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Comparative study on chemical composition, thermal properties and microstructure between the muscle of hard shell and soft shell mud crabs

Soottawat Benjakul *, Nuntapol Sutthipan

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, 15 Kanchanawanich Road, Hat Yai, Songkha 90112, Thailand

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ABSTRACT

Chemical composition, thermal properties and microstructure of the muscle from hard shell and soft shell mud crabs were studied. Both lump and claw muscle of soft shell mud crabs contained a lower protein content but higher moisture and salt contents than those from hard shell mud crab ($p < 0.05$). Calcium and magnesium were the major minerals in the muscle of hard and soft shell mud crabs (240.5– 699.2 ppm). Approximately one-third of calcium was observed in the muscle of soft shell mud crab, compared to that of hard shell mud crab. Copper, iron and zinc were the trace minerals with the amount less than 50 ppm. Hydroxyproline content ranged from 7.92 to 8.88 mg/g wet muscle in all samples, except claw muscle from soft shell mud crab, which contained considerably low hydroxyproline content (2.75 mg/g wet muscle). Sarcoplasmic and alkali-soluble proteins were the major constituents in all muscles, except claw muscle from soft shell mud crab, in which sarcoplasmic protein was the dominant component. T_{max} from myosin of lump muscle were 47.58–48.08 °C with enthalpy of 0.20–0.21 J/g, whereas myosin from claw muscle had lower T_{max} (45.00–47.48 °C) with lower enthalpy (0.17–0.18 J/g). Lump muscle bundles of hard and soft shell mud crabs aligned orderly, while claw muscles of both crabs had partial disintegrations and the porous structure was observed in that from soft shell crab.

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1. Introduction

Mud crab (Scylla serrata) has become a popular seafood and is farmed on a commercial scale in many tropical countries. Mud crab is now monocultured in increasing density to supply the growing market demands [\(Catacutan, 2002\)](#page-5-0). Naturally, the crab grows by molting process during its life time and the crab that has just molted is called soft shell crab. After molting, soft shell crab has to be taken out from seawater immediately to prevent hardening ([Dassow, 1968\)](#page-5-0). Generally, crab grows by successive molts of its shell during its lifetime. Crabs are segmented animals with a chitinous exoskeleton. Beneath this rigid cover, the internal organs like gut, nervous system and glands are embedded in the heamolymph of the body cavity [\(Yoshinaka, Sato, Itoh, Nakajima, & Sato,](#page-6-0) [1989\)](#page-6-0).

Soft shell crab becomes more popular and is usually purchased at the higher value, compared to hard shell crab. Hard and soft snow crabs are mainly distinguished on the basis of visual appearance and physical feel when touched by hand. The crust of a soft crab is much more transparent and softer than that of hard snow crab. The most pronounced characteristic of a soft snow crab is a considerable amount of free body fluid in its appendages, which can flow out from appendages when the crust is out. Soft snow

crab muscle has a higher water content and a lower protein content than hard shell crab muscle. Salinity of the free body fluid recovered from the meropodites of the soft snow crab was significantly higher than that of the hard snow crab [\(Mizuta, Kobayashi,](#page-5-0) [& Yoshinaka, 2001\)](#page-5-0).

Commercially, soft crabs can be obtained by holding hard shell crabs in floats until the molt occurs. The soft crabs are removed from the water within a few hours and are graded for size. The crabs may be held in cool storage for 2–3 days before processing. The crabs then are killed, eviscerated, washed, wrapped individually with parchment, packed in a carton, and frozen [\(Dassow, 1968](#page-5-0)). Though the consumption of soft shell mud crab has been increasing continuously in Thailand and ASEAN countries, no basic information on the chemical composition as well as the properties of soft shell mud crab has been reported. Therefore, the objective of this study was to compare chemical composition and properties between the muscles of hard and soft shell mud crabs.

