denaturation. During preparation of mince, the products were always kept in ice cooled container. Immediately after mincing, it was washed with chilled water containing 0.1% NaCl for two times. In every time, dewatering is done by pressing. Pressure was adopted on the mince kept in a flat cotton cloth bag at the rate of 5kg/cm^2 for 10 minutes and final pressing was done at 10kg/cm^2 for 15 minutes. Then washed mince was ground with 3% NaCl by a previously cooled (4°C) mortar for 25 minutes. Due to this grinding with salt the mince transformed into viscous paste. The salt ground paste was then carefully stuffed into heat stable polyvinyledene chloride cylinder manually and the both ends of the cylinder were wrapped with para film and polyethylene paper.

Preparation of Gel: The paste 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 one is two-step heating. All heating treatments were triplicate. In one-step heating, samples were heated for 120 min in water at 50°C. In two-step heating, the first heating was done for 120 min in water at 50°C; this first heating will be conveniently called pre-heating from now on. After this preheating treatment, they were immediately heated for another 30 min in water at 80°C. After heat treatments, the samples were taken out from the water bath, kept in iced water for 1 hour. After that cylinders were kept at refrigerated temperature (4±1°C) for overnight. Then cylinders were taken out from refrigerator, kept at room temperature for 15-20 minutes 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 by Poon *et al*. [8] was provided for the organoleptic assessments. The gels were removed from the cylinders and subjected to puncture test, folding test 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 (6 mm diameter). The folding test measured the resistance against breaking along with the folds when samples discs of 1.0 mm thickness were folded into halves, 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.

Folding Test: For folding test were done by following Poon *et al.* [8]. The gel was graded using the scores presented in (Table 3).

Puncture Test: Puncture test was carried out by a food rheometer texture analyzer and the breaking strength (BS) in g and the breaking deformation (BD) in cm were calculated from the chart of a potentiometric recorder according to Park [9].

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 is too soft to evaluate.

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

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 teeth's according to Shimizu *et al*. [10] and the gel strength was evaluated as described by Siddique *et al*.[11] and presented as numeral scores (Table 4).

TVB-N (MG/100G) slightly modified from Gokalp *et al.* [12].

Sample Preparation: Exactly 10g of ground sample were weighed, mixed with 90 ml of 6% perchloric acid and homogenized for two minutes with a blender in cooled condition.

Steam Distillation and Titration: 100ml of extract with 4-6 drops phenolphthalein were put in kjeldahl flask and then some glass-beads and 10ml of 20% NaOH were added to the flask after placing on the distillation. The distillation continued for more or less 15 minutes. The distillate was collected in the conical flask containing 50 ml of 3% H_3BO_3 and one drop mixed indicator. Distillation was confirmed through changing the color of mixed indicator, i.e. violet to greenish.

Then the collected distillate was titrated with 0.01N HCL and regaining the violet color of mixed indicator confirms the end-point. The results were expressed as mg of TVB-N/100g sample according to the following equation.

Measurement of Muscle pH: The muscle pH of each individually identified fish fillet was measured. The pH was measured by inserting a pH probe (Checker 200) into the upper mass of the fillet, just behind the head.

Statistical Analysis: The data of such was analyzed by Randomized Completely Block Design (RCBD) and tested (significance) through Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at $P<0.05$ and the results are presented as mean [13, 14].

RESULTS AND DISCUSSION

Organoleptic Evaluation: Based on the scores the fishes were found in acceptable conditions for 120 days of frozen storage before it becomes inedible. After 150 days of storage, fish fillets were not fully unacceptable but it turns to nearly unacceptable stages. The results of the organoleptic quality assessment of pangas (*Pangasianodon hypophthalmus*) fish during frozen storage are shown in (Table 5). At the beginning of the storage odor, color and appearance of fillet was natural and fresh. However, their quality deteriorated with time. Scores given to sensory indices (odor, color of fillet, appearance and firmness of fillet) decreased with storage time. The quality of fishes was graded using the score from 1 to 5. The grades were defined in terms of the total number of defects or demerit points. The score 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 bad or rejected. The changes in quality of frozen fish during storage were assessed by organolelptic examination. Based on the scores the fishes were found in acceptable conditions for 120 days in frozen storage before it becomes inedible. The changes occurred in organoleptic quality during the storage period and this can roughly be

