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Low-temperature combined with high-humidity thawing improves the water-holding capacity and biochemical properties of *Portunus trituberculatus* protein

Jiangang Ling（凌建刚）[1,2], Xiaoting Xuan（宣晓婷）[2], Zihan Xu（徐子涵）[3], Tian Ding（丁甜）[1], Xudong Lin（林旭东）[2], Yan Cui（崔燕）[2,*] and Donghong Liu（刘东红）[1,*]

[1]College of Biosystems Engineering and Food Science, National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang University, Hangzhou, China; [2]Institute of Agricultural Products Processing, Key Laboratory of Preservation Engineering of Agricultural Products, Ningbo Academy of Agricultural Sciences, Ningbo, China and [3]College of Food, Shenyang Agricultural University, Shenyang, China

*Correspondence to: Donghong Liu, Department of Food Science and Nutrition, National Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang University, Hangzhou 310058, China. E-mail: dhliu@zju.edu.cn; Yan Cui, Institute of Agricultural Products Processing, Key Laboratory of Preservation Engineering of Agricultural Products, Ningbo Academy of Agricultural Sciences, Ningbo 315040, China. E-mail: cuiyan1605@126.com

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Abstract

This study compared the effects of conventional thawing methods (water immersion thawing (WIT, (25±1) °C), natural air thawing (AT, (25±1) °C, relative humidity (RH) (65±2) per cent), refrigerator thawing (RT, 4 °C, RH (80±2) per cent) and low-temperature (LT) combined with high-humidity thawing (LT, –1 °C to 1 °C (LT–1–1), 2–4 °C (LT2–4), 5–7 °C (LT5–7) and 8–10 °C (LT8–10), RH≥95 per cent) on the water-holding capacity, lipid oxidation and biochemical properties of *Portunus trituberculatus (P. trituberculatus)* myofibrillar protein. The results showed that WIT and AT significantly decreased the water-holding capacity while dramatically increasing lipid oxidation, protein oxidation and degeneration, resulting in serious *P. trituberculatus* quality deterioration. High humidity was beneficial for *P. trituberculatus* thawing. The thawing time of *P. trituberculatus* under the conditions of LT2–4 was only 39.39 per cent of that of conventional air thawing at 4 °C (RT), and the LT2–4 samples not only maintained better water-holding capacity but also had an obviously reduced degree of lipid oxidation, protein oxidation and denaturation. Thawed samples LT2–4 and LT5–7 provided better maintenance of *P. trituberculatus* quality than the LT–1–1 and LT8–10 samples. The best quality was exhibited after thawing at 2–4 °C. The levels of thiobarbituric acid reacting substances, carbonyl content and surface hydrophobicity observably decreased in these samples, while the total sulfhydryl contents dramatically increased compared to those of conventionally thawed samples, indicating lower lipid oxidation and protein oxidation. Moreover, the Ca^{2+}-ATPase activity of the sample thawed at 2–4 °C (2.06 μmol Pi/mg prot/h) was markedly higher than that of samples subjected to WIT and AT. The product qualities observed after thawing at –1 °C to 1 °C, 5–7 °C and 8–10 °C under LT were comparable to that observed by RT. Considering its thawing

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efficiency and product quality, LT is a suitable method for the thawing of *P. trituberculatus*, and the ideal thawing conditions were LT at 2–4 °C.

Keywords: *Portunus trituberculatus*; thawing; low temperature combined with high humidity; water-holding capacity; lipid oxidation; biochemical properties of myofibrillar protein.

Introduction

Swimming crabs (*Portunus trituberculatus*) are important economical farmed crabs that are widely distributed in the coastal areas of China. Swimming crabs are seasonal and die easily after being caught. Once dead, the protein and fat in their bodies rapidly decompose under the action of enzymes and microorganisms, leading to spoilage and deterioration. Therefore, freezing is the main long-term storage method for the market circulation of these crabs, and thawing is a necessary step before secondary processing or eating. However, an improper thawing method may greatly degrade the quality of these crabs after considering factors such as drip loss, lipid oxidation, protein denaturation, loose muscle tissue, and dark color (Lorentzen *et al*., 2020). Thus, it is necessary to choose an appropriate thawing method to maximize the freshness of frozen crabs.