2. Materials and methods

2.1. Chemicals

Sodium dodecyl sulphate (SDS), β-mercaptoethanol (BME), glycerol, high molecular weight markers, and N,N-dimethylformamide were purchased from Sigma (St. Louis, MO, USA). Coomassie

Corresponding author. Tel.: +66 7428 6334; fax: +66 7421 2889. E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

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blue R-250, penta-sodium triphosphate, sodium hexametaphosphate, Folin-Ciocalteu's phenol reagent, ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from Merck (Darmstadt, Germany). Acrylamide and N,N,N',N'-tetramethylethylenediamine (TEMED) were obtained from Fluka (Buchs, Switzerland).

2.2. Collection and preparation of samples

Alive hard shell mud crabs (S. serrata) and soft shell mud crabs with the average weight ranging from 150 to 180 g were purchased from a farm in Kantang, Trang, Thailand. Soft shell mud crabs were obtained after 24 h molting. The crabs were kept in a plastic basket covered with the wet cloth to keep the crabs moist. All samples were transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla, within 3 h. The samples were then excised into claw and lump portions. Meat obtained from each portions was pooled and used as the composite sample. Three lots of crabs were used for each study. The meat was kept in ice until analyzed.

2.3. Chemical analyses of the muscle from hard and soft shell mud crabs

Lump and claw muscles from both hard and soft shell mud crabs were subjected to the analyses of moisture, protein, fat, ash and salt contents as per [AOAC. \(1999\)](#page-5-0) with the method No. of 950.46, 940.25, 991.36, 938.08 and 937.09, respectively. Minerals including copper, calcium, magnesium, iron and zinc in crab muscle were determined by atomic absorption spectrometry (PerkinElmer, AAnalyst 100, Norwalk, CT, USA) according to the method of [AOAC \(1999\).](#page-5-0)

Hydroxyproline content was determined according to the method of [Bergman and Loxley \(1963\)](#page-5-0) with a slight modification. The samples were hydrolyzed with 6 M HCl at 110 °C for 24 h in an oil bath (model B-490, BUCHI, Flawil, Switzerland). The hydrolysate was clarified with activated carbon and filtered through Whatman No. 4 filter paper. The filtrate was neutralized with 10 M and 1 M NaOH to obtain the pH of 6.0–6.5. The neutralized sample (0.1 ml) was transferred into a test tube and isopropanol (0.2 ml) was added. The mixtures were mixed well. A 0.1 ml of oxidant solution (the mixture of 7% (w/v) chlororamine T and acetate/citrate buffer, pH 6 at a ratio of 1:4 (v/v)) was added and mixed thoroughly. A 1.3 ml of Ehrlich's reagent solution (the mixture of solution A (2 g of p-dimethylamino-benzaldehyde in 3 ml of 60% (v/v) perchloric acid (w/v)) and isopropanol at a ratio of 3:13 (v/v) was added. The mixtures were mixed and heated at 60 °C for 25 min in a water bath (Memmert, Schwabach, Germany) and then cooled for 3 min in running water. The solution was diluted to 5 ml with isopropanol. Absorbance was measured against distilled water at 558 nm. A hydroxyproline standard solution with the concentration ranging from 10 to 60 mg/kg was also included. Hydroxyproline content was calculated and expressed as mg/g sample.

2.4. Fractionation and determination of nitrogenous constituents of muscle from hard and soft shell crabs

Fractionation of muscle from hard shell and soft shell mud crabs was carried out according to the method of [Hashimoto, Watanabe,](#page-5-0) [Kono, and Shiro \(1979\)](#page-5-0). All fractions obtained including (1) nonprotein nitrogen, (2) sarcoplasmic protein, (3) myofibrillar protein, (4) alkali-soluble protein, and (5) stroma fractions were subjected to the analysis of nitrogen content using Kjeldahl method [\(AOAC,](#page-5-0) [1999\)](#page-5-0). Pattern and molecular weight of proteins in all fractions were determined by SDS-PAGE according to the method of [Lae](#page-5-0)[mmli \(1970\)](#page-5-0) using 4% stacking gel and 10% running gel.

