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Changes in gel forming ability and protein solubility of pangasius (*Pangasianodon hypophthalmus*) muscle at different rigor stages during storage at room temperature (28 to 32°C) and in ice

MD. ISMAIL HOSSAIN, FATEMA HOQUE SHIKHA* AND SABINA YEASMIN
Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
*Email: shikhafh@bau.edu.bd

Abstract. Gel forming ability of fish muscle is greatly influenced by the storage temperature and rigor stages. The present study was carried out to assess the changes in gel forming ability and protein solubility of pangasius (*Pangasianodon hypophthalmus*) muscle stored at room temperature (28 to 32°C) and in ice. Rigor-mortis in fish sample kept at room temperature (RT) and in ice started 1hr after death. The rigor-index reached a maximum 73.33% within 7 hrs after death at RT and a maximum 87.09% within 4 hrs in ice storage. At pre-rigor stage, the breaking force (BF) was 646.00 (±2.08g) which decreased to 266.67 (±3.53g) at post rigor stage at RT and from 660.33 (±1.45g) to 420.67 (±1.45g) in ice in one step stage. At RT, grade of folding test (FT) decreased from "AA" to "A" and score of teeth cutting test (TCT) from 8 to 4. In ice also grade of FT and score of TCT decreased. Similarly, in two-step heating gels, BF decreased from 994.33 (±2.33g) to 403.00 (±1.15g) at post rigor stage at RT and 995.33 (±1.45g) to 552.67 (±3.71g) in ice. Immediately after catch myofibrillar protein solubility was 86% which declined below 30% after 24h of storage at RT and below 30%after 28 h of storage in ice. The study concludes that the rigor period of pangasius may be extended up to 18 h by storing the fish in ice while at RT this period is only about 5 h, which might contribute to slow down the gel degradation process.
Keywords: Gel forming ability, Pangasius muscle, Rigor stages, Icing

Introduction

There are a number of factors that influence the quality of fish, of which the most important one is the post-mortem changes that take place soon after death due to enzymatic action. A large number of different enzymes are naturally present in the flesh which are engaged in normal life processes such as tissue building and muscular contraction and relaxation. On death, however, they become involved in predominantly degradation reactions. One of these reactions is the gradual hydrolysis during the first few hours of glycogen to lactic acid, resulting when the process is complete in a fall in pH from about 7.0 to 6.8 depending on species and the condition of the fish. The decline in pH is accompanied by the natural post-mortem stiffening called rigor mortis.

Post mortem change is one of the most important aspects in maintaining the keeping quality of fish under various storage conditions. The state of rigor in association with other biochemical changes influences the meat quality of fish and higher animals (Penny 1967). There are three stages of rigor mortis: pre-rigor, in-rigor, post-rigor. Immediately after death the muscle of an animal become soft and limp, and can easily be flexed; at this time the flesh is said to be in pre-rigor condition. Eventually the muscles begin to stiffen and harder, and the animal is then said to be in-rigor; after some hour or day the muscle gradually being to soften and become limp again. The animal has now passed through rigor and muscle is in the post-rigor condition. The progress of rigor-mortis in association with ATP depletion and lactate accumulation is dependent

on temperature and varies from species to species. It is generally accepted that low temperature delays the onset of rigor-mortis but several tropical fish species, such as tilapia, red sea bream and plaice are reported to have a shortened pre-rigor period when stored at 0°C (Iwamoto *et al.* 1985,1987, Iwamoto and Yamanaka 1986).

The qualitative characteristics of meat products are closely related to the functionality of muscle proteins. Myofibrillar proteins (MPs), comprising approximately 50% of total muscle proteins, are generally considered to be insoluble in solutions of low ionic strength (< 0.2 M), requiring high concentrations of salt (> 0.3 M) for solubilization. These soluble proteins are the ones which determine many functional properties of meat products, including emulsification and thermal gelation (Chen *et al.* 2015). 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 is still controversy on the influence of temperature on the onset and duration of rigor-mortis of fish. The objective of the present study was to follow the rigor-mortis of pangasius (*Pangasianodon hypophthalmus*) fish at room temperature (28 to 32°C) and in iced condition.

Materials and Methods

***The experimental fish*:** Pangasius catfish, commonly called freshwater-pangasius(*P. hypophthalmus*), was used as raw material for the present study. The fish were collected live from K R market, Bangladesh Agricultural University campus, brought to the laboratory and kept both at room temperature (28 to 32°C) and in ice in an insulated box (fish: ice ratio 1:1). Ice was replaced at each 5-6 hours intervals in the insulated box (Plate I).

