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Effects of Handling Processes on the Quality and Biochemical Changes in Tissue of Mud Crab, (*Scylla serrata,* Forsskål, 1755) During Emersion Storage

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Abstract

The effects of handling processes were investigated in the mud crab, *Scylla serrata,* using pre-cooling and chelae tying during emersion storage.Muscle glycogen, muscle lactate, muscle yield, muscle pH, volatile basic nitrogen (VBN), and chemical composition were analyzed. More than 90% of the glycogen in muscle of non pre-cooling without chelae tying and non pre-cooling with chelae tying disappeared within 3 days of emersion storage, while the concentration of glycogen following non pre-cooling with chelae tying and pre-cooling with chelae tying decreased by only about 70% within the same period of time. Lactate content increased from the initial level - 1.20 ±0.20mmol/kg - to 14.81±0.28mmol/kg in non pre-cooling without chelae tying, while it increased to 11.34±0.58mmol/kg, 11.12±0.27mmol/kg and 10.08±0.62mmol/kg during treatment including non pre-cooling with chelae tying, pre-cooling without chelae tying, and pre-cooling with chelae tying, respectively. The muscle pH of all treatments remained almost unchanged, irrespective of treatment. Percentage loss of muscle yield after 7 days was 30.10±1.20%, 20.95±3.90%, 22.32±4.26% and 17.29±4.93% in non pre-cooling without chelae tying, non pre-cooling with chelae tying, pre-cooling without chelae tying, and pre-cooling with chelae tying, respectively. The volatile basic nitrogen (VBN) levels of the non pre-cooling without tying was 60.12±3.36 mgN/100g which is significantly different from the levels of the pre-cooling with chelae tying treatment (48.00±4.38 mgN/100g). None of the treatments resulted in any significant changes in mean of moisture, protein, fat, or ash content. The results indicated that the handling process including pre-cooling with chelae tying could reduce metabolic activity and metabolic stress, which could delay loss of quality and physiological changes of mud crab during emersion storage.

***Keywords*:** biochemistry; freshness; emersion storage; handling process; lactate; glycogen; mud crab; *Scylla serrata*

1. Introduction

Mud crabs have a popular and delicious taste as compared with other aquatic animals. They are sold primarily in the live state. During the process of preparing cultured mud crabs for the market, various procedures may induce stress responses that may lead to reduced quality of the crabs. Stress responses of the animals will change over time as long as a stimulus is present, and may result in changes of functional properties that can be quantified (Harding *et al*., 2003).

Transport of live mud crab often involves lowering of temperature to reduce metabolism and spoiling of the animals. The physiology of lowered temperature as a preserving effect during seafood shipment has received some limited attention (Paterson, 1993; Samet *et al.,*1996). The method of chilled packing has been applied to minimize respiratory requirements, anaerobiosis, and to improve crab meat quality during shipping (Morris and Oliver, 1999a). Moreover, improved handling can reduce physiological changes of animals and thereby delay loss of quality. Taylor and Whiteley (1989) showed that reducing O_2 consumption of prawns by cooling makes them less susceptible to stress. Samet *et al*., (1996) reported that using long pre-cooling periods will prolong the survival of prawns during transport. In addition, Paterson (1993) reported a rise of lactate concentration in live prawns stored at high temperature. Thus, the lactate concentration that occurs in prawns stored out of water can be used as an index of physiological stress. Chiou and Huang (2004) reported acceptable quality of the live mud crabs up to about 4 days when stored at 10 °C and 2 days at 25 °C.

The present study, therefore, investigated the changes in the quality of freshness as reflected by some biochemical changes during emersion storage using pre-cooling and chelae tying. The aim was to improve the methods for live transportation of this crab.

2. Materials and Methods

2.1. Animals

Adult intermolt *Scylla serrata* (male) with an average body weight of 150 g (145 -155 g) and an average carapace width of 9 cm (range 8-10 cm) were obtained from commercial farms in Chantaburi Province, Thailand. The crabs were transported to and maintained in the laboratory of the Department of Food Science and Technology, Rambhai Bharni Rajabhat University. All crabs were acclimated for one week prior to experimentation in polypropylene tanks with aerated seawater, mean salinity of 33±2 ppt, mean temperature of 27±1 °C under natural light-dark condition. During this period, the crabs were fed every day with chopped mussel tissue, and the seawater was changed every few days.