Generally, thawing with air, in water and at low temperature (4 °C in a refrigerator) are the traditional methods; however, these methods face the problems of low efficiency and easy microbial contamination (Chandirasekaran and Thulasi, 2013). Therefore, new thawing methods have been used to overcome these problems, such as low-temperature combined with high-humidity thawing (LT) (Li *et al*., 2014a, 2014b), microwaves (Manios and Skandamis, 2015), ultrahigh pressure (Truong *et al*., 2015), ohmic heating (Richa *et al*., 2017), and high-voltage electrostatic fields (Mousakhani-Ganjeh *et al*., 2016). Among these new thawing methods, the LT method is cost-effective because it requires little equipment, improves the thawing efficiency, and maintains the quality of frozen products (Li *et al*., 2014a).

The LT method was developed from refrigerator thawing, and the thawing temperature is usually set within the range of 2–8 °C, together with the relative humidity adjusted to above 90 per cent. Currently, this technique has been widely used in the cattle, sheep, chicken and other livestock and poultry product thawing fields to realize industrialization (Zhang *et al*., 2013; Li *et al*., 2014a, b; Zhang *et al*., 2017). Li *et al*. (2014b) found that, compared with conventional 4 °C air thawing, high humidity can accelerate thawing and form a water film on the meat surface to isolate oxygen, thus slowing the drip loss and protein oxidation of meat during thawing. In recent years, studies have shown that thawing at low temperatures with high humidity also has great advantages in thawing aquatic products such as pomfret (Cui *et al*., 2018) and eel (Ersoy *et al*., 2008). The thawing time of pomfret under LT (2–4 °C) is only 2/3 that of conventional 4 °C air thawing. After thawing, pomfret not only maintained good water retention and texture but also had an obviously reduced degree of protein oxidation and denaturation. In addition, Ersoy *et al*. (2008) indicated that high relative humidity during low-temperature thawing was required to minimize weight loss, and this theory was demonstrated by Li *et al*. (2014b). However, until now, there have been few reports on LT in the field of aquatic products and the thawing of swimming crabs.

In this paper, the effects of the LT method on the thawing efficiency, drip loss, pH, and total mercapto and carbonyl contents of swimming crabs were examined. This study aims to systematically investigate the effects of LT on the water retention and protein biochemical characteristics of crab muscle, and provide a theoretical basis for the application of the LT method in aquatic crab products to control quality.

Materials and Methods

Materials and reagents

Fresh swimming crabs ((300±20) g average body weight) were purchased from Lulin Aquatic Market in Ningbo, China, and transported alive under an oxygen supply to the laboratory within 30 min in August. After cleaning, the live crabs were painlessly killed using ice water. The water on the surface of the crabs was rapidly drained with a fanner. Then, the crabs were rapidly packed and frozen at –80 °C, and subsequently stored at –18 °C until the subsequent thawing experiment. The Ca^{2+}-ATPase kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All reagents used were of analytical grade and obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

Sample treatment

Fresh silver pomfret samples were set as a blank control (fresh). The 210 frozen swimming crabs were randomly divided into seven groups and subsequently treated by water immersion thawing (WIT, (25±1) °C), natural air thawing (AT, (25±1) °C, relative humidity (RH) (65±2) per cent), refrigerator thawing (RT, 4 °C, RH (80±2) per cent) or LT. WIT was carried out in a (25±1) °C water bath. AT was carried out in a (25±1) °C room. RT was carried out using a refrigerator at 4 °C. LT was carried out at –1 °C to 1 °C (LT–1–1), 2–4 °C (LT2–4), 5–7 °C (LT5–7) and 8–10 °C (LT8–10). The relative humidity in the whole LT experiment was ≥95 per cent. The geometric center temperature of the samples was detected with a temperature sensor, and the standard for complete thawing was when the geometric center temperature reached 0–2 °C. The time required for the geometric center temperature to reach 0–2 °C was considered the thawing time (Thanonkaew *et al*., 2006).

