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Physicochemical Changes in Thai Pangas (*Pangasius sutchi*) Muscle During Ice-storage in an Insulated Box

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Abstract: Studies were conducted to evaluate the post-mortem changes in Thai pangas (*Pangasius sutchi*) during 25 days of ice storage. Rigor mortis progress was measured as parameters of rigor tension. Rigor begins within 1-1.5 h after death in ice and increases gradually with the lapse of storage period. Rigor-index reached maximum of 67.46% in 6 h and did not attain full rigor (100%) and then started relaxation. The organoleptic quality of Thai pangas (*Pangasius sutchi*) during ice storage was assessed on the basis of the sensory evaluation such as appearance, odour, texture and taste. The initial pH value was around 7.0, which gradually decreased to 5.98 during 14 days of storage period and then increase until the experiment was terminated. The extractability of myofibrillar protein gradually decreased from 88.37 to 52.87% at the end of the 20 days of ice storage. The initial TVB-N value and peroxide value were 1.37 mg/100 g and 1.1 meq kg^{-1} of oil, respectively which continuously increased with the lapse of storage period. At the end of 20 days of storage TVB-N value and peroxide value were 24.25 mg/100 g and 16.64 meq kg^{-1} of oil, respectively. There is a large fall of Ca^{+2}-ATPase activity both in presence of 0.1 and 0.5 M KCl during storage period. The overall results indicated that Thai pangas fish was found in acceptable conditions for 20 days of ice storage in an insulated box.

Key words: Post-mortem, rigor-mortis, ATPase activity, ice-storage, Thai pangas

INTRODUCTION

Fresh fish is highly susceptible to the spoilage from post mortem autolysis and microbial growth. The high ambient temperature of Bangladesh favours the spoilage rate of fish. There are several methods to preserve fresh fish, however, icing is a common method for short-term preservation.

Thai pangas (*Pangasius sutchi*) is very popular fish in Bangladesh due to its delicious taste, lucrative size, high market demand and fast growth rate, disease resistance and ability to tolerate poor quality condition. There is little or no information on the keeping quality of this fishes under various storage conditions. Sufficient knowledge on the keeping quality of the various commercially important fish species is important to reduce the post-harvest losses and promote the marketing of fish.

During icing or chilling storage of fish, chemical changes are known to take place[1,2]. 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. The state of rigor in association with other biochemical changes influences the meat quality in fish and higher animals[3]. Sensory evaluation is an important method for the assessment of freshness and quality and commonly used in fish inspection services[4]. The biochemical parameters TVB-N (mg/100 g) and peroxide value are good indicator to determine the quality of fresh fish. During cold storage condition, denaturation of myofibrillar proteins is widely studied by measuring Ca^{2+}-ATPase activity[5-7]. Although much literature is available pertaining to the effect of ice storage on the biochemical changes in fresh fish, however data on the changes occurring in the functional properties of Thai pangas muscle protein during ice storage is meagre. The broad objective of this study was to develop improve methods for post-harvest handling so as to ensure the maximum and most profitable utilization of the commercial Thai pangas (*Pangasius sutchi*) fishes. In this study, the physical, biochemical changes of Thai pangas (*Pangasius sutchi*) that leads to loss of freshness during ice storage were studied.

MATERIALS AND METHODS

Design of experiment: The live Thai pangas (*Pangasius sutchi*) were collected form the local retail market of Bangladesh Agricultural University Campus and were

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brought to the laboratory of Fisheries Technology Department, Faculty of Fisheries, BAU, Mymensingh and stored in ice in an insulated box (fish: ice ratio 1:1) in a lot. The samples were randomly taken out from storage container at selected time intervals (0, 5, 10, 15, 20 and 25 days) and used for the study of quality by determining organoleptic assessment.

Rigor-index: Rigor-index of the fish was measured according to Bito *et al.*[8]. Briefly, the fish was placed on a table with half of its body (tail part) kept out of the table. At selected time intervals, rigor-index was calculated by the following equation:

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

Where, D_0 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.