Table 5: Changes in organoleptic qualities of pangas (*Pangasianodon hypophthalmus*) fillet during frozen storage

Months of storage	Defect points	Grade	Overall qualities
0	1.25	A	Excellent
1	1.9	A	Excellent
2	2.2	B	Acceptable
3	3.2	B	Acceptable
4	4.1	B	In the limit Acceptable
5	4.9	Close to C	Nearly Rejected

divided into five phases corresponding to periods of 0 to 1, 1 to 2, 2 to 3, 3 to 4 and 4 to 5 months in frozen storage. Based on the scores the fishes were found in acceptable conditions for 120 days of frozen storage before it becomes inedible. After 150 days of storage, fish fillets were not fully unacceptable but it turns to nearly unacceptable stages. The result of the present investigation showed that, here was a gradual decrease in protein content of fish fillet during the frozen and iced storages. Glazings after freezing have significant effect on fillet shelf life [15]. Meenakshi *et al.* [16] reported that very little changes alone occur in fish stored at -20°C and -80°C. However, maximum muscle and structure value were found in gray mullet [17].

From the result of the present study, it is clearly indicated that myofibrillar proteins in pangas muscle were not stable during longer period of frozen storage at -20°C. Many workers agree that freshness and post mortem condition of fish muscle at the time of freezing has an important bearing on the rate of freeze denaturation [18- 20]. The protein content was reduced in fish storage at room temperature was 25.6% for after 24 hrs of storage, 4°C was 12.06% after 96 hrs, -20oC was 2.09 and -80°C was nil the spoilage up to 96 hrs of storage [21]. The stability of fish muscle in frozen storage also varies with the season and other biological conditions such as nutritional status, degree of fatigue and spawning status [22, 23]. It has also been reported that temperature and duration of frozen storage influence the denaturation of fish muscle [24].

Lakshmanan *et al*. [25] reported that, from 36 to 44 weeks, frozen samples of whole and fillet were still acceptable quality but showed loss of characteristic flavors and texture in rock cod (*Epinephelus* spp.) at –20°C. There was no significant difference in texture, taste, appearance and overall acceptability, but off-flavors developed during storage period in the frozen Nile perch at –27°C for a period of 12 weeks [26]. Our findings show the same results. Our studies showed similar results and at the end of five months treatments fillet were in acceptable quality, but there was a significant decline ($p > 0.05$) in all sensorial quality. Tokur *et al.* [26] reported that the scores did not exceed acceptable levels of filleted trout (*Oncorhynchus mykiss*) during frozen storage at–18°C for 12 month. Differences may be result from differences between fish species. Acceptable quality of anchovy only lasted three months of frozen storage at –18°C [23, 27]. This study important because it shows that decline of storage temperature provides extent of storage time of fish.

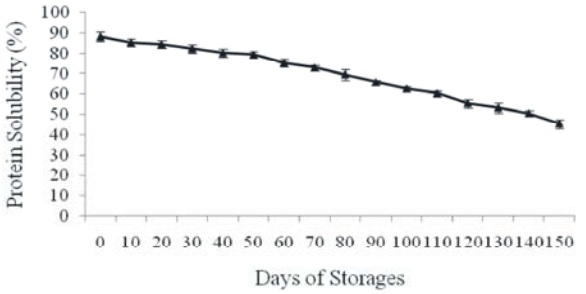

Fig. 1: Changes in muscle protein solubility of pangas (*Pangasianodon hypophthalmus*) fillet during frozen storage

Protein Solubility: In order to determine the extent of denaturation during frozen storage, myofibril was examined for solubility during storage. The storage period was 150 days. Changes in myofibrillar protein solubility of pangas fillet during frozen storage at -20°C for 150 days (Fig.1) In case of fresh fillet, myofibrillar solubility was 88.21%, which gradually decreased to 45.29% after 150 days of frozen storage.