2.5. Differential scanning calorimetry of muscle proteins from hard and soft shell crabs

Thermal denaturation of crab muscle proteins was investigated by monitoring T_{max} of transition and denaturation enthalpy. All samples were scanned at 10 \degree C/min over the range of 0–100 \degree C using differential scanning calorimetry (DSC) (model DSC 7, PerkinElmer, Norwalk, CT, USA). The system was calibrated using indium.

2.6. Scanning electron microscopy of the muscle from hard and soft shell crabs

Microstructure of crab muscle was determined using scanning electron microscopy according to the method of [Nip and Moy](#page-5-0) [\(1988\).](#page-5-0) The muscle tissue was fixed in 0.1 M sodium phosphate buffer containing 2.5% glutaraldehyde and the fixed specimen was washed in 0.1 M sodium phosphate buffer (pH 7.2). The sample was dehydrated in a graded series of ethanol (from 50%, 70%, 85%, 95% to 100%). Dehydrated sample was coated with gold–palladium and viewed with a scanning electron microscope (model JSM5800LV, JEOL, Tokyo, Japan).

2.7. Statistical analysis

Data was subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's Multiple Range Test ([Steel & Torrie, 1980\)](#page-6-0). Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Chemical composition

Chemical composition of hard and soft shell mud crabs is shown in Table 1. Both lump and claw muscles of soft shell mud crab contained the lower protein content but higher moisture content than those of hard shell mud crab ($P < 0.05$). Claw muscle of hard and soft shell mud crabs contained a higher moisture content (84.38% and 94.76%, respectively) than lump muscle of both crabs (78.69% and 82.37%). Therefore, water was considered to be the main constituent in the soft shell mud crab muscle, which varied depending on the location of the muscle. Claw muscle of soft shell mud crab had the highest moisture content (94.76%). This result was in agreement with [Mizuta et al. \(2001\)](#page-5-0) who reported that

* Mean ± standard deviation from triplicate determinations.

**Values with the different letters in the same row are significantly different $(P \le 0.05)$.

water content in the muscle of the soft snow crab was significantly higher than that of the hard snow crab. A soft snow crab contained a considerable amount of free body fluid (FBF) in its appendages which was 33.80% to 37.30% (w/w), while hard snow crab appendages contained 5.00–6.08% (w/w) FBF. Moisture, protein and total mineral contents of green crab meat were 78.7%, 17.1% and 2.2%, respectively [\(Skonberg & Perkins, 2002\)](#page-6-0). [Naczk, Williams, Brennan,](#page-5-0) [Liyanapathirana, and Shahidi \(2004\)](#page-5-0) reported that raw green crab meat contained 80.6–83.5% protein and 3.6–4.8% lipid. Salt content in the soft shell mud crab muscle varied from 1.10% to 1.70%, whereas the hard shell mud crab muscle had a lower salt content ranging from 0.70% to 1.38%. A high content of inorganic substances was observed in the claw soft shell mud crab as indicated by high ash content (2.17%). High moisture, ash, salt contents but low protein content of the soft shell mud crab muscle seemed to be partially caused by the absorption of seawater. For fat content, it ranged from 0.12% to 0.24% in the soft shell mud crab muscle and from 0.18% to 0.28% in the hard shell mud crab muscle. During post-molt stage, salvaged inorganic salts are rapidly redeposited to help thicken and harden the new shell. Hardening process stops if the crab is removed from the water and will take approximately two to four days to fully harden ([Havens and McConaugha, 1990\)](#page-5-0). As the crab slowly grows inside its new shell, tissue water is replaced with protein. Once there is no more room left to grow inside this shell, the whole molting process starts over again ([Havens and](#page-5-0) [McConaugha, 1990\)](#page-5-0).

