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# Muscle changes in hard and soft shell crabs during frozen storage

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ABSTRACT

Chemical and physicochemical changes of muscles from hard and soft shell mud crabs (*Scylla serrata*) were monitored during 12 weeks of storage at −20 °C. $Ca^{2+}$-ATPase activity of natural actomyosin (NAM) from both crabs decreased continuously during storage, regardless of muscle types. After 8 weeks of storage, $Ca^{2+}$-ATPase activity of NAM from lump muscle of soft shell crab decreased to a greater extent than that of hard shell crab ($P < 0.05$). An increase in disulfide bonds was observed with the coincidental decrease in sulfhydryl group content during extended storage ($P < 0.05$). Surface hydrophobicity of all samples increased up to 8 weeks, being this sampling time followed by a gradual decrease. Formaldehyde content of all samples increased throughout the storage ($P < 0.05$). Slightly higher formaldehyde content was found in soft shell crab muscle, compared with hard shell counterpart ($P < 0.05$). Claw muscle generally contained a greater amount of formaldehyde than lump counterpart ($P < 0.05$). Protein solubility of all samples decreased continuously throughout the 12 weeks of frozen storage ($P < 0.05$). Lipid oxidation took place during the extended storage as evidenced by the increase in thiobarbituric acid reactive substances. The pH of all samples generally decreased during frozen storage. Cooking loss of all crab muscles increased as storage time increased ($P < 0.05$) and was more pronounced in claw muscle, particularly from soft shell crab.



## 1. Introduction

Frozen storage of shellfish is an important preservation method; however deterioration in texture, flavor, and color of muscles frequently occurs with poor conditions during the process (Hale & Waters, 1988). Freezing slows enzyme activity and inhibits microorganism growth. However, lipid hydrolysis and oxidation still occur. The extent of quality loss of marine frozen food is dependent upon many factors, which include storage temperature and time, packaging, rate of freezing–thawing, and temperature fluctuations and freeze–thaw abuse (Srinivasan, Xiong, & Blanchard, 1997). Prolonged frozen storage of Egyptian shore crabs (*Carcinus maenas*) at −10 °C affected the chemical characteristics of crab meat (Aman, Moustafa, Zoueil, & Ghaly, 1983). However, the sensory quality of blue crab (*Callinectes sapidus*) was maintained at −18 °C for 10 months. Muscle proteins of freshwater prawn tails are susceptible to freezing–thawing processes, particularly during the first month of frozen storage (Srinivasan et al., 1997). Ice crystals and the increase in ionic strength of the system during frozen storage induced myosin denaturation and the disruption of the actin–myosin complex, as indicated by the decrease in both $Ca^{2+}$-ATPase and $Mg^{2+}$–$Ca^{2+}$-ATPase activities, respectively (Benjakul & Bauer, 2000, 2001). Ang and Hultin (1989) reported that crosslinking of cod myosin stored at −25 and −80 °C correlated with the decrease in ATPase activity. Lipid hydrolysis and lipid oxidation generally take place in fish muscle during the extended frozen storage (Aubourg, 1999). Subramanian (2007) reported that the lipid hydrolysis and oxidation took place in raw marine crab (*Portunus pelagicus*) during 120 days of frozen storage at −41 °C.

Mud crab (*Scylla serrata*), a popular seafood, is farmed on a commercial scale in many tropical countries. Mud crab is now monocultured with increasing density in the pond to supply the growing market (Catacutan, 2002). Naturally, the crab grows by molting during its life and the crab that has just molted is called a soft shell crab. After molting, soft shell crabs have to be removed from the seawater immediately to prevent hardening (Dassow, 1968). Soft shell crabs have become more popular, in part, because they are sold at the higher value, compared to hard shell crab. Freezing is an essential method commonly used to prevent the deterioration of soft shell crabs during storage or distribution. However, no information about the changes in chemical and physicochemical properties during frozen storage of both types of crabs has been reported. The objective of this study was to monitor the chemical and physicochemical changes of muscles of hard and soft shell mud crabs during frozen storage at −20 °C.

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## 2. Materials and methods

### 2.1. Chemicals

Bovine serum albumin, β-mercaptoethanol (βME), glycerol, ammonium molybdate, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), adenosine 5′-triphosphate (ATP) and thiobarbituric acid were purchased from Sigma (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane, Folin–Ciocalteu's phenol reagent, ethylene diamine tetraacetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from Merck (Darmstadt, Germany).

### 2.2. Collection and preparation of hard and soft shell crabs

Alive hard shell mud crabs (*S. serrata*) and soft shell mud crabs with an average weight ranging from 150 to 180 g were purchased from a farm in Kantang, Trang, Thailand. Soft shell mud crabs were obtained 24 h after molting. The crabs were kept in the plastic basket covered with wet cloth to keep the crabs moist. All samples were transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla, Thailand within 3 h. Crabs were washed with running water and subjected to analyses and freezing. Prior to analyses, the initial fresh samples and subsequent frozen samples were excised and meat was collected, chopped finely and pooled as the composite sample. For frozen samples, the samples (8 crabs) were randomly taken and thawed using running water until the core temperature reached 0–2 °C. Three different lots with total 192 crabs for each type of crab (64 crabs for each lot of one type of crab) were employed for the whole study.