(a) (b)

Plate I. Pangasius (*P. hypophthalmus*) used for the study (a) Pangasius bought for the study, (b) Pangasius stored in ice.

Analytical Methods

***Rigor-index*:** "Rigor-index" was measured essentially according to Bito *et al.* (1983) and used as a parameter of rigor tension. Briefly, the fish was placed on a horizontal table with half of its

body (tail part) kept out of the table (Plate-II). At selected time intervals, rigor-index was calculated by the following equation:

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

Where D_o and D represent the distances of the base of caudal fin from horizontal line of the table at the start of the experiment that is in pre-rigor and at subsequent storage periods respectively.

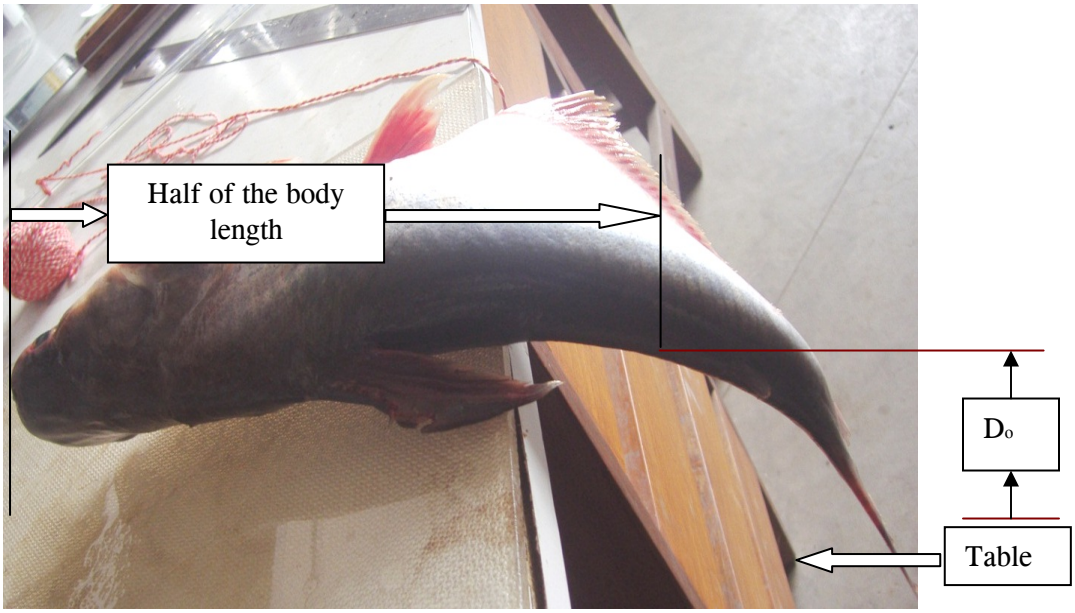


Plate II. Determination of rigor-index of pangasius (*P. hypophthalmus*)

***Estimation of changes in gel forming ability*:** The fishes were washed in chilled freshwater before they were beheaded 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 included washing with water, beheading and gutting, filleting, deboning mincer, deboned mince, grinding with 3% NaCl for 20 min, and meat paste.

***Preparation of gel*:** The paste in plastic casing cylinders(diameter 2 cm, height 12 cm) was heated to produce gel. Some samples were heated once only in well stirred water bath (One step heating), whilst the rest were heated twice (two step method) in triplicates. In one-step heating, samples were heated for 120 min in water at 40°C. In two-step heating, the first heating was for 120 min in water at 40°C, conveniently called pre-heating. After this preheating treatment, they were immediately heated for another 30 min in water at 85°C. After heat treatments, the samples were taken out from the water bath, kept in iced water for 1 hr and used for further tests.

***Measurement of gel-strength*:** The gel strength of the products was assessed by objective and organoleptic methods. A five person panel as described by Poon *et al.* (1981) was followed for the organoleptic assessments. The gel was removed from the cylinder 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. The folding test measured the resistance against breaking along with the folds when sample 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 of2 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 quarter by the help of the thumb and index finger. The gel was graded using the scores presented in Table I.

Table I. Grades 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, disc gels of the same size 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 numeral scores presented in Table II as suggested by Shimizu *et al.* (1981).