Organoleptic assessment: Sensory evaluation was carried out according to the guidelines described by EC freshness grade for fishery products[9], which is shown in Table 1 and 2.

Analytical Methods:

Measurement of muscle pH: pH value of muscle homogenate prepared by blending 10 g meat with 40 mL chilled water was measured by pH meter (Corning Model 250).

Chemical analysis: Peroxide value was described by Egan *et al.*[10] and adopted from Wood and Aurand[11]. Total volatile basic nitrogen (TVB-N) levels were performed in perchloric acid extracts, according to the method of Pearson[12].

Preparation of myofibrils: Myofibrils were prepared from ordinary muscle immediately after excision according to Perry and Grey[13] with slight modification. The muscle was chopped by meat grinder and chilled minced muscle (50 g) was homogenized for 1 min in 5 volumes of 39 mM borate buffer (pH 7.1) containing 25 mM KCl and 0.1 mM DTT. The homogenate was centrifuged for 15 min at 600×g. The residue obtained was again homogenized and centrifuged for 15 min. The light colored upper layer of residue consisting mainly of myofibril was recovered with small volume of 39 mM DTT. The suspension was centrifuged for 15 min to remove the supernatant. Myofibrils were diluted with 4 volumes of 39 M borate buffer (pH 7.1) containing 0.1 M KCl and 0.1 mM DTT and coarse materials were removed by centrifugation again for 15 min at 600×g to sediment myofibrils. After the pellet was washed three times in the same way, myofibrils were suspended with a desired volume of 39 mM borate buffer (pH 7.1) containing 0.1 M KCl to make a concentration of 10-15 mg mL^{-1}.

Myofibrillar protein solubility: Two mL of myofibrillar suspensions (5 mg mL^{-1}) were homogenized with 2 mL of 1 M KCl plus 100 mM phosphate buffer (pH 7.0) using a homogeniger. The homogenate was allowed to stand at refrigerated temperature (4°C) for overnight. The suspension was centrifuged for 30 min at 400×g in cold condition. The protein in supernatant was determined by the Biuret method[14].

Table 1: 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

Characteristics of whole fish	Defect characteristics	Defect points	Grade
Odour of neck when broken	a) Natural odour	2	Acceptable
	b) Faint or sour odour	5	Reject
Odour of gills	a) Natural odour	1	Excellent
	b) Faint sour odour	2	Acceptable
	c) Slight moderate sour odour	3	Acceptable
	d) Moderate to strong sour odour	5	Reject
Colour 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 colour covered with mucus	3	Acceptable
	d) Bleached; thick yellow slime	5	Reject
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
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
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 colour	5	Reject
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

Assay of specific ATPase activity: The reaction mixture for the Ca^{2+}-ATPase assay contained 25 mM Tris, 5 mM $CaCl_2$ 0.1 M KCl or 0.5 M KCl and 0.25 mg myofibril/mL. The ATPase activity was measured at 25°C for 6 min. After preparation of the reaction mixture, an appropriate quantity of myofibril suspension was pipetted to the reaction mixture followed by 2 min pre-incubation. The reaction was started by the addition of 1 mM ATP and 2 mL portion of the reaction mixture was withdrawn at different time intervals. The reaction was terminated by adding 1 mL of 15% trichloroacetic acid. The supernatant obtained by 5 min centrifugation at 3000×g was analyzed for the liberation of inorganic phosphate (Pi) according to the method described by Fiske and Subbarow[15].