Almost all muscle tissue proteins are soluble at a pH of 3.5-10.5. The actual solubility, however, may vary somewhat, depending on the species and the muscle type. More than 98% of cod and mackerel light muscle proteins are solubilised. The solubility of the proteins of mackerel red muscle varies from 75 to almost 100%. The variability may be related to post mortem age and time of exposure to pH values below 6.6 [28]. Lowering the temperature decreased the rate of protein denaturation. Soluble myofibrillar protein in the total protein extract of unstored whitefish muscle represented about 72% of the total muscle protein [29] which dropped to 22% over the 16 week frozen storage period [30].

During the first 4 weeks of storing cod muscle at -12°C, no changes in extractable myofibrils (actomyosin) was evident but a drop from 65 to 30% occurred in the next 4 weeks [30]. The solubility of myofibrillar protein was greatly reduced when polyunsaturated lipid in the protein-lipid system was oxidized. The decrease in solubility of myofibrillar proteins in white fish muscles was accompanied by a rise in FFA content [29, 31]. No significant change in the amount of native actin that could be extracted from frozen stored muscle during a storage period in which the amount of total extractable myofibrillar protein fell from its initial value to a minimum [29]. This result suggested that myosin was more sensitive to freezing induced denaturation than actin.

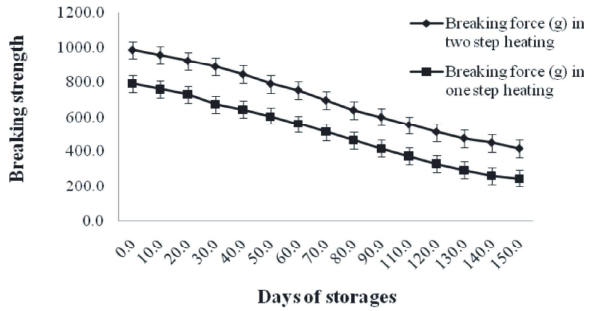

Fig. 2: Changes in breaking force (g) of pangas (*Pangasianodon hypophthalmus*) meat past in one step and two step heating during frozen storage

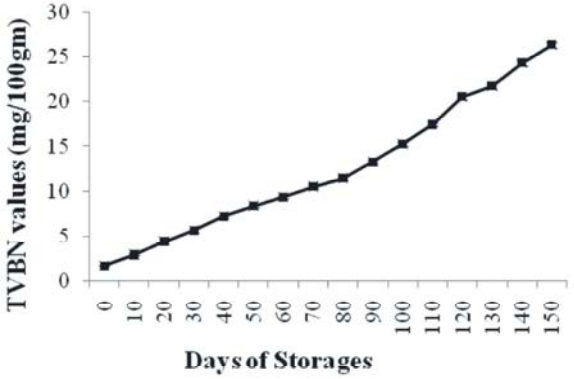

Fig. 3: Changes of TVBN-value (mg/100g) of fish muscle of pangas (*Pangasianodon hypophthalmus*) fillet during frozen storage

Gel Forming Ability

One-step Heating: Gel forming ability of fish where initial gel strength was (790± 4.472) g, which decreased to (245± 0.56) g at 50°C for 120 minutes after 150 days of frozen storage (Fig. 2). The initial folding test (FT) was 'AA', which decreased to 'B' and teeth cutting test (TCT) was 9, which decreased to 4 after 150 days of frozen storage in one-step heating. The results obtained from the study clearly indicated that the gel forming ability decreased with increasing the storage period result of denaturation of myofibrillar protein. The study observed that the gel-strength was highest in two steps heating than one-step heating [32].

