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Characterization of collagenolytic enzyme from the hepatopancreas of blue crab

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Abstract Hepatopancreas is an important source of collagenolytic enzyme, which is the enzyme responsible for muscle softening and gapping in crustaceans. The hepatopancreas collagenase of blue crab and monitor changes in total proteolytic and collagenolytic activities of the blue crab hepatopancreas during iced storage was characterized. The optimum activity of hepatopancreas collagenase was found at pH 9.0 and 40 ℃. The collagenase showed high stability over a pH range of 7.0-10.0 for up to 8 hours with over 70% activity remaining. The collagenolytic enzyme was stable at temperatures below 50 ℃ for 8 hours with over 50% of activity remaining. Moreover, the collagenolytic activity constantly decreased with increased in concentration of NaCl (0-20% w/v). After the hepatopancreas had been stored in ice for 5 days, its pH value slightly altered, but the pH stayed in the 6.7-7.5 range. The total proteolytic and collagenolytic activities from hepatopancreas exhibited a similar pattern. Both activities were increased their highest levels within 2 days of storage. Thereafter, the activities were constantly decreased until the end of storage.

Keywords: Collagen, Crustacean, Hepatopancreas, Iced storage, Muscle softening

Introduction

Blue crabs, one of the most popular kinds of seafood in Thailand, are highly perishable. The texture softening is an important problem during storage and transportation because the crab muscle normally becomes mushy soon after death. However, Balasaraswathy *et al*. (2008) observed that blue crabs could be kept in ice for up to 10 days, during which time the crab meat remained at an acceptable quality. The histamine level, which is a reliable quality index for crab, was reduced by storing in ice and showed the highest levels after 72 h (Xu *et al*., 2009). Biogenic amines forming bacteria and other susceptible perishing factors related to the formation of biogenic amines were inhibited by storing the crab *Portunus pelagicus* at a low temperature (Arulkumar *et al*., 2017).

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Moreover, storage in ice is a simple and cheap method used for blue crab storage and domestic transportation by local fishermen. However, there is little information available on the appropriate time of iced storage, which is mediated by the mechanisms of endogenous enzymes and other spoilage-related factors.

As it is the case with other seafoods, the deterioration of blue crab is associated with proteolytic degradation by endogenous and bacterial enzymes that cause loss of freshness and quality (Sriket, 2014). The process of autolysis is the initial cause of proteolytic enzyme release from digestive organs, especially from the hepatopancreas in crustaceans (Pushparajan *et al*., 2013; Singh and Benjakul, 2018; Singh and Singh, 2020). The crustacean hepatopancreas is a major digestive gland that plays a role in the synthesis and secretion of digestive enzymes, food absorption, nutrient storage, and vitellogenin and sex steroid hormone production (Ramadevi *et al*., 1990; Ferre *et al*., 2012; Wang *et al*., 2014). Among the digestive enzymes, collagenolytic enzymes are responsible for collagen degradation, which affects the softening and gapping of muscles (Kubota *et al*., 2001, 2003; Brauer *et al*., 2003; Hernandez-Herreroa *et al*., 2003; Ghaly *et al*., 2010). Collagenolytic enzymes can be divided into two types: metallocollagenases and serine collagenases (Park *et al*., 2002; Daboor *et al*., 2010). Metallocollagenases, which are matrix metalloproteinases, are zinc-containing enzymes and act at a neutral pH (Carmeli *et al*., 2004; Doboor *et al*., 2010). On the other hand, serine collagenases are considered a serine protease. Their catalytic sites contain a serine residue. Serine collagenases are generally active at neutral and alkaline pH. They are probably involved in food digestion (Kim *et al*., 2002; Doboor *et al*., 2010; Sriket, 2014). A better understanding of the biochemical characteristics of endogenous collagenolytic enzymes should lead to the development of ways to extend the iced storage duration of blue crabs during distribution. Therefore, the research was undertaken to characterize the biochemical properties of hepatopancreas collagenase from the blue crabs. The change of hepatopancreas collagenolytic activity of blue crab was monitored during the iced storage period.

Materials and methods

Sample preparation

The live blue crabs were obtained from the port of Pathiu, a district of Chumphon province in Thailand. They were kept in seawater and transported to the laboratory at King Mongkut's Institute of Technology Ladkrabang Prince of Chumphon Campus within 15 min. The crabs were knocked unconscious in the

ice, and the hepatopancreas was then manually collected and stored at -70℃ before crude enzyme extraction took place.

Crude enzyme extraction

The crude enzyme from hepatopancreas was extracted according to the method of Kim *et al*. (2002). Briefly, the hepatopancreas was homogenized in 50 mM Tris-HCl (pH 7.5) in the ratio of 1:3 (w/v). The homogenate was centrifuged at 10,000xg, and 4℃ for 60 min. The supernatant was collected and defined as "crude enzyme extract".