2.2. Handling conditions and Muscle sample preparation

The experimental live crabs were divided into four groups with three replications in each group. They were exposed to four different processing activities: (1) non pre-cooling without chelae tying (control), (2) non pre-cooling with chelae tying (following the commercial method), (3) pre-cooling at 5 °C for 5 min without chelae tying, or (4) pre-cooling at 5 °C for 5 min with chelae tying. The process of chelae tying included: (1) laying the string in front of the shell and onto the base of the claws; (2) putting a string loop under the base of claws and pulling it out to the front of the crab and then looping the string back over the base of pincer, in front of the spines, and (3) running the string in front of the swimming legs and continuing the string onto the shell, and finally, pulling the end of the string as tight as possible and tying a knot on top of the shell. Each group of crabs was accommodated in a styrofoam case that was stored at room temperature (27±1 °C) for 1-7 days. Approximately 60% of the crabs in the control treatment group survived, 80% in non pre-cooling with chelae tying treatment and pre-cooling without chelae tying treatment, and 100% in pre-cooling with chelae tying treatment for 7 days storage.)

Muscle samples were taken rapidly to avoid any muscular contraction. These samples were taken from the region of abdomen and leg (pereiopods and chelipeds) and placed on trays. Then, they were frozen by passing into liquid nitrogen, using spray a control system with a -70 °C cryogenic quick freezer. Frozen samples were wrapped in aluminum foil and stored in liquid nitrogen until analyzed.

2.3. Muscle glycogen

Muscle glycogen was measured by the colorimetric method (Carroll *et al*., 1956). The frozen muscle samples from 2.2 were freeze-dried for 24 h before grinding to a powder using a sterile pestle and mortar. Approximately 20 mg of freeze-dried ground muscle was added to 400 µl of 30% KOH before boiling for 10-20 min in a water bath maintained at 95-100 °C. Samples were cooled and added to 700 µl absolute ethanol before being placed on ice for 2 h. Samples were then centrifuged at 14,000-17,000 rpm for 10 min and the supernatants discarded. One ml of water was added to each sample prior to sonication. Fifty µl of each sonicated sample were incubated at 95-100 °C in 1 ml of anthrone reagent (Sigma, St. Louis, MO, USA) for 10 min. The absorbance of samples was measured in a spectrophotometer at 600 nm and converted to total glycogen concentrations using a series of dilutions of known concentrations.

2.4. Muscle lactate

For determination of lactate in muscle, approximately 0.8 g of frozen muscle was homogenized in 6 ml ice-cold 0.6 M perchloric acid. The homogenates were place on ice and left to extract for 30 min, then centrifuged at 11000 rpm for 5 min. The supernatant solution was neutralized by addition of 1:10 vol. 2 M potassium bicarbonate. The solution was centrifuged for 15 min at 11000 rpm for phase separation. The 50 µl of sample solutions were added to tubes containing 5 µl of 0.06 ml/ml lactate dehydrogenase (LDH), 50 µl of nicotinamide adenine dinucleotide (NAD; 2 mg/ml) and 1 ml of 0.4 M hydrazine hydrate buffer (pH 9.5) and incubated for 2 hr at 37 °C. The absorbance of sample solutions was measured in a spectrophotometer at 340 nm and converted to lactate concentrations using a series of dilutions of known concentrations (Engel and Jones, 1978; Ridgway *et al*., 2006). All chemicals were obtained from Sigma, St. Louis, MO, USA.

2.5. Muscle pH

Solutions of homogenized muscle was prepared using distilled water at a ratio of 1:10 (w/v) (Chiou and Huang, 2004). The pH of the homogenized muscle was measured by a pH meter at room temperature.

2.6. Muscle yield

Muscle tissue removed from all parts of each crab body were combined. After storage, crabs were placed in a freezer at -10 °C for 1 hr and then they were

cooked with stream at 90-100 °C for 8 min. After cooking, the total body weight of crab was recorded. All muscles were separated from the exoskeleton and weighed. The muscle yield was calculated according to Chiou and Huang (2003) as follows:

$$\text{Muscle yield (\%)} = \frac{\text{(muscle weight)}}{\text{(total body weight)}} \times 100\%$$

2.7. Volatile basic nitrogen (VBN) and Sensory properties

The volatile basic nitrogen content in the muscle samples was determined by the microdiffusion method of Conway (1950). One ml of a of trichloro acetic acid muscle extract was placed in an outer ring of a Conway unit. The 1% boric acid was then placed in an inner room of the unit. Just after adding 1 ml saturated K_2CO_3 solution to outer ring and mixing gently, the unit was covered air-tight and stood at 37°C for 60 min. The boric acid solution was then removed and titrated with 0.02 N HCl.