Two Step Heating: The gel forming ability of fish where initial gel strength was (980± 2.683) g, which decreased to (420±4.12) g, at 50°C and 80°C for 120 and 30 minutes respectively after 150 days of frozen storage (Fig. 2). The initial folding test (FT) was 'AA', which decreased to 'B' and teeth cutting test (TCT) was 9, which decreased to 4 after 150 days of frozen storage in two-step heating. The comparative study between one-step heating and two steps heating have been showed in (Fig.2). The study observed that the gel-strength was highest in two steps heating than one-step heating.

TVB-N (mg/100g): Initial TVB-N content was 1.68 mg/100g. There was a slow growth of TVB-N concentration with increasing storage time, after 150 days. TVB-N content in pangas flesh was 26.24 mg/100 g. TVB-N is used for determination of the spoilage level of fish during the storage period [29, 30]. The lowest TVB-N value (17.23 mg/100 g) was determined in whiting, the highest value in anchovy (22.55 mg/100 g) [31]. Total Volatile Base Nitrogen (TVB-N) value of fish stored in frozen condition exceeds 30mg TVB-N/100g after 75 days of storage, which indicates the deterioration of the product [32, 33].

The level of 35 mg/100 g has been considered the upper limit, above which fishery products are considered spoiled [34, 35]. TVB-N values of three fish species increased during the storage period. According to the TVB-N values, the differences between the fish species were significantly important ($p<0.05$) whereas treatments did not show significant differences [23]. During the storage period, according to TVB-N value whiting kept its freshness better than gray mullet and anchovy [36]. Anchovy showed more increase than the other species at 9th month of storage period. According to TVB-N value, while whiting and gray mullet were very good quality, anchovy was within acceptable limit end of storage period. Pons-Sanchez-Cascado *et al.* [37] determined the levels of TVB-N values for fresh anchovies ranging from 3.79 ±0.30 mg/100 g to 13.7±0.46 mg/100 g and lower than our findings. Lakshmanan *et al*. [25] found that after 52 weeks, TVB-N values (18.2 mg/100 g) in fillet rock cod (*Epinephelus* spp.) frozen at -20°C were the lowest from the all frozen fish (29.4 mg/100 g) [38]. Our results were similar with [39, 40]. TVB-N increased from 5.60 to 27.20 mg/100 g in frozen hilsa fish at –20°C throughout 75 days [41]. The other researchers stated that TVB-N value was 4.19 at the beginning [42] but increased to 14.90 at the end of 7th month of storage in pike fish at -18°C [43]. However, a relatively smaller increase (from 15.81 mg/100 g average value to 24.83 mg/100 g) was determined [44]. This lower increasing in TVB-N value may result from lower storage temperature [45]. Initial TVB-N content was 1.68 mg/100g during 0-3 days of frozen storages at -25°C [46]. There was a slow growth of TVB-N concentration with increasing storage time (Fig. 3), after 150 days, TVB-N

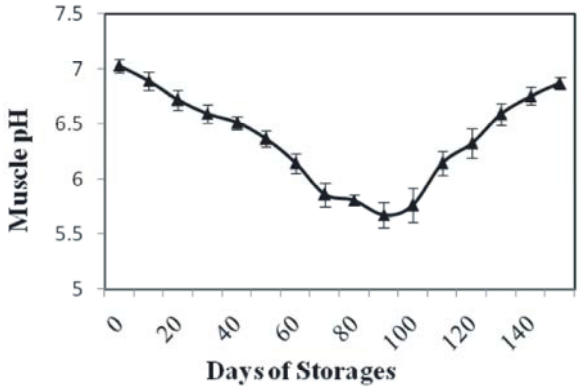

Fig. 4: Changes in pH of pangas (*Pangasianodon hypophthalmus*) fillet during frozen storage

content in pangas flesh was 26.24 mg /100 g. As the safety limit for TVB-N in fish is 30 mg/100 g fillet, the concentrations observed can be considered within the normal and acceptable range [47].