Table II. 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*:** Myofibrils are a part of muscle protein. Myfibrillar protein solubility indicates the denaturation rate of protein. Myofibrils were prepared from ordinary muscles immediately after excision according to Perry and Grey (1956) with slight

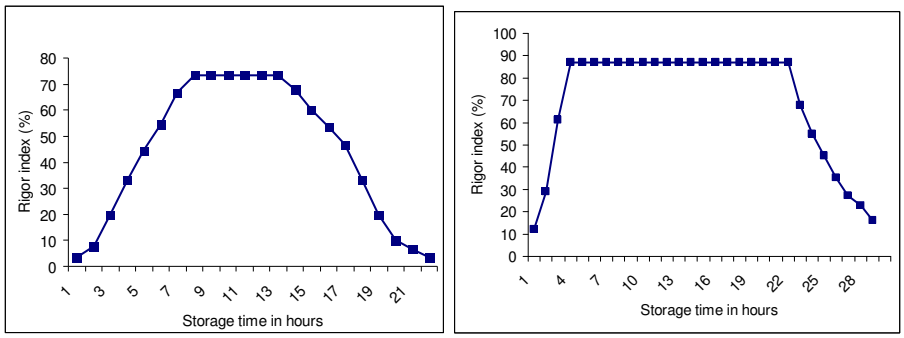
modifications. The muscle was chopped by a meat grinder and chilled minced muscle (50g) was homogenized for 1 min in 5 volumes of 39mM borate buffer (pH 7.1) containing 25Mm KCl and 0.1mM DTT. The homogenate was centrifuged for 15 min at 600×g. The residue obtained was again homogenized and centrifuged for 15 min at 600xg. The light colored upper layer of the residue consisting mainly of myofibril was recovered with small volume of 39mM borate buffer (pH 7.1) containing 0.1M KCl and 0.1mM DTT. The suspension was centrifuged for 15 min at 600xg to remove the supernatant. Myofibrils were diluted with 4 volume volumes of 39mM borate buffer (pH 7.1) containing 0.1M KCl and .1mM DTT and coarse materials removed by centrifugation at 400×g. The suspension was centrifuged for 15 min at 600×g to sediment myofibril. After the pellet was washed three times in the same was, myofibril were suspended with a desired volume of 39mM borate buffer (pH 7.1) containing 0.1M KCl to make a concentration of 10-15mg/ml.

***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).

Results and Discussion

***Changes in rigor index*:** The progress of rigor mortis was more rapid in fish stored at room temperature (RT) (28 to 32°C) than in ice (Fig. 1). In fish stored at RT, rigor started 1h after spiking and it reached to a maximum of 73.33%, within 7 h after death. The rigor period lasted for about 5 hand then post rigor started. The muscle fully relaxed after 22 h of storage. Rigor also started within 1 h of death for fish stored in ice. Gradual progress in rigor was observed with the lapse of storage time. Rigor reached to a maximum of 87.09% within 4 h; the rigor period continued for 22hand then muscle started to relax. The muscle relaxed up to 29 h of storage in ice without emitting any foul odor.

Time involved in each stages of the rigor development, duration and subsequent resolution of rigor-mortis depends on many factors such as species, size, and catching method, handling of the fish, temperature and the physical condition of the fish. Rigor-mortis is known to be dependent on temperature which influences the onset and the rate of progress of rigor. The results obtained in the present study are more or less similar to the findings of other researchers. Watabe *et al.* (1989) reported the progress of rigor up to 80% during storage at 10°C for mackerel and Hossain (1995) reported the progress of rigor up to 78% for mrigal (*Cirrhinus mrigala*) within 6 h after death at room temperature. In iced condition it reached to maximum value after about 10 h of death.



(a) **(b)**

Fig. 1. Changes in rigor- index (%) ofpangasius muscle; (a) room temperature (28 to 32°C) and (b) ice

***Changes in gel characteristics of one and two step heating gels*:** At the heating temperature of 20°C one step heating gels were too soft to measure the gel characteristics whereas for two step heating gels the breaking force (BF) was found 596.3±(3.28), folding test value "A" and teeth cutting test (TCT) 7. The highest BF was found 669.33±(0.67) in one step heating gel at 40°C. At this temperature folding test (FT) result showed grade "AA" indicating no crack visible when disc was folded into quarter and TCT also showed the highest score 9, indicating very strong gel. In case of two step heating gels highest BF was also obtained at 40°C.but the value was quite high (898.00±4.04) indicating the effect of further heating of the one step heating gels at high temperature for shorter period. Here, in two step heating gels also similar results for FT and TCT were observed at 40°C though the obtained results for these two parameters were comparatively higher than one step heating gels at 30° and 80°C temperatures.