The degree of freshness and decomposition of the stored crab muscle was evaluated by laboratory panelists and classified into three stages mainly based on its odor : acceptable stage (no smell), stage of initial decomposition (faintly putrid smell) and stage of advanced decomposition (putrid smell) according to the method reported by Yamanaka and Shimada (1996).

2.8. Chemical composition

Moisture (AOAC 990.20), crude protein (AOAC 920.123), crude fat (AOAC 989.04), and ash (AOAC 990.20) were determined. Moisture was calculated by the weight loss of sample dried at 105 °C until constant weight. Crude protein was estimated from the total nitrogen by the Kjeldahl method. Crude fat was extracted with chloroform-methanol (2:1) mixture and dried in an air-oven. Ash of the muscle was determined after exposure to 500 °C in an oven (AOAC, 2000).

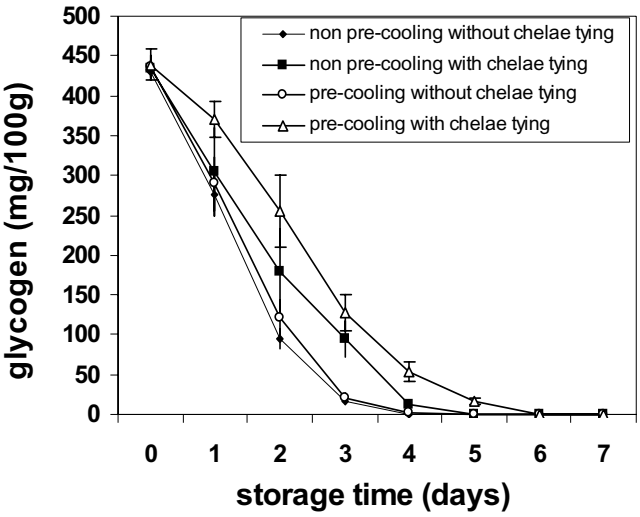

Figure 1. Changes in glycogen content (means± SD, n=12) of muscle tissue from mud crabs exposed to different conditions during emersion storage at room temperature (27±1 °C).

2.9. Statistical analysis

Statistical analysis was performed using the Analysis of Variance (ANOVA) followed by Duncan's new Multiple Range test ($p < 0.05$).

3. Results and Discussion

3.1. Changes in muscle glycogen and lactate

The initial glycogen content in muscle of mud crab averaged 435±13.9 mg/100 g. The value decreased rapidly on the first day of emersion storages (Fig. 1). The mean value of non pre-cooling without chelae tying (control) decreased from the initial value to 275±26.0 mg/100g. However, this average is not significantly different from those of pre-cooling without chelae tying (289±34.6 mg/100g) and non pre-cooling with chelae tying (304±55.2 mg/100g), but is significantly different from that of pre-cooling with chelae tying (370±22.7 mg/100g). More than 90% of the glycogen in muscle of non pre-cooling without chelae tying and non pre-cooling with chelae tying disappeared within 3 days of emersion storage while about 70% disappeared during non pre-cooling with chelae tying and pre-cooling with chelae tying within the same period of time. A rapid decrease of glycogen was also found in the snow crab muscle at 0 °C and the mud crab muscle at 25 °C (Chiou and Huang, 2004).

Emersion storage resulted in a progressive increase of lactate content in muscle of all treatment groups (Fig. 2), the increase being greatest during non pre-cooling without chelae tying. The content of lactate increased from the initial level of 1.20±0.20 mmol/kg to 14.81±0.28 mmol/kg on the seventh day of emersion storage in treatment of non pre-cooling without chelae tying, while in treatment of non pre-cooling with chelae tying, pre-cooling without chelae tying and pre-cooling with chelae tying the lactate content increased to 11.34±0.58 mmol/kg, 11.12±0.27 mmol/kg and 10.08±0.62 mmol/kg, respectively, during the same period of time. These three means were not significantly different from each other but significantly different from non pre-cooling without chelae tying. Similar results have been reported by Morris and Olive (1999a) who measured the lactate concentration in muscle of lobster, *Jasus edwardsii.* Before chilling,