All treatments were performed in triplicate. After thawing, a portion of the samples were immediately kept in an ice bath. The thawing properties and quality indexes were measured within 2 hours. The remaining samples were frozen and stored at –80 °C for biochemical assays.

Determination of thawing loss

Thawing loss of the frozen swimming crab was calculated as the difference between the weight of frozen sample (M_0) and weight of thawed sample (M_1), and expressed as a percentage of M_0 (Xia *et al*., 2012):

$$\text{thawing drip loss}\,(\%) = \frac{M_0 - M_1}{M_0} \times 100\%$$

Determination of cooking loss

Cooking loss was evaluated according to the method reported by Huang *et al*. (2020). Thirty grams of the crab muscle (W_1) was

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accurately weighed and placed in a plastic bag and then cooked in an 80 °C water bath for 30 min. After cooling, filter paper was used to absorb the surface moisture, and the crab muscle was weighed again (W_2). The cooking loss was expressed as the percent weight difference between the thawed and cooked crab muscle (W_1–W_2) based on the weight of the thawed sample (W_1).

Determination of water-holding capacity (WHC)

WHC was determined using the centrifugation method according to Liu *et al.* (2020). Muscle samples (approximately 10 g, M_M) were accurately weighed and placed into centrifuge tubes with cotton and filter papers at the bottom. After centrifugation at 4500 rpm and 4 °C for 5 min, the samples were removed and reweighed (M_N). The WHC was expressed as centrifugal loss, which was calculated using the following equation:

$$\text{WHC}\ (\%) = \frac{M_M - M_N}{M_M} \times 100\%$$

Determination of pH

Minced muscles were homogenized with 9 volumes of precooled physiological saline. After standing for 30 min, the filtrates were immediately used to assay the pH values with a PB-10 pH meter (Sartorius, Gottingen, Germany).

Determination of thiobarbituric acid reacting substances (TBARS)

TBARS was determined according to the method described in our previous study (Cui *et al*., 2018). Briefly, a crab muscle sample (10.0 g) was homogenized with 50 mL of 7.5 per cent trichloroacetic acid (containing 0.1 per cent elhylene diamine tetraacetic acid). After extraction for 30 min, the mixture was filtered. Five milliliters of filtrate was added to 5 mL of 0.02 mol/L thiobarbituric acid solution. The mixture was heated in a boiling water bath for 40 min followed by cooling and centrifugation at 5500 rpm for 25 min. The supernatant was blended with 5 mL of chloroform, and the absorbance of the supernatant was measured at 532 nm and 600 nm by using a 752S spectrophotometer (Shanghai Lengguang Technology Co., Ltd., Shanghai, China). The TBARS value was calculated using the following equation and is expressed as mg of malonaldehyde/kg of muscle sample (mg MDA/kg):

$$\text{TBARS}\ (\text{mg MDA/kg}) = \frac{(A_{532} - A_{600}) \times V \times 10^{-3}}{\varepsilon \times d \times m} \times 72.06 \times 10^3 \times 1000$$

where A_{532} and A_{600} are the absorption values at 532 nm and 600 nm, respectively; V is the total reaction volume (mL); ε is the molar extinction coefficient of MDA, 155 000 L/(mol·cm); d is the optical path, 1 cm; and m is the amount of sample participating in the reaction (g).

Myofibrillar protein preparation

Myofibrillar protein was prepared from crab muscle according the method described by Xiong *et al.* (2009) and Lin *et al.* (2015). The whole preparation process was carried out at 4 °C. The obtained myofibrillar protein solution was aliquoted and stored at −80 °C before analysis. The protein concentration was determined by the Biuret method using bovine serum albumin as the standard.