RESULTS AND DISCUSSION

Organoleptic assessment: The quality of fishes was graded using the score from 1 to 5. 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 (Table 3). The changes in quality of chilled fish during storage were assessed by daily organolelptic examination. On the basis of the scores the fishes were found in acceptable conditions for 20 days in ice storage before it becomes inedible. The changes occurred in organoleptic quality during the storage period and this can roughly be divided into five phases corresponding to periods of 0 to 5, 5 to 10, 10 to 15, 15 to 20 and 20-25 days in ice. In phase 1 the fishes were found excellent with grade A. At this stage the fishes had the characteristics of excellent quality. In phase 2 there was little deterioration apart from some slight loss of natural flavour. At this stage there was little loss of the characteristic odour and the flesh was neutral but had no off-flavour. In phase 3 there were signs of early spoilage with sour of flavour. In the beginning of this phase the off-flavour was slightly sour, sickly sweet, fruity of like dried fish but the fishes were judged as acceptable quality. In phase 4, the fish begins to taste stale, its appearance and texture begins to show obvious signs of spoilage and the gills and belly cavity had an unpleasant smell. In phase 5, the fish become putrid by all of the characteristics.

The available reports suggest that the quality of the fishes varies considerably depending on species and storage conditions. Kamal *et al.*[16] reported that the Hilsa fish (*Hilsa ilisha*) transported immediately after catch in the insulated box in ice remained in acceptable condition up to 18 days in ice storage. Rodriguez *et al.*[17] reported that the storage of European hake specimens at subzero temperature allowed a better quality up to the 19 days of

Table 3: Changes in organoleptic qualities of Thai pangas (*Pangasius sutchi*) ice storage in an insulated box

Days of storage	Defect points	Grade	Overall qualities
0	1.25	A	Excellent
5	1.90	A	Excellent
10	2.20	B	Acceptable
15	3.50	B	Acceptable
20	4.50	B	In the limit acceptable
25	5.00	C	Rejected

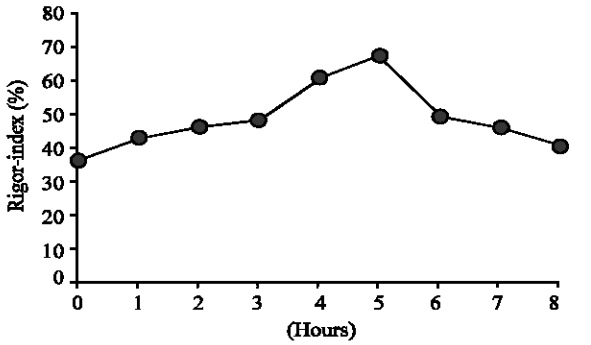

Fig. 1: Changes in rigor-mortis-index (%) of Thai pangus (*Pangasius suchi*) during ice storage

ice storage. White sardines were reported to be acceptable for human consumption up to 9 days in ice storage[18]. Bandyopadhyay *et al.*[19] suggested that *Catla* and *Labeo fimbriatus* could be kept in ice for 18 days before it becomes inedible.

Progress of rigor-mortis: To study the post-mortem quality in Thai pangas (*P. sutchi*) during ice storage, the earliest changes were observed by determining the progress of rigor-mortis in fish. Rigor-index of Thai pangas (*Pangasius sucthi*) stored in ice was shown in Fig. 1. Rigor started 1 h after spiking and it reached up to 61.11% in 5 h and it reached a maximum of 67.46% in 6 h after death and then started relaxation. The rigor relaxed to only 40.47% after 8 h and at this stage there was no apparent change in appearance and flavour. Among the many factors, rigor mortis is known to be dependant on temperature, which influence the onset and the rate of progress of rigor. It is widely believed that lower temperature delays the rigor-mortis progress. In the present study, Thai pangas (*Pangasius sucthi*) samples did not reach full rigor state. However, several fishes such as tilapia (*Tilapia mossambica)* are reported to enter onset of *rigor mortis* after 2 h, reach full *rigor mortis* after 7½ h and resolve after 11½ h[20]. In the same study freshwater Mrigal (*Cirrhina mrigala*) were shown to enter onset of *rigor mortis* after 5½ h, reach full *rigor mortis* after 13 h and resolve after 56 h when kept in crushed ice at 2°C. It is widely believed that lower temperature delays the rigor-mortis progress. There appears to be a difference