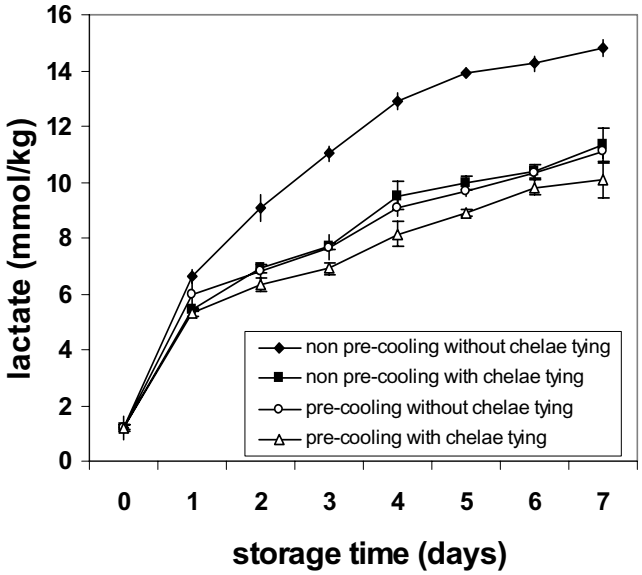

Figure 2. Changes in lactate (means ± SD, n=12) of muscle tissue from mud crabs exposed to different conditions during emersion storage at room temperature (27±1 °C).

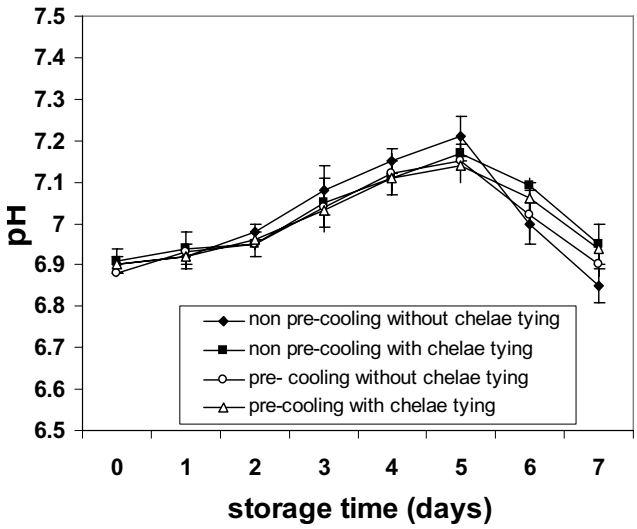

Figure 3. Changes in pH (means ± SD, n=12) of muscle tissue from mud crabs exposed to different conditions during emersion storage at room temperature (27±1 °C).

the lactate concentration was 3.9 mmol/kg. In the non-chilled and chilled (lobster was dipped in 5 °C seawater for 2 min before being packaged), the concentration had increased to 9.76 mmol/kg and decreased to 0.90 mmol/kg after 10 hr emersion storage, respectively. The lactate accumulated in the muscle comes from anaerobic metabolism (Morris and Olive, 1999a). Thus, this result indicated that the pre-cooling process could reduce accumulation of lactate and glycogen change during emersion period by reducing O_2 demand and thereby delaying the onset of anaerobiosis.

3.2. Changes in muscle pH

The mean levels of pH in muscle of mud crab in all treatments changed slightly during emersion storage (Fig. 3). The initial pH of muscle of mud crab averaged 6.90 and increased slowly in all treatments with average of 6.93, 6.96, 7.05, 7.12, and 7.17 on 1-5 days storage, respectively. The pH following all treatments decreased slightly to 6.85, 6.95, 6.90, and 6.94 on the seventh day in non pre-cooling without chelae tying, non pre-cooling with chelae tying, pre-cooling without chelae tying and pre-cooling with chelae tying, respectively. However, there was no significant difference in pH between the treatments. Chiou and Huang (2004) also reported that the pH level of abdominal muscle of mud crab *Scylla serrata* increased after 2 days of storage. The changes in pH of muscle during emersion storage may result from the metabolic alkalosis and the metabolic acidosis by mechanisms enabling metabolic compensation of acidosis induced by emersion condition. An immediate metabolic compensation in emersed, non chilled animals has been observed in some crabs and lobsters, *Jasus edwardsii*, *Homarus gammarus* in air at 10 °C (Burnett and Mcmahon, 1987; DeFur and McMahon, 1984 and Whiteley and Taylor, 1990). In the crab *Scylla serrata*, an increased pH during emersion resulted from changes in the conditions of the gill lamellae (Varley and Greenaway, 1992). Metabolic compensation can be caused by changing the strong ion difference between tissue compartments within the animal. The changes in strong ion difference is accounted for by the elevation during the period from 24 hr emersion to 7 d emersion (DeFur and McMahon, 1984). The strong ion differences of the tissues or haemolymph may result from exchange of ions between the haemolymph and tissue compartments (Varley and Greenaway, 1992). In aquatic crustaceans, the gills play the major role in buffering pH changes through ion exchange with the environment (Morris and Oliver, 1999b).