Optimum pH and stability of collagenolytic activity from hepatopancreas extract

The collagenolytic activity was carried out using a modified method from Kim *et al*. (2002) and Brauer *et al*. (2003), with 5 mg/ml collagen used as a substrate. The collagenolytic activity from the hepatopancreas extract was determined at 37℃, over a pH range of 4.0-11.0 (100 mM Citrate buffer for pH 4.0-6.0, 100 mM Tris-HCl for pH 7.0-9.0, 100 mM Glycine-NaOH for pH 10.0-11.0). The crude enzyme (0.1 ml) was incubated with 1 ml of 5 mg/ml collagen at 37℃. After 30 min of incubation, the reaction was terminated by the addition of 0.2 ml of 50% (w/v) trichloroacetic acid and allowed to sit at room temperature for 10 min. The mixture was then centrifuged at 1,800xg, 4℃ for 20 min. The supernatant (0.2 ml) was mixed with 1 ml of 1% (w/v) ninhydrin solution and then boiled for 20 min. The mixture was cooled at room temperature. Thereafter, 5 ml of 50% (v/v) ethanol was added to the mixture, and the absorbance was measured at 570 nm. One unit of collagenolytic enzyme was defined as the amount of enzyme releasing 1 μmol L-leucine per minute. The pH stability of collagenolytic activity was investigated by pre-incubation of the crude enzyme at various pH (4.0-11.0) using several buffers as previously described, at 4℃. Samples were taken at 1, 2, 4, and 8 h after incubation. The residual activity was determined under standard conditions.

Optimum temperature and stability of collagenolytic activity from hepatopancreas extract

The collagenolytic activity of crude enzyme from hepatopancreas was analyzed at different temperatures (0, 4, 30, 37, 40, 50, 60, 70, and 80℃). The collagenolytic activity was assayed as previously described at pH 7.5. The thermal stability of the collagenolytic activity of hepatopancreas extract

was determined by keeping the crude enzyme at various temperatures (0, 4, 30, 40, 50, 60, and 70 ℃) for 1, 2, 4, and 8 h. The residual activity was assayed under standard conditions.

Effect of NaCl on collagenolytic activity

The influence of NaCl on the collagenolytic activity of hepatopancreas extract was examined by adding NaCl to the reaction mixture to obtain a final concentration of 0-20% (w/v). The collagenolytic activity was determined under standard conditions.

Change of total proteolytic and collagenolytic activities of blue crab hepatopancreas during iced storage

All of the crabs obtained from the port were stored in polystyrene boxes with ice using a crab/ice ratio of 1:2 (w/w) (Sriket *et al*., 2011b). To maintain the ratio, the liquid from the melting ice was drained and replaced with an equal amount of ice. The box was kept in a laboratory room that had an uncontrolled temperature (26-35 ℃). Samples were taken every day for up to 5 days. The hepatopancreas was isolated for analysis of total proteolytic and collagenolytic activities. The experiment was performed in triplicates. The total protease activity was measured using 1% (w/v) casein as a substrate according to a modified method from Mehrotra *et al*. (1999) and Chaijaroen and Thongruang (2016). The hepatopancreas extract (1 ml) was mixed with 1% casein (1 ml) and incubated at 37 ℃ for 15 min. The enzymatic reaction was terminated by adding 2 ml of 20% (w/v) trichloroacetic acid (TCA) and the mixture was allowed to stand on ice for 60 min and then centrifuged at 10,000xg for 10 min at 4 ℃. The supernatant was collected and measured at 280 nm. One unit of protease activity was defined as the amount of enzyme releasing 1 μmol of tyrosine/min. The collagenolytic activity was analyzed under standard conditions as mentioned above.

Results

Optimum pH and stability of hepatopancreas collagenolytic activity

The effect of pH on the collagenolytic activity of hepatopancreas extract from blue crabs was investigated over the pH range of 4.0-11.0 as shown in Figure 1A. The highest activity of collagenase was observed at pH 9.0. The activities suddenly decreased when the pH was increased to above pH