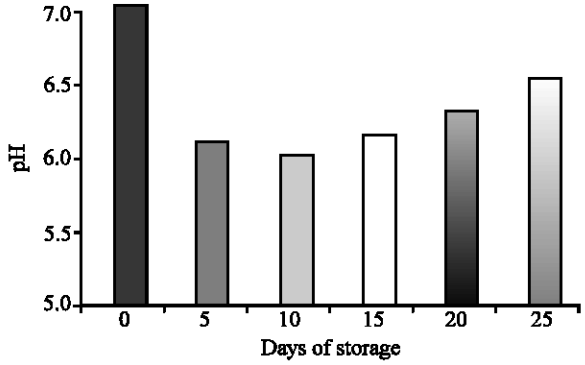

Fig. 2: Changes in pH of Thai pangus (*Pangasius suchi*) during ice storage

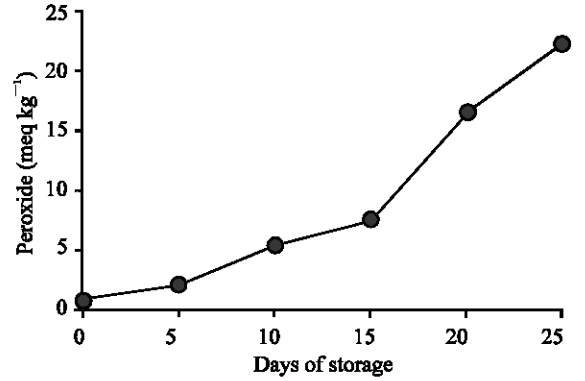

Fig. 3: Changes in peroxide value of Thai pangus (*Pangasius suchi*) during ice storage

in *rigor mortis* onset between temperate and tropical fish species. Temperate freshwater fish are reported to go faster into *rigor* with increasing temperatures[21]. Abe and Okuma[22] found full *rigor mortis* in carp after 24 h on ice when acclimatised to 30°C.

Changes in muscle pH: The pH of Fish muscle immediately after death was around 7.0, which decreased gradually to 5.98 after 14 days in ice (Fig. 2). Then it increased gradually up to 6.50 after 25 days when the fish were organoleptically unacceptable. The muscle pH of pangas fish immediately after death was close to neutral. Pacheco-Aguilar *et al.*[23] reported that live sardine muscle has a pH 7.2. After death, the muscle pH decreases to 6.8 after 2 h, to 6.2 after 8 h and to 5.8 after 24 h[24]. El Marrakchi *et al.*[25] found that the pH value of sardine (*Sardina pilchardus*) stored in ice was 5.8 at day 0, 6.36 at day 9 and 6.57 at day 18. At present study, we also found that after 15 days of ice storage pH gradually increased due to formation of basic compounds and finally reached up to 6.50 after 25 days when the fish were organoleptically unacceptable.

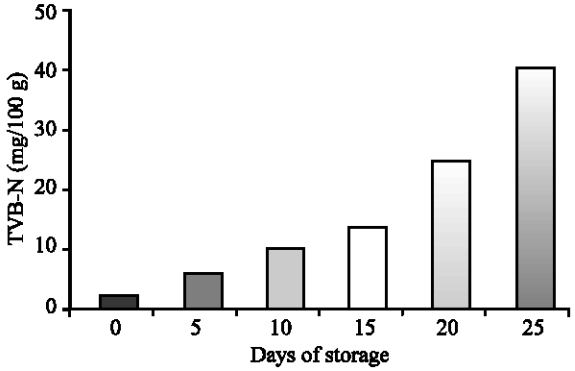

Fig. 4: Changes in TVB-N of Thai pangus (*Pangasius suchi*) during ice storage

The correlation between pH and organoleptic scores in this study suggests that pH can be used as a reliable index of quality.