Determination of total sulfhydryl (T-SH) content

The T-SH content in the myofibrillar protein was determined by Ellman's method (Ellman, 1959; Cui *et al.*, 2019). A molar extinction coefficient of 13 600 L/(mol·cm) was utilized to calculate the T-SH content. The T-SH levels in the myofibrillar protein are expressed as nmol/mg prot.

Determination of carbonyl content

The carbonyl content of myofibrillar protein was determined by the 2,4-dinitrophenylhydrazine colorimetric method (Oliver *et al.*, 1987; Zhu *et al.*, 2017). The molar extinction coefficient of 22 000 L/(mol·cm) was used to calculate the carbonyl content, and the results are expressed as nmol/mg prot.

Determination of surface hydrophobicity

The surface hydrophobicity of myofibrillar protein was determined by the binding of bromophenol blue to hydrophobic amino acid residues on the protein surface (Chelh *et al.*, 2006; Wang *et al.*, 2017). A 1 mL aliquot of myofibrillar protein samples was reacted with 200 µL of 1 mg/mL bromophenol blue solution for 10 min at room temperature. After centrifugation at 10 000 rpm for 15 min, the supernatant was diluted 10-fold, and the absorbance at 595 nm was measured. Tris–maleate (0.6 mol/L KCl-20 mmol/L, pH 7.0) was used as a blank sample. The surface hydrophobicity value was expressed by the amount of bromophenol blue bound to each milligram of protein (µg/mg prot). The calculation formula was as follows:

$$\text{Surface hydrophobicity}\ (\mu\text{g/mg prot}) = \frac{A_0 - A_1}{A_0 \times C}$$

where A_0 and A_1 are the absorbance values at 595 nm of the blank sample and the sample, respectively, and C is the protein concentration (mg/mL) of the sample.

Determination of Ca^{2+}-ATPase activity

Samples were homogenized with 9 volumes of precooled physiological saline. After centrifugation at 2500 rpm for 10 min, the supernatants were diluted and immediately used for Ca^{2+}-ATPase activity measurement. The Ca^{2+}-ATPase activity determination was carried out according to the protocol of the corresponding kit, and the results are expressed as the amount of inorganic phosphorus released by the ATPase decomposing ATP per milligram of protein per hour (µmol Pi/mg prot/h).

Statistical analysis

All measurements were performed in triplicate and the data were expressed as means±standard deviations. The data were compared and analyzed by one-way analysis of variance and Duncan's test with SPSS 18.0, and the correlation between each index was analyzed at the same time. Differences were considered significant at the level of $P<0.05$.

Results and Analysis

Effects of the thawing method on thawing time

The thawing times of the swimming crabs under different thawing conditions are shown in Figure 1A. High humidity contributed favorably to the thawing of the swimming crabs, and the thawing time under high humidity was much shorter than that under traditional air thawing, including the RT and AT groups. In the LT–1–1, LT2–4, LT5–7 and LT8–10 groups, the thawing time were 54.01 per cent, 63.61 per cent, 76.21 per cent and 83.06 per cent lower than that of the RT group (4 °C, RH (80±2) per cent) and

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5.49 per cent, 25.21 per cent, 51.11 per cent and 65.18 per cent lower than that of the AT group ((25±1) °C, RH (65±2) per cent), respectively. Under high-humidity conditions, water vapor condensed to release a large amount of latent heat and attached to the surface of the crab body to form a water film. Further, as water has a higher thermal conductivity than air, the accelerated heat transfer led to accelerated melting of the ice crystals so that the thawing time of the crabs was shortened in the LT groups (Peng *et al.*, 2019). Zhu *et al.* (2019) found that high humidity (RH 90 per cent) can significantly shorten the thawing time of pork, which was only 9 h at 4 °C (47.06 per cent shorter than that of refrigerator thawing at 4 °C and a RH of 65–73 per cent). Some studies have found that high humidity can also increase the thawing rate of beef (Di and Jin, 2015) and butterfish (Cui *et al.*, 2018), which is consistent with the results of this experiment.

