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Effect of storage temperature on the quality of live mud crabs

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Abstract The chilling storage effect on the quality of live mud crabs was examined. The results showed that the chilling storage at 20℃ could decrease delayed loss of quality change with high remained glycogen (7.40±2.30 mg/100g), low accumulated lactate (9.75±0.12 mmol/kg) and 45.57±3.59 mgN/100g of VBN, while the pH decreased slightly average to 7.01±0.09. This condition is maintained muscle yield of 15.63±0.58% after 7 days. The total proximate compositions changed slightly during storage, the averaged between 78.10-79.10% of moisture content, 16.85-16.91% of protein, 0.10-0.22% of fat, and 1.4-1.54 % of ash on 4-7 days storage. Thus, result indicated that the 20℃ chilling storage can be benefit to the handling process and transport of mud crabs.

Keywords: Mud crab, Chilling storage, Glycogen, Lactate

Introduction

Live mud transport crab concerns lower temperature to reduce the metabolism and spoiling. The low temperature affects the seafood shipment wguch limited attention (Baylo, 2013 ; Syafaat *et al*., 2021). The chilled packing method is known to minimize anaerobiosis, respiration and extended shipping (Syafaat *et al*., 2021). The improved handling was to reduce physiological changes of animals leading to delay a loss of quality. Nurdiani and Zeng (2007) stated that reducing O_2 consumption of prawns can be done by cooling which leading to less susceptible and stress. Ninlanon and Tangkrock-Olan (2008) stated that long pre-cooling periods to prolong the survival of mud crabs during transport. The pre-cooling technique is to decrease a stress response and to delay the loss of muscle yield for mud crabs (Ninlanon, 2011). Biochemical changes occurs in mud crab that can be stored out of water by using as an index of physiological stress. The research finding was to find the changes in the quality of freshness which is reflected by biochemical changes during different cooling storage, which would be improved the method for live transportation of crab.

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Materials and methods

Animals

Adult intermolt of *Scylla serrata* (male) was used and averaged the body weight of 150 g (145 -155 g), and averaged carapace width of 9 cm (range 8-10 cm). Those were reveived from commercial farms in Chantaburi province, Thailand. The crabs were taken and maintained in the laboratory, Department of Food Science and Technology, Rambhai Bharni Rajabhat University. All crabs were acclimated for one week prior to test in polypropylene tanks with aerated seawater. The means of salinity test was 32±2 ppt, temperature was 27±1°C under naturally light-dark conditions. The crabs were fed in each day which chopped the mussel tissue during this period. The seawater was changed every three days.

Experimental design and muscle sample preparation

The live crabs were set up to five groups with three replications in each group. Each crab test was accommodated in a Styrofoam case of 20x 33x18 cm, and kept at 10, 15, 20, 25 and 30 °C served as control for 7 days which was internal temperature of cases. The crabs were moved everyday, then the muscle samples were analyzed for pH level, glycogen, lactate, muscle yield, VBN together with chemical compositions such as ash, fat, moisture, and protein . Muscle samples were rapidly taken to avoid the muscular contraction. Samples were then taken from region of abdomen, and placed in trays, then frozen by quick freezer. Frozen samples were wrapped with aluminum foil and kept in cold air at -20°C until analysis.

Analyses

Muscle glycogen was performe by the colorimetric method which modifired from (Barton and Iwama 1991). The samples of frozen muscle were freeze-dried for 24 h prior to grind into power form by sterile pestle and mortar. of freeze-dried ground muscle at approximately 20 mg which added to 400 µl of 30% KOH prior to boil for 10-20 min in a water bath and maintained the temperature at 95-100°C. The tested samples were cooled and added into 700 µl absolute ethanol prioer to place in ice for 2 h. The tested samples were then centrifuged at 14,000-17,000 rpm for 10 min, and the supernatants was discarded. One ml of water was added into each sample prior to sonicate. The fifty µl of each sonicated sample was incubated at temperature of 95-100°C in

1 ml anthrone reagent for 10 min (Sigma, St. Louis, MO, USA). The absorbance was measured the sample in a spectrophotometer at 600 nm, and converted to total glycogen concentrations by using a dilution series of known concentrations.