In the manufacture of heat-processed fish products produced from washed fish mince or surimi, two-step heating has been considered tohave tremendous gel enhancing effects (Ishioroshi *et al.* 1982, Niwa *et al.* 1991, Nowsad *et al.*1999,Park *et al.* 1996). Salt-ground fish mince paste added with ingredients if incubated at 30-50°C for 1-2 h before final cooking at 90-100°C, the gel strength is increased by about 1.5 to 2 folds (Niwa *et al.* 1991). Reppond *et al.* (1995) noticed a 3-fold increment of gel strength in set and cooked products due to two-step heating treatment of Pacific herring. At low temperature around 30-50°C in fish, helical tail portion of myosin heavy chain unfolds and helps in intense cross-linking of proteins during high cooking temperature around 80-95°C (Ishioroshi *et al.*1982).

Table III. Changes in gel characters of one and two step heating gels prepared from pangasius muscle

Type of heating	Gel character-istics measured	Heating temperature(°C)						
		20	30	40	50	60	70	80
One step	BF	*	245.33± (1.45)	669.33± (0.67)	647.67± (1.45)	600.67± (1.45)	299.00± (1.00)	209.67± (0.88)
	FT	*	A	AA	AA	AA	A	A
	TCT	*	6	9	8	7	6	5
Two step	BF	596.3± (3.28)	631.00± (1.00)	898.00± (4.04)	607.33± (4.26)	541.67± (6.01)	349.33± (2.96)	303.67± (2.19)
	FT	A	AA	AA	AA	AA	A	A
	TCT	7	8	9	8	7	6	6

***** Gel too soft BF = Breaking force (Mean± SE); FT = Folding Test; TCT = Teeth Cutting Test

***Changes in gel characteristics at different stages of rigor*:** Different stages of rigor has effect on the characteristics of gel. At pre-rigor stage BF was 646.00 (±2.08g) in one step heating gel which decreased to 266.67 (±3.53g) at post rigor stage at RT. The grade of FT and score of TCT also decreased from "AA" and 8 to "A" and 4, respectively at the post rigor stage. Similarly, in ice, BF decreased from 660.33 (±1.45g) to 420.67 (±1.45g), grade of FT, "AA" to "A" and score of TCT, 6 to 4indicating that- at the later phase of rigor (at in rigor and post rigor stage) the gel strength decreases (Table IV).

Table IV. Changes in gel characteristics of one and two step heating gels prepared with pangasius (*P. hypophthalmus*) muscle at different stages of rigor

Types of sample	Heating time and temperature	Rigor stages	Measurement	Obtained results	
				One-step heating gel	Two-step heating gel
Room temperature	40°C; 120 min (one step heating) 40°C; 120 min + 80°C; 20 min (two step heating)	Pre-rigor	BF	646.00 ± (2.08)	994.33±(2.33)
			FT	AA	AA
			TCT	8	9
		In-rigor	BF	513.00 ± (1.73)	798.00±(1.73)
			FT	AA	AA
			TCT	6	8
		Post-rigor	BF	266.67 ± (3.53)	403.00±(1.15)
			FT	A	A
			TCT	4	4
Iced condition	40°C; 120 min (one step heating) 40°C; 120 min + 80°C; 20 min (two step heating)	Pre-rigor	BF	660.33 ± (1.45)	995.33± (1.45)
			FT	AA	AA
			TCT	6	9
		In-rigor	BF	605.33 ± (2.60)	906.33±(2.73)
			FT	AA	AA
			TCT	6	9
		Post-rigor	BF	420.67 ± (1.45)	552.67±(3.71)
			FT	A	A
			TCT	4	4