Increases of TVB-N values and that when these values reach the highest tolerance level of 25–35 mg/100 g of flesh the fish is considered spoiled due to increased bacterial activity and total volatile bases [1]. Bacterial activity in fish muscle is responsible for ammonia accumulation and accumulation of volatile bases in flesh of the fish. Studies on storage of different frozen fish species, it was suggested that TVB-N values might change depending on the spoilage flora and analysis method [48]. The TVB-N value was more useful in assessing the degree of deterioration than in evaluating the changes occurring during the first stages of storage [2]. It is suggested that the TVB-N value depends on the species, catching methods, season and region, age and sex of [1, 20]. The results obtained from the present study showed that there was a gradual increase in TVB-N levels during long-term storage in frozen condition, which suggests that this may occur due to slight aulolytic and bacterial activity during frozen storage.

Muscle pH: Changes in muscle pH of frozen stored fish are shown in (Fig. 4). The pH of Fish muscle immediately after death was around 7.07, which decreased gradually to 5.78 after 90 days of frozen storage. Then it increased gradually up to 6.43 after 150 days. The muscle pH of fish immediately after death was close to neutral. Increasing the storage period, a decline was observed in the pH values of fish species. Differences between the species and treatments were significant ($p<0.05$) in terms of pH value. Similarly storage time caused significant differences in pH value of frozen sardines (*Sardinella aurita*) [44] whereas Simeonidou *et al.* [38] reported that the pH values significantly different in frozen whole and fillet of horse mackerel (*Trachurus trachurus*). The highest pH value was obtained in whiting comparing with other species. However, when the treatments were compared, the highest pH value was found in fillets (6.56) and the lowest pH (6.50) value was found gutted fish. Olgunoglu *et al*. [40] found that pH value increased from 6.80 to 7.02 in frozen pike perch fillets at -20°C throughout 7 months however, Lakshmanan *et al*. [25] reported that there was a decline in pH after 36 weeks in whole, gutted and fillet frozen rock cod (*Epinephelus* spp.) at -20°C. Catfish muscle pH after frozen storage for various times was not significantly different from fresh muscle pH. In our research, a slightly decrease was observed in fish species throughout 5 months storage. Similarly, the pH value of filleted trout decreased significantly during frozen storage ($p<0.05$) [48]. pH of fresh and frozen fillets samples were 6.5 and 6.6, respectively while for fresh and frozen shrimp were 6.6 and 7.5, respectively [23]. Fresh seafood had a significant lower ($p<0.05$) pH values in compared to frozen products [49]. The pH value of seafood varies from 5.8- 7.2 depending on struggling at the time of harvesting but the normal variation is of pH 5.8-6.5[38]. The increasing pH values could be associated with the production of basic components induced by the growth of bacteria [45]. The pH changes are in agreement with the findings of [23, 39]. Changes in the pH of fish muscle have been considered one of the causative factors in the denaturation of fish protein during frozen storage [10]. However, it was reported that the extent of changes in pH in frozen fish flesh was small, as the buffer, action of unfrozen liquid was quite strong and the pH change would therefore not be the main factor in protein freeze denaturation.

CONCLUSION

After filleting, changes in fillet quality during frozen storage was studied by evaluating the changes in organoleptic quality, protein solubility, gel-forming ability, Total volatile base nitrogen (TVB-N) and P^H. Organoleptic quality of fillets show that the fishes were found in acceptable conditions for 120 days of frozen storage before it becomes inedible. The results of protein solubility during frozen storage showed that at the end of 150 days of frozen storage when the fishes were organoleptically unacceptable for consumption. The solubility decreased continuously with the increasing of storage period. One-step heating and two

steps heating showed that the gel strength was highest in two steps heating than one step heating. The result of the TVB-N during frozen storage showed that gradual increase in TVB-N value with lapse of storage period. The pH of fish muscle immediately after death was around neutral, which decreased gradually and after a certain period it increase again.

The following conclusions can be drawn from the results of the experiment conducted in this work.