Lactate in muscle was dertermined by approximately 0.8 g of freeze muscle which homogenized in 6 ml ice-cold 0.6 M perchloric acid. The homogenates were placed in ice and extracted for 30 min, and centrifuged at 11000 rpm for 5 min. Supernatant was neutralized by adding at a ratio of 1:10 vol in 2 M potassium bicarbonate. The solution was centrifuged for 15 min at 11000 rpm to separate. 50 μl of sample solution was added into tubes which contained 5 μl of 0.06 ml/ml lactate dehydrogenase (LDH), 50 μl of nicotinamide adenine dinucleotide (NAD; 2 mg/ml), 1 ml of 0.4 M hydrazine hydrate buffer (pH 9.5), and incubated at temperature of 37° C for 2 h were done. The absorbance was measured the sample solution in a spectrophotometer at 340 nm. and converted to lactate concentrations using the dilution series which compared with the known concentrations (Engel and Jones, 1978 ; Ridgway *et al*., 2006).

Homogenized muscle solution was prepared by using sterile distilled water at a ratio of 1:10 (w/v) according to Chiou and Huang (2004). pH of homogenized muscle was evaluated by pH meter at room temperature.

Volatile basic nitrogen (VBN) content of muscle samples were evaluated by the microdiffusion method which modified from Conway (1950). One ml of trichloro acetic acid of muscle extract was placed into an outer ring of a conway unit. 1% boric acid which further placed in an inner room of the unit. Thereafter adding 1 ml saturated K_2CO_3 solution to outer ring was done. It was gently mixed to homogenize. The unit was covered air-tight and kept at temperature of 37°C for 60 min. The boric acid solution was done by titration with 0.02 N HCl.

Muscle yield was measured. The total body weight of crab were recorded before cooking. Crab was freezed at temperature of -10°C for 1 hr and cooked with stream at temperature of 100°C for 8 min. All muscles were separately performed with the exoskeleton and then weighed. The muscle yield was calculated with modification from Chiou and Huang (2003) as follows:

Muscle yield (%) is calculated as muscle weight × 100/ total body weight, and Muscle loss (%) is calculated by weight loss of muscle yield x 100%/ initial weight of muscle yield.

Ash, crude protein, fat, andmoisture were determined according to AOAC (1984). Moisture was calculated by the weight loss of dried sample at temperature of 105°C until constant weight. Crude protein was estimated by the total nitrogen in a factor of 6.25, and the muscle was analyzed by the

Kjeldahl method. Crude fat was perform the extraction with chloroform-methanol at a ration of 2:1 v/v, and dried in hot air-oven. Ash was determined after exposure to 500°C in hot air oven.

Statistical analysis

Statistical analysis was done using the Analysis of Variance (ANOVA) and mean in each treatment was computed to compare by Duncan's Multiple Range Test at $p < 0.05$.

Results

Changes in muscle glycogen and lactate

The glycogen in muscle was decreased in the first day of storage at all tested temperatures. The glycogen concentration was reduced with similar trends in all temperatures (Figure 1). The glycogen disappeared over 90 % in 4 days after storage at temperatures of 10 ℃, and 25 ℃ compared to the control, while the temperartures of 15 and 20 ℃ showed the glycogen disappeared in 5 days after storage. The concentrations of muscle glycogen decreased from initial averaged 435.7±10.0 mg/100g to 9.80±5.97, 11.20±2.28, and 4.40±3.36 mg/100g at temperature of 10 ℃, 25 ℃, and control, respectively in 4 days after storage. 50 days after storage at temperartures of 15 ℃ and 20 ℃ which averaged 3.00±2.92 and 7.40±2.30 mg/100g, respectively. The glycogen concentrations was observed at temperatures of 15 and 20 ℃ which were significantly differed when compared to the control in 2-4 days, and 25 ℃ in 3-4 days, but there was significantly differed from the temperature of 10 ℃ at all storage times. While the 10 ℃ was not significantly differd from the control at all periods of storage.