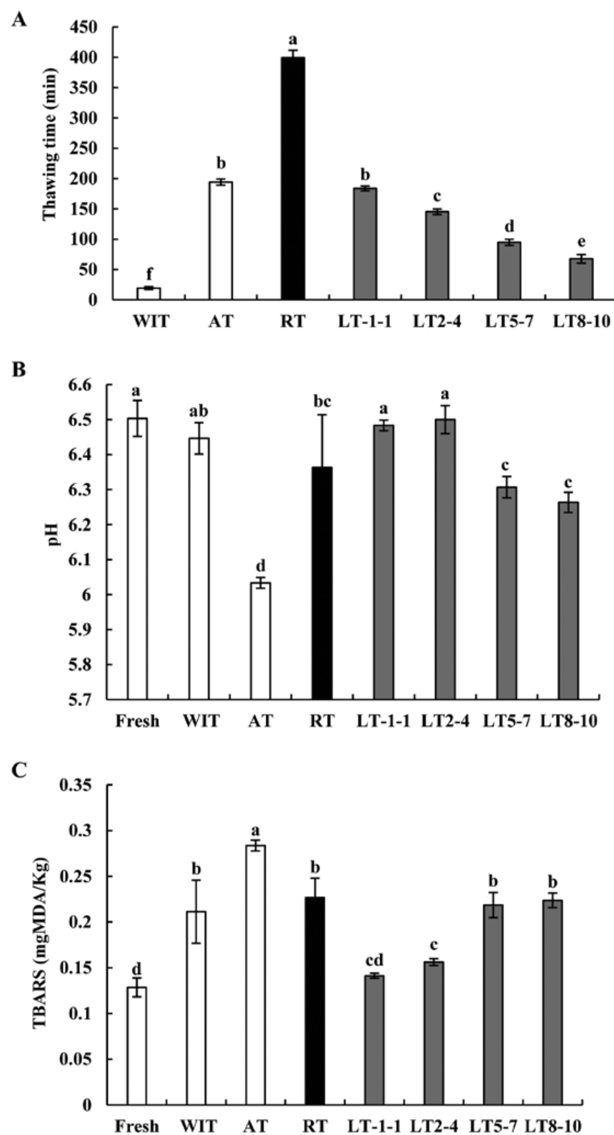

Figure 1. Effects of thawing methods on the thawing time (A), pH (B) and lipid oxidation (C) of *Portunus trituberculatus.* Values followed by different Superscripts indicate significant differences (P<0.05). AT, air thawing; LT, low-temperature combined with high-humidity thawing; RT, refrigerator thawing; TBARS, thiobarbituric acid reacting substances; WIT, represents water immersion thawing.

Effects of the thawing method on water retention of swimming crab muscle

Drip loss is an important indicator of muscle water retention, which directly affects the quality, weight and sensory properties of the product. To explore the effects of low-temperature high-humidity thawing on the water retention in swimming crab muscle, the drip loss after thawing, cooking and centrifugation was determined. As shown in Table 1, there were significant differences in thawing loss among the different thawing methods. The thawing loss of the LT2–4 group was the lowest (3.38 per cent) and was significantly lower than that of the WIT and AT methods (P<0.05), but not significantly different from that of RT. Compared with WIT and AT, the thawing loss in the LT–1–1 and LT5–7 groups decreased significantly (P<0.05), indicating that low-temperature high-humidity thawing is beneficial to reduce the nutritional loss of swimming crabs during thawing. From the point of view of cooking loss, the freezing–thawing process led to an accelerated drip loss in swimming crab muscle during cooking, and the cooking loss of each group increased to varying degrees, among which the lowest cooking loss of 27.55 per cent was found in the LT2–4 group. Compared with that of fresh swimming crabs, the cooking loss after low-temperature high-humidity thawing (LT–1–1, LT2–4 and LT5–7) showed no significant change but significantly decreased by 11.72–18.05 per cent (P<0.05) when compared with that of WIT, indicating that the low-temperature and high-humidity environment can reduce the nutritional loss in the cooking process of thawed swimming crabs. After thawing, the centrifugal loss from swimming crabs showed an upward trend, in which the centrifugal loss of the LT5–7 group was the lowest (12.26 per cent), followed by the LT2–4 group, which was significantly lower than that of the three traditional thawing methods (WIT, RT and AT); additionally, no significant change was recorded when compared with the fresh sample (P>0.05). This finding may be closely related to the lower thawing temperature and shorter thawing time.