### 2.3. Study on chemical and physicochemical changes of hard and soft shell mud crab muscles during frozen storage

Crabs (8 crabs) were packed as one layer in a polyethylene bag, heat sealed and stored at −20 °C in an air-blast freezer. The samples were randomly taken for analyses after 0, 1, 2, 4, 6, 8, 10 and 12 weeks of storage. Prior to analyses, the samples were thawed using running water as previously described. Thawed samples were excised and claw and lump meats were collected for analyses.

### 2.4. Analyses

#### 2.4.1. ATPase activity

$Ca^{2+}$-ATPase activity was determined according to the method of Benjakul, Seymour, Morrissey, and An (1997). Natural actomyosin (NAM) from the claw of soft shell crab could not be precipitated using distilled water. As a result, NAM pellet could not be recovered for further analyses.

NAM from all crab muscles, except from claw muscle of soft shell crab, was prepared as described by Benjakul et al. (1997). To determine $Ca^{2+}$-ATPase activity, NAM was diluted to 2.5–6 mg/ml with 0.6 M KCl, pH 7.0. Diluted NAM solution (1 ml) was added to 0.6 ml of 0.5 M Tris-maleate, pH 7.0. $CaCl_2$ was added to obtain a final concentration of 10 mM $CaCl_2$ with a total volume of 9.5 ml. To each assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction was conducted for 8 min at 25 °C and terminated by adding 5 ml of chilled 15% (w/v) trichloroacetic acid. The reaction mixture was centrifuged at 3500 × g (Hettich Micro 20, Tuttlingen, Germany) for 5 min and the inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as μmol inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP.

#### 2.4.2. Total sulfhydryl group content

Total sulfhydryl (SH) group content was measured using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) according to the method of Ellman (1959) as modified by Benjakul et al. (1997). To 1.0 ml of NAM solution (4 mg/ml), 9 ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA was added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB was added and incubated at 40 °C for 25 min. The absorbance at 412 nm was then measured using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). A blank was prepared by replacing the sample with 0.6 M KCl, pH 7.0. The total SH group content was calculated using a molar extinction coefficient of 13,600 $M^{-1}\,cm^{-1}$.

#### 2.4.3. Disulfide bond content

Disulfide bond content in NAM was determined using 2-nitro-5-thiosulfobenzoate (NTSB) assay according to the method of Thannhauser, Konishi, and Scheraga (1987). To 0.2 ml of NAM sample (4 mg/ml), 3.0 ml of freshly prepared NTSB assay solution was added. The mixture was incubated in the dark at room temperature (25–27 °C) for 25 min. Absorbance at 412 nm was measured. Disulfide bond content was calculated using the extinction coefficient of 13,900 $M^{-1}\,cm^{-1}$.

#### 2.4.4. Surface hydrophobicity

Surface hydrophobicity of NAM was determined according to the method described by Benjakul et al. (1997) using 1-anilinonapthalene-8-sulfonic acid (ANS) as a probe. NAM was dissolved in 10 mM phosphate buffer, pH 6.0, containing 0.6 M NaCl to obtain different concentrations and then mixed with ANS. The fluorescence intensity of ANS–protein conjugates was measured at an excitation wavelength of 374 nm and an emission wavelength of 485 nm using an RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan). The initial slope of the plot of fluorescence intensity versus NAM concentration was referred to as SoANS.

#### 2.4.5. Formaldehyde content

Formaldehyde content in crab muscle was determined according to the method of Nash (1953). Fish muscle (5 g) was placed in 50 ml beaker and 20 ml of 5% trichloroacetic acid (TCA) was added. The mixture was homogenized (IKA Labortechnik, Selangor, Malaysia) at a speed of 13,000 rpm for 2 min. The homogenate was then filtered using Whatman paper No. 41 (Whatman International Ltd, Maidstone, England). The extraction was repeated by homogenizing the residue with 10 ml of 5% TCA, followed by filtering. The filtrate was combined and neutralized to pH 6.0–6.5 with 1 or 2 N NaOH. The final volume was made up to 50 ml using distilled water. To neutralized filtrate (3 ml), 3 ml of acetylacetone reagent was added and mixed thoroughly. The reaction mixture was kept at 60 °C for 15 min and cooled in running water. The absorbance was measured at 412 nm. The blank was prepared by using 5% trichloroacetic acid instead of filtrate. Formaldehyde content was calculated from a standard curve (0–24 μg formaldehyde) and expressed as μg/g sample.

#### 2.4.6. Protein solubility

Protein solubility was determined as described by Benjakul and Bauer (2000) with some modifications. To a 2 g sample, 18 ml of 0.6 M KCl was added and the mixture was homogenized for 30 s at a speed of 13,000 rpm. The homogenate was stirred at room temperature (25–27 °C) for 4 h using a magnetic stirrer (IKA-WERKE, Staufen, Germany), followed by centrifuging at 12,000 × g for 20 min at 4 °C using a Sorvall RC 26 Plus refrigerated centrifuge (Sorvall, Norwalk, CT, USA). To 10 ml of the supernatant, cold 50% (w/v) TCA was added to obtain the final concentration of 10%. The precipitate was washed with 10% TCA and solubilized in 10 ml of 0.5 M NaOH. The muscle was also solubilized using 0.5 M NaOH to