3.2. Mineral content

Mineral composition of hard and soft shell mud crabs are shown in [Table 1](#page-1-0). Among all minerals of hard and soft shell crabs, calcium and magnesium were shown to be the major minerals ranging from 240.5 to 699.2 mg/kg wet tissue and from 403.6 to 428.2 mg/kg wet tissue, respectively. The levels of Cu, Fe and Zn were not higher than 50 mg/kg wet tissue. One-third of calcium was obtained in soft shell mud crab, compared to that found in hard shell mud crab. However, no marked changes in magnesium were observed between both crabs. Approximately 50% of Fe content was found in soft shell mud crab, compared to that found in hard shell mud crab. Slightly lower Ca and Zn contents were observed in soft shell mud crab, compared to those in hard shell mud crab. Growth stage in the life cycle of mud crabs also affected the mineral contents in the muscle. This result was in agreement with [Scott-fordsmand and Depledge \(1997\)](#page-6-0) who reported the lower whole body calcium concentration in newly molted shore crabs (Carcinus maenas) compared to premolt counterpart. Additionally, Cu and Zn contents of postmolt stage of shore crab decreased by 25% and 24% on the whole body basis as crabs entered the paper shell stage from the newly molted stage. S. Serrata is a large portunid crab, which matures and spawns in seawater, spends post-larval and juvenile phases in brackish water, and then returns to the sea as a pre-adult ([Davenport & Wang, 1987](#page-5-0)). Changes in salinity may disrupt the osmotic balance of decapod crustaceans ([Chen &](#page-5-0) [Chia, 1997](#page-5-0)). [Chen et al. \(2007\)](#page-5-0) reported that Chinese mitten crab meat was rich in zinc, iron, copper and phosphorus.

3.3. Hydroxyproline content

[Table 1](#page-1-0) shows the content of hydroxyproline in the muscle of hard and soft shell mud crabs. Based on wet basis, hydroxyproline contents in muscle of hard shell mud crab were generally higher than those of soft shell mud crab muscle ($P < 0.05$). When comparing the hydroxyproline content in lump and claw muscles of both crabs, hydroxyproline content in the lump muscle was higher than that in the claw muscle. Among all samples, lump muscle of hard shell mud crab had the highest hydroxyproline content. The claw muscle of soft shell mud crab had the lowest hydroxyproline content ($P < 0.05$), ranging from 2.80 to 2.70 mg/g wet tissue. Low hydroxyproline content might be due to the lowest protein content with the highest moisture content in the claw of soft shell mud crab. Based on dry matter, similar hydroxyproline content was found between soft and hard shell mud crab claw muscle and between soft and hard shell mud crab lump muscle. However, claw muscle contained higher hydroxyproline content than lump muscle ($P < 0.05$). Lump muscle is located at the joint connected between leg and abdominal muscle and its collagen is involved in the flexibility and maintaining the balance during locomotion. Claw muscle collagen, on the other hand, is involved in the functions to grip the prey [\(Yoshinaka et al., 1989](#page-6-0)). The musculature of several crustaceans was found to contain collagenous proteins ([Yoshinaka et al., 1989](#page-6-0)). Collagen in the marine animal muscle plays an important role in maintenance of meat texture ([Sato,](#page-6-0) [Yashinaka, Sato, & Shimizu, 1986\)](#page-6-0). [Sivakumar, Suguna, and Chand](#page-6-0)[rakasan \(2000\)](#page-6-0) found the levels of hydroxyproline in abdominal and leg muscles of mud crab at 0.28 and 0.38 mg/g wet tissue, respectively. The crab leg muscle collagens were highly crosslinked and stabilized by more bound carbohydrates, as compared to the abdominal muscle collagen. Crab leg muscle collagen was characterised as type V collagen with more lysine hydroxylation and slightly reduced glycine content [\(Yoshinaka et al., 1989\)](#page-6-0).