In case of two step heating gels, at pre rigor stage, BF was found 646.00 (±2.08g), which decreased to 266.67 (±3.53g) at post rigor stage while stored at RT. The grade of FT and score of TCT decreased from "AA" and 9 to "A" and 4, respectively. For the samples stored in ice, BF decreased from 660.33 (±1.45g) to 420.67 (±1.45g), grade of FT from "AA" to "A" and score of TCT value from 9 to 4, indicating that the progress of rigor though affect the gel characterizes but storage of fish at low temperature contribute to increase the gel strength (Table IV). Hossain *et al.* (2019)applying both one and two step heating methods observed decrease of BF of gels prepared with Silver jewfish, Ribbon fish and Bombay duck during 10 days of ice storage. For the same fishes both in one and two step heating, the initial folding test (FT) grade decreased from 'AA' to 'B' or 'A' and teeth cutting test (TCT) scores decreased from 8 to below 3. 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 in ice with is in accordance with the present study. Hossain *et al.* (2005) found a maximum breaking force from both washed and unwashed mince of queen fish (*Chorinemus lysan*) at the incubation temperature of 50°C. The gel-strength of both unwashed and washed meat paste gradually declined with lapse of storage period which is quite similar to

the findings of the present study. Varghese and Mathew (2017) observed that, the water holding capacity of the muscle *Anabas testudineus* decreased when the fish muscle entered in post rigor stage while cook loss and expressible water content were significantly increased. .The histochemical studies and textural profile analysis of fish muscle in their study proved that, the degradation of both collagen and myofibrillar protein induced the post-mortem tenderization and the resultant quality deterioration of iced stored *Anabas testudineus* fish. These results describe well the reason of decrease of breaking force and solubility of pangasius muscle with the progress of rigor in the present study during storage in ice.

Different stages of rigor have influence on the solubility of protein. Protein solubility of the samples stored at RT was found 86% at pre rigor stage of the fish. After that, solubility decreased continuously throughout in-rigor and post rigor stages (Fig. 2).While post rigor started, after 12 h of storage myofibrillar protein solubility decreased below 50%. The solubility decreased further with the lapse of storage period and after 24 h of storage the solubility declined below 30%. In the case of ice stored samples also the protein solubility was found 86% at the beginning of rigor. Afterward the myofibrillar protein solubility decreased continuously with the progress of storage period. The post rigor started after 22 h of ice storage and the protein solubility declined below 50%after 28 h of storage the solubility declined below 30%. Protein solubility (%) of three marine fish species (Silver jewfish, Ribbon fish and Bombay duck) were determined during ice storage by Hossain *et al.* (2019) which is quite similar to the results obtained in the present study. Seki *et al.* (1979) reported that the solubility of carp myofibrils decreased from 95% to 20% during ice storage of 2-3 weeks. Mehta et. al. (2014) observed, 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.

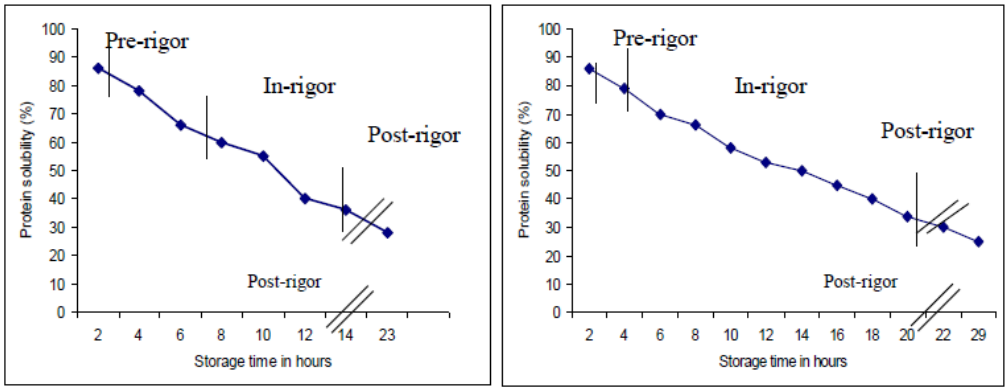


Fig. 2. Changes in protein solubility (%) of pangasius (*P. hypophthalmus*) muscle at different stages of rigor; (a) at room temperature (28 to 32°C) and (b) in ice.

Rigor-mortis in fish sample kept at both room temperature and iced condition started 1h after death. It was observed that rigor-index reached maximum 73.33% within 7h after death at room temperature and 87.09% within 3h after death in ice. The breaking force of the resulting gels was highest at 40°C at an incubation of 120 min at pre-rigor stage both at room temperature and in ice. Immediately after catch myofibrillar protein solubility was 86%. The solubility decreased continuously with the progress of time and reached to 30% after 24 h of storage at room temperature and 28 h in ice. From the obtained results this study it can be concluded that ice storage of muscle extend the in rigor stage of pangasius muscle from 5 h to 18 h which might contribute to slow down the degradation process of protein.

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