There was an increased in lactate concentration in both temperature and of period of storage (Figure 2). The miud crabs were greatest increased which exposed to higher temperature and longer period of storage. All tested temperatures led to increase lactate concentration after the first day of storage. The lactate concentration was steadily increased in 7 days after storage, which increased lactate cocentrartion of 9.96±0.09 mmol/kg of 25 ℃ which there was not significantly differed when compared to the control. Throughout the storage, lactate concentration was largest increased in iactate at the control treatment by 10.00±0.08 mmol/kg in 7 days after storage. The other treatments showed that the lactate concentrations were increased significantly lower than temperature at 25 ℃. The lactate contents was steadily increased with averaged 6.83±0.23 mmol/kg at 10 ℃ for 4 days after storage, which was 8.59±0.36

mmol/kg at temperarture of 15℃ in 6 days after storage, and 9.75±0.12 mmol/kg at 20℃ in 7 days after storage.

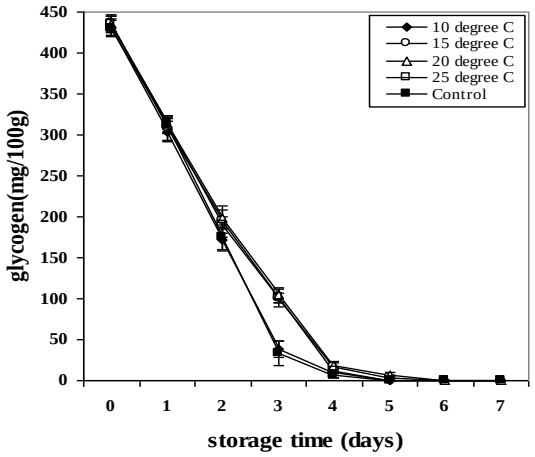

Figure 1. Changes in glycogen content (means ± SD, n=15) of muscle tissue from mud crabs exposed to different temperature during emersion storage

Crabs were died approximately 40% at temperature of 10℃ in 4 days and reached to 100% in 5 days after storage. Crabs were died approximately 30% and 100% at tempersrture of 15 ℃ in 5 and 7 days after storage. All crabs were survived 100% at temperatures of 20 ℃, 25 ℃ and control in 7 days after storage.

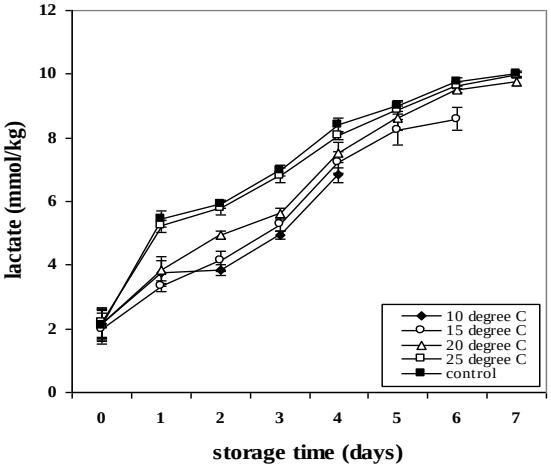

Figure 2. Changes in lactate (means ± SD, n=15) of muscle tissue from mud crabs exposed to different temperature during emersion storage

Changes in muscle pH

The muscle pH was changed and varied with different temperatures. The pH in muscle of all treatments was slowly changed with the initial pH which averaged 6.85±0.05 (Figure 3). They were slowly increased the muscle pH in all treatments with averaged of 7.02±0.04, 7.03±0.04, 7.06±0.05, 7.11±0.04, and 7.15±0.03 in 1-6 days after storage at temperatures of 10℃, 15℃, 20℃, 25℃ and control, respectively. The muscle pH was slightly decreased which averaged to 7.01±0.09, 7.04±0.04, and 7.00±0.11 in 7 days after storage at temperatures of 20℃, 25℃, and control, respectively but it observed all crabs were died that temperastures of 10℃ and 15℃ but there were not significantly differed in muscle pH in treatments. The change of muscle pH was higher with high temperature of 25℃ and in control.