According to the comprehensive analysis, thawing at a low temperature (2–7 °C) and high humidity (LT2–4 and LT5–7) can effectively maintain the water-holding capacity of swimming crabs, which is similar to the results found in the study of thawing of butterfish and pork at low temperature combined with high humidity (Cui *et al.*, 2018; Zhu *et al.*, 2019). In fact, glycolysis and protein oxidation during thawing can cause the destruction of the myofibrillary network structure, resulting in drip loss from muscle (Xia *et al.*, 2010). Therefore, the low thawing temperature in the LT method helped to inhibit the activities of these related enzymes and slow glycolysis. In addition, the LT method can shorten the thawing time and form a water film; to isolate oxygen and effectively protect the protein hydration surface, slow down the degree of muscle contraction, and prevent the evaporation of water on the surface and dry consumption (Cui *et al.*, 2018). As a result, the drip loss of thawed juices was reduced after LT treatment on swimming crabs.

Effects of the thawing method on pH values of swimming crab

After thawing, the change in pH of the swimming crabs in each treatment group is shown in Figure 1B. The pH of the fresh swimming crabs was 6.50, which is weakly acidic. After quick freezing–thawing, the pH of the swimming crabs decreased to varying degrees, which was due to glycolysis of the muscle after the death of the swimming crabs. Additionally, substances such as ATP and creatine phosphate decomposed to produce acidic substances such as phosphoric acid, resulting in a decrease in the pH value (Ryu *et al.*, 2005). Moreover,

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Table 1. Effects of thawing methods on the thawing drip loss, cooking loss and centrifugal drip loss of *Portunus trituberculatus*

Thawing treatment	Thawing loss (%)	Cooking loss (%)	Centrifugal loss (%)
Fresh	—	27.51±1.13[a]	12.26±0.36[a]
WIT (25 °C)	6.32±0.37[c]	33.62±0.53[c]	25.53±0.83[d]
AT (25 °C)	8.67±0.50[d]	30.13±1.75[b]	25.96±1.27[d]
RT (4 °C)	3.53±0.53[a]	28.07±1.43[ab]	21.69±0.75[c]
LT–1–1 (–1–1 °C)	4.50±0.36[b]	29.56±0.91[ab]	22.80±1.67[c]
LT2–4 (2–4 °C)	3.38±0.64[a]	27.55±1.98[a]	13.91±0.92[a]
LT5–7 (5–7 °C)	3.55±0.64[a]	29.68±0.61[ab]	12.26±0.84[a]
LT8–10 (8–10 °C)	6.03±0.48[c]	30.22±0.74[b]	19.18±0.69[b]

WIT, represents water immersion thawing; AT, air thawing; RT, refrigerator thawing; LT, low-temperature combined with high-humidity thawing.
Values followed by different Superscripts in the same column indicate significant differences ($P<0.05$).