3.3. Muscle yield

Changes in muscle yield are shown in Table 1. We found a loss of muscle yield following all types of treatments, increasing with storage time. On the first day of emersion storage, the loss of muscle yield in all treatment was in the range 2-4%. After seven days of emersion storage, the loss of muscle yield had increased to 30.10±1.20, 20.95±3.90, 22.32±4.26, and 17.29±4.93% in non pre-cooling without chelae tying, non pre-cooling with chelae tying, pre-cooling without

Table 1. Percentages loss of muscle yield (means ± SD, n=12) of mud crab during emersion storage at room temperature (27±1 °C)

Handling methods	Percentages loss of muscle yield						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Non pre-cooling without chelae tying	[a]4.07±3.07	[a]8.84±5.56	[a]10.47±1.75	[a]15.21±4.34	[a]20.30±4.09	[a]26.73±9.97	[a]30.10±1.20
Non pre-cooling with chelae tying	[a]3.40±1.48	[a]6.33±1.91	[a]8.30±2.21	[a]10.62±2.12	[ab]13.24±6.36	[b]17.45±5.03	[b]20.95±3.90
Pre-cooling without chelae tying	[a]2.07±1.58	[a]5.13±3.28	[a]7.28±1.86	[a]10.74±2.03	[ab]13.05±1.95	[b]16.41±1.92	[b]22.32±4.26
Pre-cooling with chelae tying	[a]2.04±1.17	[a]4.80±2.93	[a]6.17±2.66	[a]10.06±2.66	[b]12.45±2.97	[b]12.88±6.94	[b]17.29±4.93

[a,b] different letters in the same column indicate significant differenes ($p < 0.05$)

chelae tying, and pre-cooling with chelae tying, respectively. However, the statistical test showed significantly difference only between non pre-cooling without chelae tying and all other treatments. Corresponding to muscle yield losses, death of crabs also occurred when approximately 20% of the yield had been lost on the fifth to the seventh days of storage. Loss of muscle yield may result from high metabolic rate of crabs during emersion storage and reactions that lead to chemical changes of proteins and lead to an increase in drip loss (Samet *et al*., 1996; Yamagata and Low, 1995), glycogen loss and lactate accumulation. It has also been reported that body weight losses of prawns occurred after 18 hr of air exposure; and the prawns died when there was approximately 16% loss of the initial body weight. This loss may result from a reduction of haemolymph volume as well as a slight decrease of the muscular water content. The increase in the viscosity of the haemolymph may cause problems on circulation and oxygen delivery which may result in death (Samet *et al*., 1996).

3.4. Changes in VBN and Sensory evaluation

Changes in VBN are presented in Fig. 4. There was an increase in VBN in all treatments throughout the period of storage. The initial levels of VBN averaged 3.98 mgN/100g. Non pre-cooling without chelae tying increased the VBN levels more than all other treatments throughout the period of storage. On the seventh day, the VBN levels of the non pre-cooling without chelae tying was 60.12±3.36 mgN/100g which is significantly different from the levels of the pre-cooling with chelae tying treatment (48.00±4.38 mgN/100g), but the level was not significantly different from the pre-cooling without chelae tying and non pre-cooling with chelae tying treatments (49.71±5.03 mgN/100g and 50.00±2.78 mgN/100g, respectively).