9.0. Simultaneously, the collagenolytic activity consistently decreased when the pH level was below 9.0.

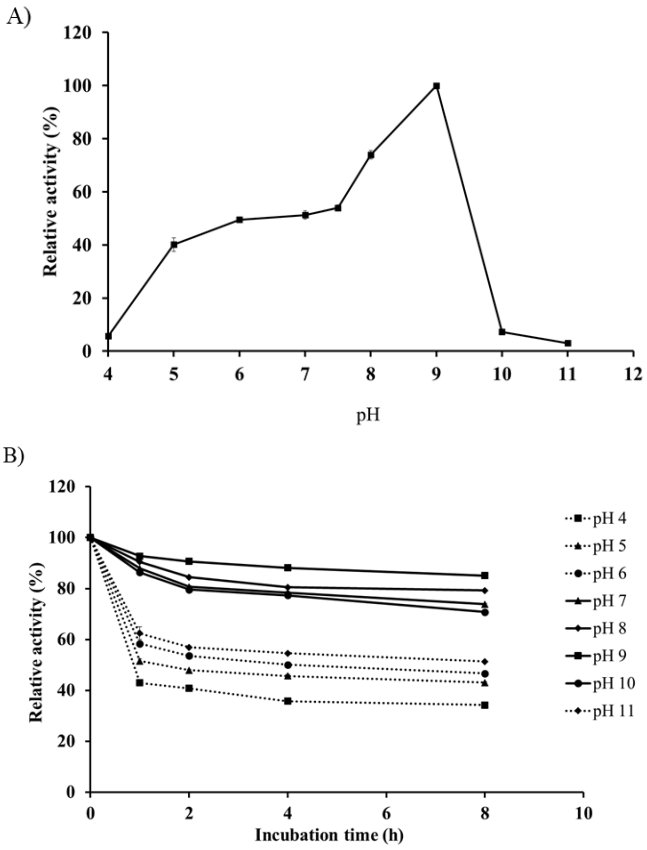

Figure 1. (A) pH profiles and (B) stability of collagenolytic enzyme from blue crab hepatopancreas extract (Thongpradub *et al*.)

The hepatopancreas extract was kept in various buffers with the pH values varying between 4.0-11.0 at 4 ℃ for 1, 2, 4, and 8 h. The hepatopancreas collagenolytic activity from blue crab showed the highest stability at pH 9.0 for up to 8 h, remaining at over 80% of its initial activity (Figure 1B). The collagenase also was stable for up to 8 h at pH 7.0, 8.0, and 10.0, maintaining activity level over 70% of its initial activity. At the same time, the remaining activity of the hepatopancreas collagenase was lower than 60% of initial after 1 h of incubation at the acidic pH and pH 11.0. However, the collagenase stability over all pH ranges gradually decreased after a longer incubation period. Furthermore, this result suggested the hepatopancreas collagenase from blue crabs was also stable in ice at pH level of 6.5-8.5.

Optimum temperature and stability of hepatopancreas collagenolytic activity

The influence of temperature on hepatopancreas collagenolytic activity from blue crab is shown in Figure 2A. The hepatopancreas extract demonstrated the highest collagenolytic activity at 40 ℃. The crude enzyme showed activity above 80% at 37, 40, and 50 ℃. The activity of hepatopancreas collagenase decreased with increasing temperature (60-80 ℃) with the remaining activities of approximately 75%, 72%, and 36%, respectively. Moreover, the collagenolytic activity suddenly decreased at low temperatures (0 and 4 ℃) with remaining activities of 2% and 25%, respectively.

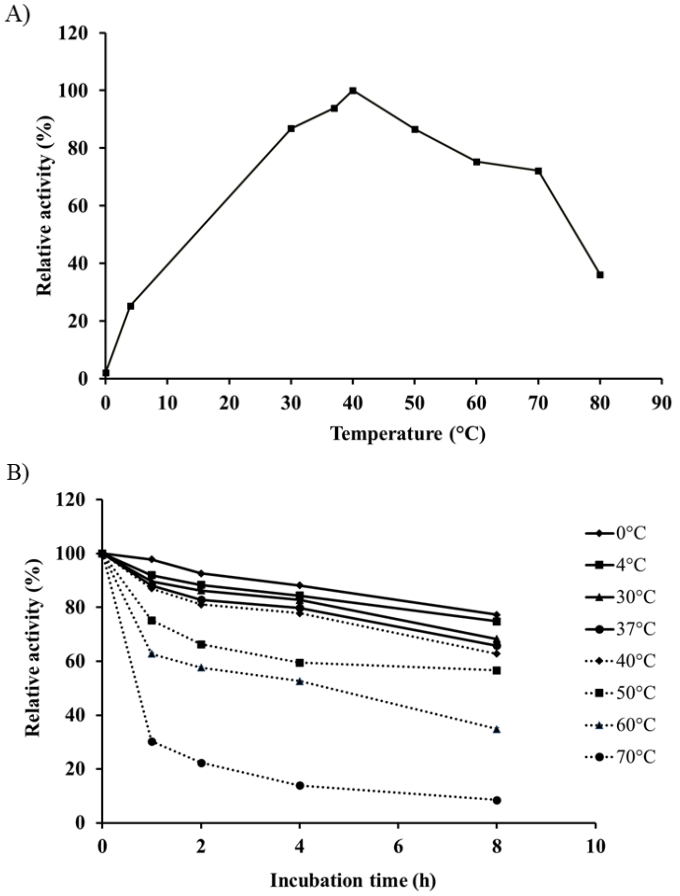

Figure 2. (A) Thermal profiles and (B) stability of collagenolytic enzyme from blue crab hepatopancreas extract (Thongpradub *et al*.)