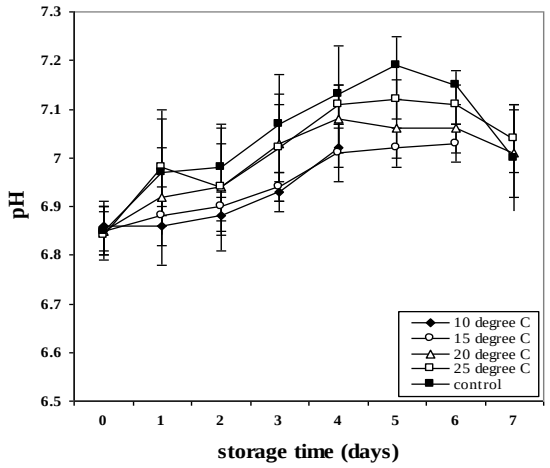

Figure 3. Changes in pH (means ± SD, n=15) of muscle tissue from mud crabs exposed to different temperature during emersion storage

Changes in muscle yield

The effects of temperatures and storage duration resulted to decrease the muscle yield with the initial weight averaged 151.1 g (Table 1). The muscle yield lost in all treatments during storage period with the temperatures increased. The muscle yield was lost at lower temperatures but less than at higher temperature during storage perio. It found that the storage at temperarture of 10˚C was not significantly differed compared to the temperastures of 15℃ and 20℃ throughout the storage period. The muscle yield was greatest loss in mud crabs at control treatment to 16.35±0.67% in 7

days after storage but it was not significantly differed at temperatures of 20 ℃ and 25 ℃ which averaged 15.63±0.58 and 16.27±0.29% in 7 days after storage, respectively. There were not significantly changed at temperartures of 10 ℃ and 15 ℃ after storage. It found that tempersture of 20 ℃ were averaged 9.86±0.24% in 4 days and 12.16±0.20% in 6 days of storage period, respectively.

Table 1. Percentages loss of muscle yield (means ± SD, n=15) of mud crab during emersion storage at different temperatures

Storage temperature	Percentages loss of muscle yield						
	Day 1	**Day 2**	**Day 3**	**Day 4***	**Day 5**	**Day 6**	**Day 7***
10 ℃	^{b}2.10±0.12	^{a}4.09±0.10	^{a}5.65±0.34	9.86±0.24	-	-	-
15 ℃	^{a}1.95±0.09	^{a}4.08±0.12	^{a}5.33±0.27	9.73±0.35	^{a}11.11±0.64	^{a}12.16±0.20	-
20 ℃	^{a}1.99±0.08	^{a}4.02±0.09	^{a}5.33±0.33	9.68±0.32	^{a}11.11±0.75	^{a}12.25±0.19	15.63±0.58
25 ℃	^{ab}2.06±0.08	^{b}4.34±0.17	^{b}6.09±0.31	10.10±0.55	^{ab}11.94±0.53	^{bc}12.53±0.21	16.27±0.29
Control	^{b}2.13±0.02	^{b}4.57±0.25	^{b}6.11±0.31	10.20±0.62	^{b}12.12±0.57	^{c}12.86±0.23	16.35±0.67

^{a,b,c} different letters in the same column indicate significant differences ($p< 0.05$)
^{ns} not significantly differences ($p> 0.05$)