- The organoleptic quality changes in Thai pangas fillet during frozen storage have indicated those fish fillets were remained in an acceptable condition for 150 days.
- The gel forming ability of Thai pangas fillet suwari-gels in two steps heating was considerably higher than one-step heating.
- The results obtained from the present study has indicated that there was a gradual increase in TVB-N levels during long term storage in frozen storage, which suggests that this may occur due to slight aulolytic and bacterial activity during frozen storage.
- Studies on the changes in the pH of fish muscle has indicated that the muscle pH of fish immediately after death was close to neutral but it gradually decreased to acidic condition due to different biochemical changes in fish muscle.

REFERENCES

1. DOF, 2013. National Fish Week 2013 compendium (In Bengali). Department of Fisheries, Ministry of Fisheries and Live Stock, Bangladesh, pp: 144.
2. FAO, 2013. Globefish. DOI:http:// www.globefish.org/ pangasius-june-2013.html
3. Mitch, T., 2013. BBC Food Techniques. How to fillet round fish. http:// www.bbc.co.uk/ food/ techniques/ filleting_round_fish Date: 25 September 2013.
4. Howgate, P.A.J. and J.K. Whittle, 1992. Multilingual Guide to EC Freshness Grades for Fishery Products. Torry Research Station, Food safety Directorate, Ministry of Agriculture, Fisheries and Food, Aberdeen, Scotland.
5. Perry, S.V. and T.C. Grey, 1956. A study of the effects of substrate concentration and certain relaxing factors on the magnesium-activated myofibrillar adenosine triphosphatase. Biochem. J., 64: 184.
6. Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Denaturation of serum proteins by means of the Biuret reaction. J. Biol. Chem., pp: 751-766.
7. Stoscheck, C.M., 1990. General methods for handling proteins and enzymes: Quantitation of protein. In M.P. Deutscher (ed.), Methods in enzymology: Guide to protein purification, pp: 40-42. New York Academic Press.
8. Poon, K.H., P.Y. Lim, M.C. Ng and P.C. Ng, 1981. The suitability of leached meat of small demersal fish for making fish jelly products. Singapore J. Primary Indus., 9: 28-37.
9. Park, J.W., 2004. Surimi Gel preparation and texture analysis for better quality control. pp: 333-341. In: Sakaguchi M. editor. More efficient of fish and fisheries products, Hardbound.
10. Shimizu,Y. and T. Fujita, 1985. Stability of unwashed and washed fish minces during frozen storage. Bull. Japan. Soc. Sci. Fish., 51: 1187.
11. Siddique, M.A., A.A.K.M. Nowsad, M.N. Islam, M.S. Uddin, M.A.A. Faroque and S. Yeasmine, 2013. Quality and shelf life of fish sausage and fish ball prepared from Bombay duck. Bangladesh Res. Pub. J., 8(1): 41-48.
12. Gokalp, H.Y., M. Kaya, Y. Tulek, O. Zorba, 1993. ET veet ürünlerinde kalite kontrolü ve laboratuvar uygulama klavuzu. Atatürk Üniversitesi yayın no: 751, Ziraat Fakültesi yayın no: 318, Ders kitapları serisi, 69: 214-217.
13. Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for Agricultural Research. Second edition. Intl. Rice. Res. Inst. A. Willey Int. Sci. Pub., pp: 28-192.
14. Shil, R.N. and S.C. Debnath, 1992. An Introduction to the Theory of Statistics.pp.240-320. 2nd Edition, Publisher: Minati Shil and Anita Debnath, Mymensingh.
15. Peter, Z., 2011. The effect of glaze on the quality of frozen stored Alaska Pollack (*Theragra chalcogramma*) fillets under stable and unstable conditions. Acta vet. Brno., 80: 299-304.
16. Meenakshi, V., K.R. Narayanan and R. Venkataraman, 2010. Evaluation of organoleptic and biochemical status of the Fish, *Cyprinus carpio* at different storage temperatures. Biomed. Sci. and Res., 2(4): 254-257.
17. Orak, H.H. and S. Kayisoglu, 2008. Quality changes in whole, gutted and filleted three fish species (*Gadus euxinus*, *Mugil cephalus*, *Engraulis encrasicholus*). At frozen storage period (-26°C). Acta Sci. Pol., Technol. Aliment., 7(3): 15-28.
18. Dyer, W.J., 1951. Protein denaturation in frozen and stored fish. Food Res., 16: 522-527.