The thermal stability profile is demonstrated in Figure 2B. The hepatopancreas collagenase from blue crabs was highly stable at low temperatures (0 and 4 ℃) for up to 8 h, and maintained 75% of its initial activity. The crude extract was quite stable at 30-50 ℃ with its activity remaining above 50% of its original activity after 8 h incubation. The collagenolytic activity was unstable at 70 ℃ after incubation for 1 h, with lower than 30% of its original activity remaining. This result revealed that the hepatopancreas collagenase was highly stable at the chilling temperature used for blue crab local distribution (0 and 4 ℃).

Effect of NaCl concentration on collagenolytic activity

The influence of NaCl on the hepatopancreas collagenolytic activity is presented in Figure 3. The activity of the crude enzyme continuously decreased with increment of NaCl concentration up to 20%. The remaining activities of 73%, 64%, and 51% were found in the presence of 5%, 10%, and 15% NaCl, respectively. The collagenolytic activity was lowest with the addition of 20% NaCl.

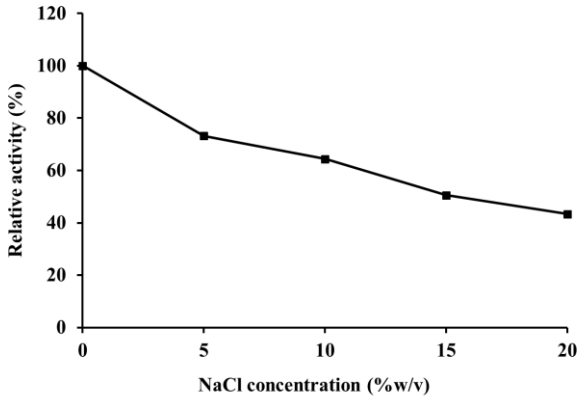

Figure 3. Influence of NaCl concentration on the activity of collagenolytic enzyme from hepatopancreas (Thongpradub *et al*.)

Effect of iced storage on hepatopancreas pH, total proteolytic and collagenolytic activities

The pH level of hepatopancreas from blue crabs kept in ice for 5 days is demonstrated in Figure 4A. The initial pH of the hepatopancreas was 6.7±0.1. A gentle increase in the pH level was observed within the first 3 days of iced storage, which increased to 7.5±0.1. Thereafter, a slight change in the

pH values was found when the storage time increased until at the end of 5 days (pH 7.3±0.1). A gradual increment of pH value in crab muscle *(P. pelagicus)* was observed during iced storage for 96 h.

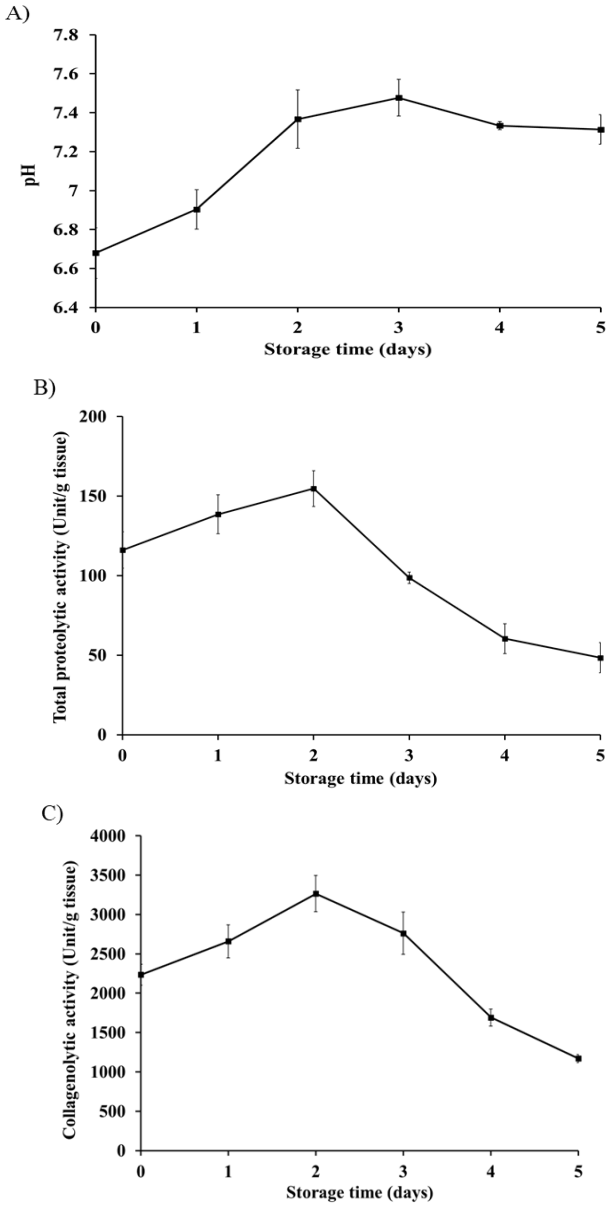

Figure 4. Change in (A) pH, (B) total proteolytic and (C) collagenolytic activities of hepatopancreas from blue crabs during iced storage for 5 days (Thongpradub *et al*.)