3.4. Nitrogenous compositions and protein patterns

Nitrogenous compositions of hard and soft shell mud crab muscles are shown in Table 2. Surprisingly, myofibrillar protein was not the major constituent in the muscle of hard and soft shell mud crabs. Its content was lower than alkali-soluble and sarcoplasmic proteins for both lump and claw muscles of hard shell mud crab. The lowest content of myofibrillar proteins was found among other protein components in the muscle of soft shell mud crab. [Hashimoto et al. \(1979\), Mackie \(1994\), and Suzuki \(1981\)](#page-5-0) found that myofibrillar protein was the main nitrogenous component in fish muscle. Sarcoplasmic protein seemed to be the major component in the muscle of hard and soft shell mud crabs, compared to

Table 2

* Numbers in parenthesis represent percentage distribution.

 $*$ Mean \pm standard deviation from triplicate determinations.

**Values with the different letters in the same column are significantly different ($P \leqslant 0.05$).

other nitrogenous proteins. Sarcoplasmic protein content in soft shell mud crab muscle was 78.27% for the claw muscle and 44.65% for the lump muscle. For hard shell crab muscle, sarcoplasmic protein content (38.00–39.33%) was similar to the content of alkali-soluble protein (39.80–40.73%). Alkali-soluble proteins were most likely cross-linked myofibrillar proteins. It was suggested that proteins underwent the changes during molting period, in which the content of myofibrillar and alkali-soluble proteins decreased while sarcoplasmic and stroma protein contents increased. This might be caused by the activities of proteinases in crab muscle, especially in the molting period.

Protein patterns of hard and soft shell crab muscle determined using SDS-PAGE are shown in Fig. 1. Myosin heavy chain, appeared at MW of 200,000 dalton, was not a major muscle protein in both crab muscles as found in many fish species. Nevertheless, similar protein patterns of hard shell crab lump (H1), hard shell crab claw (H2) and soft shell crab lump (S1) were observed. Actin appeared at MW of 45,000 dalton was considered to be the major protein band.

For the protein pattern of soft shell crab claw (S2), not only the lowest intensity of myosin heavy chain but also actin, paramyosin (MW of 95,000 dalton) and tropomyosin (MW of 34,000 dalton) were hardly found. Claw of soft shell crab had the protein with MW of 75,000 dalton as the major protein. This protein might play an important role in making the differences in properties of the muscle between hard shell crab and soft shell crab.

Protein patterns of different fractions from different muscles are shown in [Fig. 2.](#page-4-0) Actin was the dominant protein in myofibrillar fraction of H1, H2 and S1 samples while myosin heavy chain was found to be the second abundant protein of those samples as shown in [Fig. 2](#page-4-0). However, similar band intensity between actin and myosin heavy chain in S2 sample were observed ([Fig. 2](#page-4-0)). Patterns of sarcoplasmic proteins of all samples were not different. Low molecular weight proteins were dominant and might be associated with high water solubility of this fraction. Protein patterns of alkali-soluble and stroma protein of S2 sample were different from the others [\(Fig. 2](#page-4-0)). The result suggested that most of proteins in alkali-soluble and stroma fractions of S2

had low molecular weight. Alkali-soluble fraction contained cross-linked actin and paramyosin, which could not be dissolved in salt solution.

3.5. DSC thermal transition of hard and soft shell mud crab muscle proteins

DSC peak transition temperature (T_{max}) and enthalpy of hard and soft shell mud crab muscle are shown in [Table 3](#page-4-0). Three major peaks with T_{max} values of 45.00–48.08 °C, 72.41–76.27 °C and 83.11–87.34 \degree C were obtained. The first two peaks were postulated to represent the transition of myosin and actin, respectively. The third peak with T_{max} of 83.11–87.34 °C was possibly attributed to the most heat stable protein of mud crab muscle. DSC analysis was used to determine the thermal transition or unfolding temperature of protein and also to quantify the enthalpy of conformational transition ([John & Shashtri, 1998](#page-5-0)).