For the degree of freshness by panelists, initial decomposition was found on the second days of emersion storage in the non pre-cooling without chelae tying (VBN = 16.32±2.00 mgN/100g) and on the third day in non pre-cooling with chelae tying (VBN = 14.84±0.40 mgN/100g), pre-cooling without chelae tying (VBN = 14.52±0.98 mgN/100g) and pre-cooling with chelae tying (VBN = 14.10±0.26 mgN/100g), respectively. The stage of initial decomposition may result from the bacterial enzymes and autolysis enzymes in the muscles, for example, phosphorylase, pyruvate kinase, and phosphofructokinase which are utilized for both aerobic and anaerobic metabolism (Yamanaka and Shimada, 1996). Our results show that pre-cooling and chelae tying could delay decomposition of muscle of mud crab during emersion storage. Similar results reported in snow crab indicate that the initial decomposition of the muscle started after two to seven days of storage (Miyagawa *et al*., 1990). In Kuruma prawn and Japanese spiny lobster the decomposition of muscle started after four days to five days of storage at 5 °C, and already during the

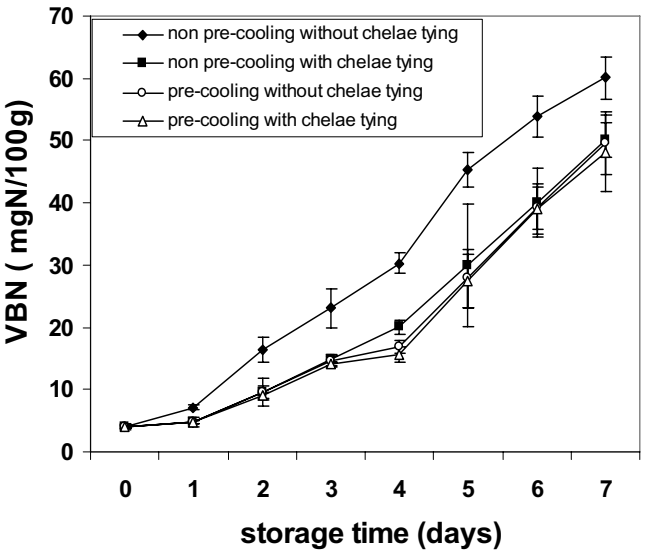

Figure 4. Changes in VBN (means ± SD, n=12) of muscle tissue from mud crabs exposed to different conditions during emersion storage at room temperature (27±1 °C).

Table 2. Percentage of chemical composition (means ± SD, n=12) of muscle tissue of mud crab during emersion storage on the seventh day at room temperature (27±1 °C)

Handling methods	Percentage of proximate composition (%)			
	moisture	protein	fat	ash
Non pre-cooling without chelae tying	80.69±1.16	15.93±0.24	0.20±0.02	1.48±0.05
Non pre-cooling with chelae tying	79.50±0.79	16.00±0.11	0.22±0.03	1.52±0.03
Pre-cooling without chelae tying	79.20±0.82	16.00±0.87	0.19±0.01	1.50±0.05
Pre-cooling with chelae tying	79.10±0.10	16.35±0.30	0.22±0.03	1.57±0.06

first day of storage at 20 °C (Matsumoto and Yamanaka,1992). These animals did not have their chelae tied, and it is possible that this caused an increase in metabolic activity and induced stress, resulting in an increased initial decomposition of the muscle of mud crab.

3.5. Changes in chemical composition

Changes in chemical composition of the muscle tissue of the mud crab are shown in Table 2. Before storage, levels of the moisture content of all treatments averaged 77.86 %, protein 17.07 %, fat 0.20 %, and ash 1.52 %. The total chemical composition changed little during emersion storage in all treatments, and there were no significant changes in moisture, protein, fat, and ash content in all treatments. During storage, the freshness of mud crab decreased with time and slightly softer texture. The loss of texture could be due to enzyme reactions, which are probably involved in many reactions that lead to crosslinking of proteins. The chemical changes of protein lead to an increase in drip loss and to an increase in expressible moisture (Yamagata and Low, 1995). This resulted in loss of freshness with softer texture, loss of muscle yield, slight ammonia odor, and poor appearance.

In conclusion, the handling by pre-cooling and having their chelae tied seemed to decrease metabolic activity of crabs which resulted in longer survival and reduced changes of physiology and quality of crabs. This indicates that pre-cooling and chelae tying can be used for the process of preparing live mud crab before transportation in emersion storage conditions.

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