The changes in the total proteolytic and collagenolytic activities of blue crab hepatopancreas during iced storage were investigated for 5 days, and the results are shown in Figure 4B-C. The initial activity of total protease (day 0) was 116.15±11.40 unit/g tissue. The activity gradually increased to its maximum level at 2 days after storage showing 154.78±11.19 unit/g tissue, and then continuously decreased until the end of iced storage period (48.49±9.49 unit/g tissue). The collagenolytic activity exhibited a similar trend to the total proteolytic activity. The activity of collagenolytic enzyme increased from 2235.65±134.13 unit/g tissue to the highest level (3265.48±231.24 unit/g tissue) after storage for 2 days. Then, decreasing activity was observed until the end of the iced storage period (1169.60±52.76 unit/g tissue).

Discussion

In this work, crude extract from blue crab hepatopancreas was investigated for collagenolytic activity, and the biochemical properties of hepatopancreas collagenase were illustrated. The hepatopancreas collagenolytic activity was also monitored during the ice storage period. The optimum pH (pH 9.0) of hepatopancreas collagenase from blue crab was similar to that of collagenolytic proteinase from the intestines of Atlantic cod (*Gadus morhua*), which had an optimum pH between 8.0 and 9.5 (Kristjansson *et al*., 1995). The result was slightly different to those found for collagenases from the internal organs of the filefish, *Novoden modestrus* (Kim *et al*., 2002), the skeletal muscles of winter flounder, *Pseudopleuronectes americanus* (Teruel and Simpson, 1995), the viscera waste of smooth weakfish, *Cynoscion leiarchus* (Oliveira *et al*., 2017), the internal organs of mackerel, *Scomber japonicus* (Park *et al*., 2002), the pyloric caeca of tuna, *Thunnus thynnus* (Byun *et al*., 2003) and by products from peacock bass, *Cichla ocellaris* (Oliveira *et al*., 2019), which had optimum pH levels between 7.0 and 8.0. The decrease of hepatopancreas collagenolytic activity from blue crab observed at very strongly acidic and alkaline pH might have been caused by the denaturation of enzymes (Sriket *et al*., 2011a). Changes in pH levels lead to the ionization of amino acid atoms and molecules, and changes in the shape and structure of proteins; changes that interfere with the function of proteins. Very high or very low pH will lead to the complete loss of activity of most enzymes (Coopland, 2000).

The pH stability of hepatopancreas collagenase from blue crabs was stable for up to 8 h at pH 7.0-10.0, and still showed an activity of over 70% of its initial activity. The pH stability was similar to that of intestine collagenase from Atlantic cod, which was stable in the pH range 7.0-9.5 (Kristjansson *et al*., 1995). However, the collagenolytic activity of intestinal *C. ocellaris* was stable over pH between 6.5 and 11.5 (Oliveira *et al.*, 2019).

The optimum temperature of collagenolytic activity from blue crabs was 40°C. Higher temperatures (above 40˚C) probably resulted in the denaturation of the enzyme as decreased activity was observed at these higher temperatures. This may have been due to the unfolding of the enzyme molecule, which resulted in thermal inactivation (Sriket *et al.*, 2011b). Similar results were reported for collagenase from the skeletal muscles of winter flounder, *P. americanus* (Teruel and Simpson, 1995), and collagenase A2 from the hepatopancreas of Northern shrimp, *Pandalus eous* (Aoki *et al*., 2003). However, the optimum temperature of hepatopancreas collagenolytic activity from blue crabs is lower than that of collagenolytic activities from filefish (Kim *et al*., 2002), smooth weakfish (Oliveira *et al*., 2017), mackerel (Park *et al*., 2002), tuna (Byun *et al*., 2003) and peacock bass (Oliveira *et al*., 2019), which showed an optimum temperature at 55 ℃. The temperature was higher than that from fish waste mixtures of haddock, herring, ground fish, and flounder (35 ℃) (Daboor *et al*., 2012).

The hepatopancreas collagenase activity from blue crab was quite stable at chilling temperatures (0 and 4 ℃). Its remaining activity was over 75% after 8 h incubation. However, the collagenase activity from winter flounder was stable at 0-45 ℃ (Teruel and Simpson, 1995). The intestinal collagenase from *C. ocellaris* was stable in the range of 25 to 60 ℃ (Oliveira *et al*., 2019.) The visceral and liver collagenase from cod species retained a lower figure of 60% of its initial activity at 50 ℃ for 10 min (Sovik and Rustad, 2006).