When the lump muscle of hard and soft shell mud crabs were compared, T_{max} of all peaks of hard shell mud crab were higher than those of the soft shell mud crab. Therefore, proteins in the lump muscle of soft shell mud crab were more likely to be sensitive to the thermal degradation than those of the lump muscle of hard shell mud crab. This result might be due to the higher sodium chloride content in the soft shell mud crab muscle, which caused more rapid denaturation of the proteins. [Beas, Wagner, Anon, and Crupin](#page-5-0) [\(1991\) and Park and Lanier \(1989\)](#page-5-0) reported a decrease in denaturation enthalpy and the shift of T_{max} to the lower temperature of fish muscle protein with salt addition (2.5–3.0% NaCl). Nevertheless, peak 1 and peak 3 of claw muscle of soft shell mud crab had higher T_{max} and enthalpy than those of the claw muscle of hard shell mud crab. However, peak 2 was not detected in the thermogram of soft shell mud crab claw. This result was in agreement with the low intensity of actin as shown in SDS-PAGE (Fig. 1). As a result, the endothermic transition of actin from soft shell mud crab claw was not observed. Also, the highest T_{max} of peak 3 was observed in soft shell mud crab claw. Thus, the protein with a MW of 75,000 dalton was presumed to contribute to the thermal stability of soft shell mud crab claw muscle.

Fig. 1. SDS-PAGE pattern of protein in the muscle of hard and soft shell mud crabs. H1; hard shell crab lump muscle, H2; hard shell crab claw muscle, S1; soft shell crab lump muscle, S2; soft shell crab claw muscle, H; high molecular weight standard, L; low molecular weight standard.

Fig. 2. SDS-PAGE pattern of different fractions of muscle from hard and soft shell mud crabs. H; high molecular weight standard, H1; hard shell crab lump muscle, H2; hard shell crab claw muscle, S1; soft shell crab lump muscle, S2; soft shell crab claw muscle.

Table 3

Peak transition temperature (T_{max}) and enthalpy of hard and soft shell mud crab muscle

* Mean ± standard deviation from triplicate determinations.

 $\sqrt{\frac{m}{\pi}}$ values with the different letters in the same column are significantly different ($P \le 0.05$). ***Not detectable.

Fig. 3. Microstructure of lump and claw muscle of hard and soft shell mud crabs. Bar = 50 μ m.

3.6. Microstructure of muscle from hard and soft shell mud crabs

The microstructures of lump and claw muscles of hard and soft shell mud crabs are shown in Fig. 3. The bundles of lump muscle from hard and soft crabs were connected orderly. The structure of claw muscle of both crabs had more partial disintegrations than those observed in the lump muscle. Among all samples tested, sponge-like structure was found only in the claw of soft shell crab. These histological characteristics of the soft shell crab muscle suggested a structural weakening of muscle fibres or connective tissue, possibly due to physiological phenomena related to molting, such as the uptake of seawater (Mizuta et al., 2001). The looser structure was correlated well with the high moisture content and low protein content. The much more released fluid, compared to other samples, might be owing to the sponge like structure which could not hold water in such a structure. The soft shell crab muscle was juicier, saltier and less firm than the hard shell crab muscle (Mizuta, Yoshinaka, Sato, & Sakaguchi, 1994).

4. Conclusion

Muscle of soft and hard shell mud crabs were different in chemical compositions, thermal properties and microstructure. Distinguished features of soft shell mud crab muscle were high water content and low protein content. The porous structural muscle with low water holding capacity was found in claw muscle of soft shell mud crab. Muscle of soft shell mud crab was generally more susceptible to thermal denaturation than muscle of hard shell mud crab. Furthermore, claw muscle was more sensitive to denaturation than lump muscle. Thus, mild heat processing should be applied for soft shell mud crab muscle in order to minimize the quality loss associated with heating.

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