19. Gill, T.A., 1992. Objective analysis of seafood quality. Food Rev. Int., 6: 681-714.
20. Nasopoulou, C., C.A. Demopoulos and I. Zabetakis, 2012. Effect of freezing on quality of sea bass and gilthead sea bream. Eur. J. Lipid Sci. Technol., 114: 733-740.
21. Qian, C., H.M. Zhao, Y. Liu, C. and L. Mao, 2012. Comparison of superchilling and freezing on the microstructure, muscle quality and protein denaturation of grass carp (C*tenopharyngodon idellus*). Journal of Food Processing and Preservation, 37(5): 546-554.
22. Umemoto, S., K. Kanna and Iwata, 1971. Studies on the quality of frozen stored Allaska Pollock surimi-II. Changes in extractable protein of surimi during cold storage. Nippon Suisan Gakkaishi., 37: 1100-1104.
23. Veeck, A.P.L., L.O. Garcia, B. Baldisserotto, F.E. Zaniboni and T. Emanuelli, 2013. Proximate composition and lipid stability of dourado (*Salminus brasilensis*, Cuvier, 1817) fillets exposed to different levels of ammonia and oxygen *in vivo*. J. Sci. Food Agric., 93: 2590-2595.
24. Abdolaziz, N. and A. Khajeh-Rahimi, 2012. Effect of gelatin coating on fatty acid composition of grass carp (C*tenopharyngodon idella*) during refrigerated storage. World Journal of Fish and Marine Sciences, 4 (5): 462-466.
25. Lakshmanan, P.T., P.R.G. Varma, T.S.G. Iyer and K. Gopakumar, 1990. Quality changes in sea frozen whole and filleted rock cod (*Epinephelus* spp.) during storage. Fisheries Res., 9: 1-12.
26. Tokur, B., S. Calki and A. Polat, 2006. Trout (*Oncorhynchus mykiss* W., 1792) with a vegetable topping during frozen storage (-18°C). J. Fish. Aquatic Sci., 3(4): 345-350.
27. Kose, S., H. Karacam, S. Kutlu and M. Boran, 2001. Investigating the shelf-life of the anchovy dish called 'Hamsikusu' in frozen storage at -18 ±1°C. Turk. J. Vet. Anim. Sci., 25: 651-656.
28. Hultin, H.O., H.G. Kristinsson, T.C. Lanier and J.W. Park, 2005. Process for Recovery of Functional Proteins by pH shifts. J.W. Park (Ed.). Surimi and Surimi Seafood, Boca Raton; Taylor and Francis Group, pp: 107-139.
29. Connel, J.J., 1960. Changes in the adenosinetriphosphate activity and sulphydryl group of cod during frozen storage. J. Sci. Food Agr., 11(5): 245-249.
30. Fraser, D.I. and W.J. Dyer, 1959. Proteins in fish muscle and lipid hydrolysis. J. Fish. Res. Bd., *(Canada),* 16: 43.
31. Awad, A., W.D. Powrie and O. Fennema, 1969. Deterioration of freshwater whitefish muscle during frozen storage at -10°C. J. Food Sci., 34: 1.
32. Vidacek, S., T. Janci, Z. Brdek, D. Udovicic, N. Marusic, H. Medic, T. Petrak and I. Lackovic, 2012. Differencing sea bass (*Dicentrarchus labrax*) fillets frozen in different conditions by impedance measurements. International Journal of Food Science & Technolog, 47: 1757-1764.
33. Hassan, M.N., M. Rahman, M.M. Hossain, A.A.K.M. Nowsad and M.B. Hossain, 2013. Post-harvest loss and shelf life of traditionally smoked shrimp products produced in Bangladesh. World Journal of Fish and Marine Sciences, 5(1): 14-19.
34. Bahareh, S. and A. Jamshidi, 2013. Quality characteristics of fried rainbow trout (*Onchorhynchus mykiss*) fillet coated with different hydrocolloids edible film. World Journal of Fish and Marine Sciences, 5(4): 398-404.
35. Schormuller, J., 1969. Handbuch der Lebensmittel Chemie. Band 4. Fette und Lipoide [Lipids]. Springer Berlin, pp: 872-878.
36. Nowsad, A.A.K.M., M.A. Siddique, M.N. Islam, M.S. Uddin, M.A.A. Faroque and S. Yeasmine, 2013. Quality and shelf life of fish sausage and fish ball prepared from Bombay duck. Bangladesh Res. Pub. J., 8(1): 41-48.
37. Pons-Sanchez-Cascado S., M.T. Veciana-Nogues, S. Bover-Cid, A.M. Font and M.C. Vidal-Carou, 2005. Volatile and biogenic amines, microbiological counts and bacterial amino acid decarboxylase activity throughout the salt ripening process of anchovies (*Engraulis encrasicholus*). J. Food Prot., 68: 1683-1689.
38. Simeonidou, S., A. Govaris and V. Kyriakos, 1997. Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and medditerean hake (*Merluccius mediterraneus*). Untersuch. Forsch, A, pp: 6.
39. Kamal, M., B.C. Biswas, L. Yasmin, K.M. Azimuddin and M.N. Islam, 2001. Influence of temperature on the gel-forming ability of some underutilized marine fish species in Bangladesh. Pakistan J. Biol. Sci., 4(12): 1535-1542.
40. Olgunoglu I.A., A. Polat and I. Var, 2002. Chemical and sensory changes of pike perch (*Sander lucioperca* Bogustkaya and Naseka 1996) fillets during frozen storage (-18°C). Turk. J. Vet. Anim. Sci., 4: 879-884.