For preservation of seafood quality, vendors keep the products at temperatures below 4 ℃. The purpose of temperature reduction is to prevent microbial deterioration, and this is done by soaking the products in salted ice boxes. Addition of NaCl to ice causes a temperature drop that slows the melting of ice and increases the freezing rate. Our results showed that NaCl content influenced collagenolytic activity of blue crab hepatopancreas. Enzyme activity continuously decreased with increase of NaCl concentration up to 20%. The decline in activity might be due to an increase in ionic strength, which can be explained by the salting-out effect (Klomklao *et al*., 2004). The result suggested the collagenolytic activity appeared to be less sensitive to NaCl than the proteolytic activity from blue crabs (Makkapan and Narkthewan, 2019). The activity of blue crab protease was reduced with 15% (w/v) NaCl with approximately 30% of the remaining activity. Moreover, Ghaly *et al*. (2010) observed that NaCl can inactivate autolytic enzymes in marine animals. The autolytic activity of proteases in Indian anchovy (*Stolephorus indicus*) was reduced by 25% (w/w) NaCl (Siringan *et al*., 2006).

The initial pH in blue crab hepatopancreas was 6.7, and a continuous rise in pH took place for 3 days. The maximum pH value of 7.5 was measured after

3 days of storage on ice. The pH level was 6.74 after 24 h storage (Arulkumar *et al*., 2017). Balasaraswathy *et al.* (2008) reported that a slight change of pH in crab meat was encountered during an iced storage period of 14 days, which was within the range of 7.6-8.4. The highest pH was observed after storage in ice for 7 days. A report on the mean pH values of shrimp, *Penaeus monodon*, indicated increase from 6.63 to 7.28 within 10 days after iced storage (Rahaman *et al*., 2001). However, the pH of Atlantic cod (*G. morhua*) muscle showed no significant different change during ice storage for 9 days (Hernandez-Herreroa *et al*., 2003). The increase in the pH value of fish muscle during iced storage was probably dependent on several factors, one of which was the decomposition of nitrogenous compounds during storage (Pacheco-Aguilar *et al*., 2000; Benjakul *et al*., 2002).

Changes in the total proteolytic and collagenolytic activities of blue crab hepatopancreas during iced storage were observed for 5 days. The collagenolytic and total proteolytic activity followed a similar trend. Both activities increased over 2 days of storage. Subsequently, the activities of both enzymes decreased until the end of the iced storage period. The decrease of hepatopancreas enzymes with increasing storage period probably occurred because these enzymes diffused out from the hepatopancreas into other internal tissues, especially muscle. Previous findings in freshwater prawn (*Macrobrachium rosenbergii*) implied that the autolysis of hepatopancreas and internal organs within the cephalothorax during the storage caused the release of the protease into the muscle (Sriket *et al*., 2011b). In a similar way to that occurring in other crustaceans, the release of hepatopancreas proteases in blue crab results from autolysis during storage. The protease enzymes including trypsin, calpain, cathepsins and collagenases cause protein degradation that produces the nutrients for bacteria proliferation (Hernandez-Herreroa *et al*., 2003; Ghaly *et al*., 2010; Sriket, 2014).

Based on the study, it has been demonstrated that the transportation of blue crab stored in ice should be completed within 1-2 days. This corresponds with previous reports that suggested that blue crab *P. pelagicus* must be used for further processing within 24 h after death if refrigerated because the spoilage indicator putrescine was at detectable levels after 48-96 h (Arulkumar *et al*., 2017). Moreover, previous findings indicated the level of histamine, one of the quality indexes for crabs, exhibited maximum levels after 72 h at 4℃. Therefore, it was concluded that the crabs must be used within 24 h of death if refrigerated (Xu *et al*., 2009). However, Balasaraswathy *et al*. (2008) suggested that crabs could be stored in ice and remain in an acceptable condition as judged by organoleptic evaluation for a maximum period of 10 days. No significant changes in the total volatile base (TVB) and trimethylamine (TMA),

which are indexes of seafood product quality, were found throughout the storage period.

The hepatopancreas collagenase from the blue crab exhibited an optimum activity at pH 9.0 and a temperature of 40 ℃. The collagenase was most stable in the pH range of 7.0-10.0 and temperatures below 50 ℃. An increment of NaCl concentration caused a reduction of collagenolytic activity. The hepatopancreas pH values changed in the range of pH from 6.7-7.5 during the iced storage period of 5 days. Furthermore, changes of total proteolytic and collagenolytic activities were observed during storage in ice. They showed the highest levels after 2 days of iced storage. Based on these results, blue crabs kept in ice should be distributed locally within 2 days. The iced storage condition should include control of pH in the acidic range, and the presence of salt during blue crab distribution seems to be a further helpful step. Further research with joint investigation of the factors influencing autolysis in blue crabs is needed in order to improve methods of maintaining the quality of blue crab during iced storage and distribution.