Changes in peroxide value and TVB-N: Lipid oxidation limits the shelf life of oily fish. In the present study, the changes in peroxide value of Thai pangus (*Pangasius sutchi*) during ice storage were initially very low (Fig. 3). The value was 1.1 at day 0, 2.2 at day 5 and 5.46 at day 10 indicating a reduced level of oxidation in this fish. There are several reports on initial peroxide value for small pelagic fish, of 10 meq kg^{-1} lipid for sardine, just after catch[26] and from 0.8 to 1.2 for herring (*Clupea harengus*)[27]. At the end of 20 days of storage, the peroxide value was 16.64 meq kg^{-1} of oil, which were within recommended value of 10 to 20 meq kg^{-1} of oil. According to Connell[28] the recommended value of peroxide for fresh finfish were 10-20 meq kg^{-1} of oil. The value above 20, the fish was found to be emit smell and taste rancid. The peroxides were presumed to be eventually further oxidized to aldehydes and ketones which had a very disagreeable fishy or rancid odour and taste. At the end of the 25 days of storage, the peroxide value was 22.18 meq kg^{-1} of oil that exceeded the recommended value.

The initial TVB-N value was 1.37 mg/100 g; at the end of 20th day of ice storage TVB-N value increased 24.25 mg/100 g, which is within the range of recommended value of 25 to 30 mg TVB-N/100 g for fresh fish (Fig. 4). The available report suggests that the upper limit of 30 mg TVB-N/100 g is considered for finfish acceptability[28]. Fatima and Qadri[29], also reported that a level of 30 mg TVB-N of muscle has been considered the upper limit above which some fishery products are considered as spoiled and unfit for human consumption. In the present study, data shows at the end of 25th day, the TVB-N value was 40.1 mg/100 g exceeded the recommended

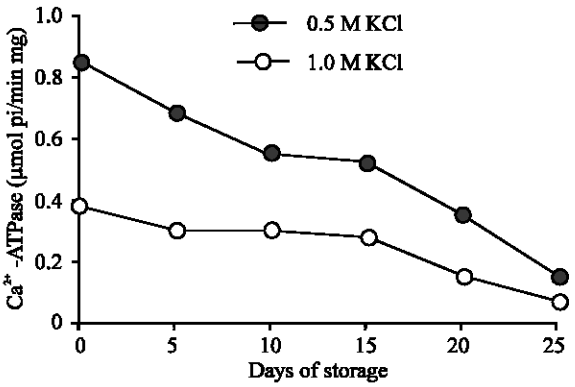

Fig. 5: Remaining ATPase activity of myofibrillar protein of thai pangus (*Pangasius suchi*) during ice storage

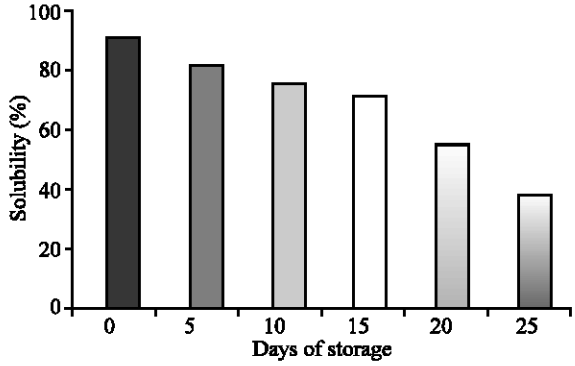

Fig. 6: Changes in protein solubility of Thai pangus (*Pangasius suchi*) during ice storage

value. The amount of TVB in fish muscle increases as spoilage progresses. TVB-N is a term that includes the measurement of trimethylamine, dimethylamine, ammonia and other compounds. Increase in TVB-N with the lapse of storage may be attributed 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[30]. 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. In the present study, at the end of 20th day the TVB-N value were 24.25 mg/100 g, which is within the range of acceptable value.