41. Anderson, A.K., 2008. Biogenic and volatile amine-related qualities of three popular fish species sold at Kuwait fish markets. Food Chem., 107: 761-767.
42. Antonacopoulos, N. and W. Vyncke, 1989. Determination of volatile basic nitrogen in fish: a third collaborative study by the West European Fish Technologists Association (WEFTA). Zeitschrift Lebensmittel Untersuchung Forschung, 189: 309-316.
43. Suarez, D.G., J.V. Puig and A.G. Cantillo, 2002. Physical, chemical and sensorial evaluation of round sardines (*Sardinella aurita* V.) during frozen storage at -18°C. Rev. Cıent. Facul. Cıenc. Vet., 12(4): 278-285.
44. Rostamzad, H., B. Shabanpour, A. Shabani and H. Shahiri, 2011. Enhancement of the storage quality of frozen Persian sturgeon fillets by using of ascorbic acid. International Food Research Journal, 18: 109-116.
45. Manthey, M., V. Hilge and H. Rehbein, 1988. Sensory and chemical evaluation of three catfish species *(Silurusgluvis, Ictuzurus puncmtus, Chrius guriepinus)* from intensive culture. Arch. Rschereiwiss, 38(3): 215.
46. Ryder, S.P., M.I. Recht and J.R. Williamson, 2008. Quantitative analysis of protein-RNA interactions by gel mobility shift. Methods Mol. Biol., 488: 99-115.
47. Fennema, O.R., 1973. Nature of the freezing process. Low-Temperature Preservation of Foods and Living Matter, 1st ed., Marcel Dekker Inc. New York, pp: 150-239.
48. Dalin, M., K. Saritha and Jansi, 2013. Postharvest handling and traditional processing of marine fishes and the quality of the end products. World Journal of Fish and Marine Sciences, 5(1): 56-62.
49. Shiva, A., Y. Peighambari, B. Shabanpure and A.R. Hosseini, 2013. Survey of quality control in whole yellowfin truna (*Thunnus albacares*) using relation of biogenic amines with psychrophilic bacteria load during frozen storage. World Journal of Fish and Marine Sciences, 5(5): 527-532.