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References

Aoki, H., Ahsan, MD. N., Matsuo, K., Hagiwara, T. and Watabe, S. (2003). Purification and characterization of collagenolytic proteases from the hepatopancreas of Northern shrimp (*Pandalus eous*). Journal of Agricultural and Food Chemistry, 51:777-783.

Arulkumar, A., Paramasivam, S., Rameshthangam, P. and Rabie, M. A. (2017). Changes on biogenic, volatile amines and microbial quality of the blue swimmer crab (*Portunus pelagicus*) muscle during storage. Journal of Food Science and Technology, 54:2503-2511.

Balasaraswathy, N., Sugumar, G., Selvan, A., Ramesh, U. and Velayutham, P. (2008). Changes in quality characteristics of cooked and uncooked crab meat (*Portunus pelagicus*) under ice storage. Asian Fisheries Science, 21:101-112.

Benjakul, S., Visessanguan, W., Riebroy, S., Ishizaki, S. and Tanaka, M. (2002). Gel-forming properties of surimi produced from bigeye snapper, *Priacanthus tayenus* and *P. macracanthus*, stored in ice. Journal of the Science of Food and Agriculture, 82:1442-1451.

Brauer, J. M. E., Leyva, J. A. S., Alvarado, L. B. and Sandez, O. R. (2003). Effect of dietary protein on muscle collagen, collagenase and shear force of farmed white shrimp (*Litopenaeus vannamei*). European Food Research and Technology, 217:277-280.

Byun, H.-G., Park, P.-J., Sung, N.-J. and Kim, S.-K. (2003). Purification and characterization of a serine proteinase from the tuna pyloric caeca. Journal of Food Biochemistry, 26:479-494.

Carmeli, E., Moas, M., Reznick, A. Z. and Coleman, R. (2004). Matrix metalloproteinases and skeletal muscle: A brief review. Muscle Nerve, 29:191-197.

Chaijaroen, T. and Thongruang, C. (2016). Extraction, characterization and activity of digestive enzyme from Nile tilapia (*Oreochromis niloticus*) viscera waste. International Food Research Journal, 23:1432-1438.

Coopland, R. A. (2000). Experimental measures of enzyme activity. In: Enzymes: a practical introduction to structure, mechanism, and data analysis, Unite State of America, Wiley-VCH, Inc., pp. 188-265.

Daboor, S. M., Budge, S. M., Ghaly, A. E., Brooks, S. L. and Dave, D. (2010). Extraction and purification of collagenase enzymes: A critical review. American Journal of Biochemistry and Biotechnology, 6:239-263.

Daboor, S. M., Budge, S. M., Ghaly, A. E., Brooks, M. S. and Dave, D. (2012). Isolation and activation of collagenase from fish processing waste. Advances in Bioscience and Biotechnology 3:191-203.

Ferre, L. E., Medesani, D. A., Garcia, C. F., Grodzielski, M. and Rodriguez, E. M. (2012). Vitellogenin levels in hemolymph, ovary and hepatopancreas of the freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) during the reproductive cycle. Revista de Biolog á Tropical, 60:253-261.

Ghaly, A. E., Dave, D., Budge, S. and Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: Review. American Journal of Applied Sciences, 7:859-877.

Hernandez-Herreroa, M. M., Duflos, G., Malle, P. and Bouquelet, S. (2003). Collagenase activity and protein hydrolysis as related to spoilage of iced cod (*Gadus morhua*). Food Research International, 36:141-147.

Kim, S. K., Park, P. J., Kim, J. B. and Shahidi, F. (2002). Purification and characterization of a collagenolytic protease from the filefish, *Novoden modestrus*. Journal of Biochemistry and Molecular Biology, 35:165-171.

Klomklao, S., Benjakul, S. and Visessanguan, W. (2004). Comparative studies on proteolytic activity of splenic extract from three tune species commonly used in Thailand. Journal of Food Biochemistry, 28:355-372.

Kristjansson, M. M., Gudmundsdottir, S., Fox, J. W. and Bjarnason J. B. (1995). Characterization of a collagenolytic serine proteinase from the Atlantic cod (*Gadus morhua*). Comparative Biochemistry and Physiology Part B, 110:707-717.

Kubota, M., Kinoshita, M., Kubota, S., Yamashita, M., Toyohara, H. and Sakaguchi, M. (2001). Possible implication of metalloproteinases in post-mortem tenderization of fish muscle. Fisheries Science, 67:965-968.