Changes in VBN

Changes in Volatile basic nitrogen (VBN) are presented in Figure 4. The VBN was continuously increased in storage with time and temperatures in all treatments. The initial VBN was 3.78±0.08 mg/100g., and in control was significantly higher than the other temperatures at 10 ℃, 15 ℃, and 20 ℃, but not significantly differed at temperstrure of 25 ℃ throughout the storage period, then it reached the maximum level of VBN in n 4 days after storage at 10 ℃ which was 9.03±0.59 mgN/100g, 6 days after storage at 15 ℃ was 15.29±1.47 mgN/100g. It found that 7 days after storage was averaged 30.42±3.33 mgN/100g. 43.42±3.56 mgN/100g, and 45.57±3.59 mgN/100g at temperartures of 20 ℃, 25 ℃, and control, respectively. It was increased VBN levels at lower temperatures than higher temperatures.

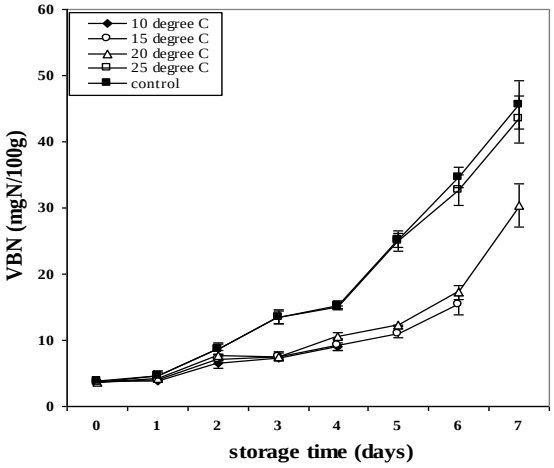

Figure 4. Changes in VBN (means ± SD, n=15) of muscle tissue from mud crabs exposed to different temperature during emersion storage

Changes in chemical composition

The proximate compositions were changed with times and temperatures (Table 2). The total proximate compositionswas slightly changed during storage period. The moisture content of all treatments was averaged between 78.10 to 79.10%, protein 16.85 to16.91%, fat 0.10 to 0.22%, and ash 1.4 to 1.54% in 4-7 days after storage. According to storage temperature, the mud crabs stored at lower temperatures of 10℃, 15℃, and 20 ℃ maintained higher proximate compositions than the higher temperature, especially in protein content. There was significantly differed at temperature of 25℃ and control treatments. The highest change was found in the control throughout the storage period.

Table 2. Percentage of proximate compositions (means ± SD, n=15) of muscle tissue of mud crab during emersion storage on 4-7 days at different temperature

Storage temperature	Percentage of chemical compositions (%)			
	Moisture*	protein	Fat*	Ash*
10˚C	78.10±0.50	[a]16.91±0.14	0.18±0.03	1.51±0.02
15˚C	78.50±0.53	[a]16.90±0.21	0.20±0.04	1.50±0.03
20˚C	79.00±0.52	[a]16.90±0.11	0.19±0.02	1.50±0.02
25˚C	79.10±0.25	[b]16.55±0.17	0.21±0.04	1.51±0.02
Control	79.10±0.20	[b]16.45±0.20	0.20±0.03	1.52±0.03

[a,b] different letters in the same column indicate significant differences ($p < 0.05$)
[ns] not significantly differences ($p > 0.05$)