Ca^{2+}-ATPase activity: To evaluate the denaturation profile more precisely, changes in remaining ATPase activity of myofibrillar protein of Thai-pangas during 25 days of ice storage at 0°C was examined. Ca^{2+}-ATPase both in presence of 0.1 M KCl and 0.5 M KCl gradually declined with the lapse of storage period (Fig. 5). The initial Ca^{2+}-ATPase activity in presence of 0.1 M KCl was 0.849 µmol pi/min mg which gradually declined during the storage period and at the end of 25 days of storage, the ATPase activity declined to 0.15 µmol pi/min mg. On the other hand the initial Ca^{2+}-ATPase activities in presence of 0.5M KCL was 0.38 µmol pi/min mg which gradually decreased to 0.07 µmol pi/min mg at the end of the 25 days of storage. There was a large fall of myofibrillar Ca^{2+}-ATPase activities during ice storage, indicating denaturation of myofibrillar protein. Seki *et al.*[31] reported that EDTA-ATPase activity of carp myofibrillar proteins decreased considerably during 16 days of ice storage. Seki *et al.*[32] also reported that sardine myofibrillar EDTA-ATPase activity lost about 70% activity in one-day storage in ice. Yasmin *et al.*[33] studied the changes in ATPase activity of Indian major caprs during ice storage and reported that myfibrillar Ca^{2+}-ATPase activities of catla in the presence of 0.1 M KCl were remained unchanged up to the first 5 days of storage and then gradually declined with the lapse of storage period and by the end of 20 days of storage the activity declined to about 40% of its initial level. On the other hand, there was little change of Ca^{2+}-ATPase activities of myofibrillar protein in the presence of 0.5M KCl throughout the storage period. In a similar study, they also reported that the initial Ca^{2+}-ATPase activities of mrigal fish in presence of 0.1 M KCl and 0.5 M KCl were 0.44 and 0.11 µmol-pi/min mg, respectively which declined rapidly during 10 days of storage and after 20 days of storage, the activity decreased to 60 and 20%, respectively. The results reported from other fishes were found to be in agreement with the present study, indicating that the ice-storage have a marked denaturing affect on myfibrillar protein. Some researchers[34,35] stated that an increase in acidity during post-mortem changes reduce activity of myofibrillar ATPase activity of at saturation concentration of Ca^{2+}.

Changes in protein solubility: Solubility of myofibrillar protein gradually decreased during the storage period. The solubility of the myofibrillar protein immediately after death was 88.37%, which decreased gradually to 36.43% at the end of 25 days of storage when the fish were organoleptically unacceptable (Fig. 6). Seki *et al.*[31] reported that solubility of carp myofibril decreased from 95 to 20% during ice storage within 2-3 weeks. Losses of protein solubility and water holding capacity in the fish muscle have been attributed to cross-linking between adjacent polypeptide by formaldehyde derived from trimethylamine oxide[36]. Jiang *et al.*[37] reported that loss in solubility in milkfish during storage was due to the aggregates formation by disulfide, hydrozen and hydrophobic bonds. Recently, Benjakul *et al.*[38] found that solubility in muscle protein of croaker, lizardfish,

threadfin bream and bigeye snapper decreased continuously during prolonged storage. The result from the present study indicated that the decrease in solubility of pangas muscle is due to the aggregation as well as denaturation of proteins caused by ice storage.

The overall quality of the present study indicated that organoleptically Thai pangas (*P. sutchi*) was found in acceptable condition up to 20 days in ice storage condition in an insulated box. The fish muscle pH immediately after killed was around 7.0 which decreased to 5.98 during the first 14 days and then it increased gradually at the end of 25 days of ice storage. Rigor-mortis in ice-stored Thai pangas (*P. sutchi*) after spiking started in 1 hr and it reached maximum 67.46% rigor within 6 h. TVB-N and Peroxide values increased with the lapse of storage period but the values were within the acceptable range up to 20 days of storage. There was a large fall of Ca^{2+}-ATPase activity both in presence of 0.1 M KCl and 0.5 KCl during storage period. The present study suggested that Thai pangas has an excellent and long shelf life up to the 20 days of ice storage in an insulated box.

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