Kubota, M., Kinoshita, M., Takeuchi, K., Kubota, S., Toyohara, H. and Sakaguchi, M. (2003). Solubilization of type I collagen from fish muscle connective tissue by matrix metalloproteinase-9 at chilled temperature. Fisheries Science, 69:1053-1059.

Makkapan, W. and Narkthewan, P. (2019). Characterization of protease activity from hepatopancreas of blue crab. IOP Conference Series: Earth and Environmental Science, 346:012032.

Mehrotra, S., Pandey, P. K., Gaur, R. and Darmwal, N. S. (1999). The production of alkaline protease by a *Bacillus* species isolate. Bioresource Technology, 67:201-203.

Oliveira, V. de M., Assis, C. R. D., Herculano, P. N., Cavalcanti, M. T. H., Bezerra, R. de S. and Porto, A. L. F. (2017). Collagenase from smooth weakfish: Extraction, partial purification, characterization and collagen specificity test for industrial application. Boletim do Instituto de Pesca, 43:52-64.

Oliveira, V. de M., Assis, C. R. D., Silva, J. C., Silva, Q. J., Bezerra, R. de S. and Porto, A. L. F. (2019). Recovery of fibrinolytic and collagenolytic enzymes from fish and shrimp byproducts: Potential source for biomedical applications. Boletim do Instituto de Pesca, 45:e389.

Pacheco-Aguilar, R., Lugo-Sanchez, M. E. and Robles-Burgueno, M. R. (2000). Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 ℃. Journal of Food Science, 65:40-47.

Park, P. J., Lee, S. H., Byun, H. G., Kim, S. H. and Kim, S. K. (2002). Purification and characterization of a collagenase from the mackerel, *Scomber japonicus*. Journal of Biochemistry and Molecular Biology, 35:576-582.

Pushparajan, N., Varadharajan, D. and Soundarapandian, P. (2013). Microbial studies of prepared curries stored in tin free steel cans. Journal of Food Processing and Technology, 4:1000238.

Rahaman, M., Yasmin, L., Kamal, M., Mazid, M. A. and Islam, M. N. (2001). Effect of delayed icing on the quality changes in brackish water shrimp *Penaeus monodon* during ice storage. Pakistan Journal of Biological Sciences, 4:1390-1394.

Ramadevi, K. R. L. S., Shyamasundari, K. and Hanumantha Rao, K. (1990). Observation on the hepatopancreas of *Ocypoda platytarsis* (Milne-Edwards) (Crustacea, Brachyura). Italian Journal of Zoology, 57:261-265.

Singh, A. and Benjakul, S. (2018). Proteolysis and its control using protease inhibitors in fish and fish products: A review. Comprehensive Reviews in Food Science and Food Safety, 17:496-509.

Singh, J. and Singh, B. (2020). Inhibition of post-mortem fish muscle softening and degradation using legume seed proteinase inhibitor. Journal of Food Science and Technology, 57:1-11.

Siringan, P., Raksakulthai, N. and Yongsawatdigul, J. (2006). Autolytic activity and biochemical characteristics of endogenous proteinases in Indian anchovy (*Stolephorus indicus*). Food Chemistry, 98:678-684.

Sovik, S. L. and Rustad, T. (2006). Effect of season and fishing ground on the activity of cathepsin B and collagenase in by-products from cod Species. LWT, 39:43-53.

Sriket, C. (2014). Proteases in fish and shellfish: Role on muscle softening and prevention. International Food Research Journal, 21:433-445.

Sriket, C., Benjakul, S. and Visessanguan, W. (2011a). Characterization of proteolytic enzymes from muscle and hepatopancreas of fresh water prawn (*Macrobrachium rosenbergii*). Journal of the Science of Food and Agriculture, 91:52-59.

Sriket, C., Benjakul, S., Visessanguan, W. and Kishimura, H. (2011b). Collagenolytic serine protease in fresh water prawn (*Macrobrachium rosenbergii*): Characteristics and its impact on muscle during iced storage. Food Chemistry, 124:29-35.

Teruel, S. R. L. and Simpson, B. K. (1995). Characterization of the collagenolytic enzyme fraction from winter flounder (*Pseudopleuronectes americanus*). Comparative Biochemistry and Physiology Part B, 112:131-136.

Wang, W., Wu, X., Liu, Z., Zheng, H. and Cheng, Y. (2014). Insights into hepatopancreatic functions for nutrition metabolism and ovarian development in the crab *Portunus trituberculatus*: Gene discovery in the comparative transcriptome of different hepatopancreas stages. PLoS ONE, 9:e84921.

Xu, Y., Xia, W., and Kim, J. M. (2009). Biogenic and volatile amines in Chinese mitten crab (*Eriocheir sinensis*) stored at different temperatures. International Journal of Food Science and Technology, 44:1547-1552.

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