Discussion

The storage period of mud crab at lower and higher temperatures resulted to change the glycogen and lactate concentration in muscle tissue. The changes in glycogen and lactate concentration of crabs during storage possible be caused by switching to anaerobic metabolism. The metabolic rate of crustacean is varied directly to change in temperature when temperature is lowered in the heart rate and decreased the rate of oxygen consumption (Morris and Oliver, 1999; Skudlarek *et al*., 2011; Tsai *et al*., 2019). The result demonstrated that accumulation of lactate was correlated to tolerate exposure to air of crab during storage period. Whereas, the change of muscle glycogen was not related to accumulate lactate, loss of glycogen which observed duringstorage period at lower and higher temperatures. Lower temperature found to be not significantly differed in muscle glycogen as compared to the control, while at higher temperatures were significantly decreased in glycogen contents, and subsequently increased in lactate contents more tha low temperature. It delayed anaerobiosis activity and lactate accumulationat lower temperature which found in last longer than the higher temperature. Ridgway *et al*. (2006) stated that lactate concentration in the haemolymph of *N. norvegicus* exposed to air at temperature of 25℃ for 4 h revealed more than double of those observed in animals which exposed at the same duration in temperatures of 10℃ and 15℃. It is well known that the metabolic rate of aerial exposure in animal would increase with temperature and resulted to increase the energy demand and leaded to greater lactate accumulation. However, the lower temperature would affect to deplete the glycogen storage in muscle due to the crabs resisted the cold temperature with increased in energy demand by low metabolic activity leading to accumulate the lactate to be lower than the higher temperatures of 25℃ and in control treatment.

Several authors reported that a low pH in crustacean haemolymph resulted from respiratory and metabolic acidosis due to increase the lactate and bicarbonate-carbonic acid during anaerobic metabolism (Tsai *et al*., 2019). Therefore, the chilled emersion storage influenced to change the muscle pH of mud crabs, and correlated to increase in the accumulated of lactate leading to delay the decomposition of muscle crabs.

The results of muscle yield loss correlated to decline glycogen which may result from loss of texture and increases in moisture in tissue. It leaded to be unacceptable on sensory quality of mud crabs. The difference in yield loss may result from the unacceptably high metabolic rate of the crabs when they are directly transferred to variuos temperatures from the optimum temperature. The elevated metabolic rate would probably be leaded to deplete the tissue for

glycogen concentration (Ridgway *et al*., 2006), and moved to be premature for exhaustion of tissues. Moreover, the observed weight loss was probably due to experimental differences of handling process and temperatures, which affected in the water lost from the body fluids of crab.

VBN was increased in the muscle that may correlate with pH. These could be due to decompose by microorganism leading to decrease in odor, texture, and color quality, and increased in VBN, pH, and indole (Ninlanon, 2011). Thus, the storage period at low temperature could delay the decomposition of muscle more than at higher temperature. The same result was reported by Chiou and Huang (2004) who found that VBN of mud crabs increased to 59.0 mg/100g in 6 days at temperarture of 10 ℃ and 43.8 mg/100g in 3 days at temperature of 25 ℃ during the initial time to decompose the muscles. Therefore, the temperature of storage was more important factor for storage period than the others at the optimum temperature of 20 ℃. It prolonged the survival of crabs during the same period of storage period resukted to give the VBN value in the lower than higher temperature.

The proximate compositions related to low temperature that may affect to low activity and less biochemical reaction changes of mud crabs. Lorenzon *et al*. (2007) stated that the change of animal compositions could be caused by aerial exposure and high temperature which related to higher energy demand and compensatory responses. As reported by Pascual *et al.* (2003) in shrimp *Litopenaeus setiferus*, stated that the general metabolism of crustacean increased in consequencely raised temperature which required an increment in energy intake. Lipids and proteins could be used to repair membranes. The changes in content may cause by biochemical reaction of bacterial and autolysis enzymes leading to decompose chemical compositions and nutritional values was then decreased (Tsai *et al*., 2019).

It concluded that mud crabs which stored at temperatures of 10 ℃, 15 ℃, 25 ℃ and control treatment affected to metabolic process. It was found that higher temperature of 25 ℃ and in control to accelerate the chemical reaction and respiration of crab leading to be unacceptable quality. While lower temperature prolonged storage time and its qualities. However, low temperatures of 10 ℃ and 15 ℃ may harmful to animals by causing stress response leading to be resistivity decreased. Thus, the optimal temperature for stored mud crabs with storage period suggested at temperature of 20 ℃ which found 100% survival rate and retained glycogen, muscle yield and nutritional values more than other temperatures. Thus, the storage temperature used for simulated live shipment is suggested at temperature of 20 ℃.

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