the change in pH value can cause an electrostatic charge effect between protein molecules, which is related to the water retention of the crabs (Zhu *et al.*, 2019). As reported, some minerals and small protein compounds would be exuded along with thawing drip loss during the freezing–thawing process, thus changing the ionic balance in the crab muscle, which is probably another reason for the reduced pH value (Leygonie *et al.*, 2012). It can be seen in the figure that the pH value of the natural air thawing group decreased the most, and the pH in this group was significantly lower than that of the other treatment groups. After thawing at a low temperature in the range of –1 °C to 4 °C under high humidity, the pH value of the swimming crabs decreased slightly, but there was no significant change compared with that of the fresh value ($P>0.05$). The results showed that compared with traditional thawing methods (WIT, RT and AT), low-temperature high-humidity thawing (–1 °C to 4°C) could better maintain the freshness and quality of swimming crabs. It has been reported that compared with traditional thawing, high humidity can significantly inhibit the increase in pH value of butterfish after thawing (Cui *et al.*, 2018), while Zhu *et al.* (2019) found that high humidity had no significant effect on the pH value of thawed pork samples, which is different from the results of this experiment. The reason for this difference may be related to the different types of samples.

Effects of the thawing method on lipid oxidation of swimming crab

TBARS is an important index to measure the degree of lipid oxidation, which is often marked by MDA, the peroxidation product of polyunsaturated fatty acids (Crowe *et al.*, 2012; Yu *et al.*, 2015). As shown in Figure 1C, different degrees of lipid oxidation occurred in all groups of swimming crabs after thawing treatment, which could be due to the destruction of muscle tissue cells during the freeze–thaw process. This leads to the inactivation of important antioxidant enzymes and the release of pro-oxidants such as Fe^{2+}, thereby accelerating the oxidation of fats (Benjakul and Bauer, 2001). Among these groups, the natural air thawing group had a higher thawing temperature and longer thawing time, resulting in a higher degree of oxidation. The TBARS value in this group (0.26 mg MDA/kg) was significantly higher than those in the other treatment groups ($P<0.05$). However, at the same temperature, the TBARS value in the low-temperature high-humidity thawing group (LT2–4) was significantly lower (68.72 per cent lower) than that of the refrigerator thawing (RT) group ($P<0.05$). The TBARS value of the LT–1–1 group reached that of fresh crabs. This result indicated that high humidity could slow the lipid oxidation of swimming crabs during thawing, which was mainly due to the high humidity accelerating the thawing and isolating oxygen, thus reducing the occurrence of fat oxidation (Cui *et al.*, 2018).

Effects of the thawing method on the protein oxidation index of swimming crab

Carbonylation is an important sign that proteins have been attacked and oxidized by free radicals (Estévez, 2011), and carbonyl content is a common index to reflect the degree of protein oxidation (Oliveira *et al.*, 2017). The effect of different thawing methods on carbonyl content is shown in Figure 2A. After thawing, except for in the LT2–4 group, the carbonyl content of the swimming crabs in each group was significantly higher than that of fresh crabs ($P<0.05$), indicating that obvious oxidative deterioration of protein occurred during thawing. This result was due to the formation of ice crystals that cause muscle cells to breakdown and release oxidase, which in turn catalyzes the oxidation reaction (Xia *et al.*, 2012). The carbonyl content of the natural air thawing group was the highest (5.63 nmol/mg prot). This result was mainly because the relatively high thawing temperature and long thawing time were beneficial for the oxidation reaction, leading to the accumulation of additional carbonyl groups. There was no significant difference in carbonyl content among the water immersion, refrigeration, LT–1–1, LT5–7 and LT8–10 groups ($P>0.05$). The carbonyl content of the LT2–4 group was the lowest, showing a 31.40 per cent decrease compared with that of the refrigeration group in the same temperature range and showing a level close to the fresh value, indicating that high humidity could inhibit the oxidative deterioration of protein amino acid residues. Li *et al.* (2014a) also found that high humidity could significantly inhibit protein oxidation during beef thawing at low temperature and high humidity, and the carbonyl content during the whole thawing process and even 12 hours after thawing was significantly lower than that after traditional refrigerator thawing.

Effects of the thawing methods on the T-SH content of myofibrillar protein in swimming crabs

The sulfhydryl group is the most active functional group in the protein structure and is easily oxidized to form disulfide bonds during frozen storage or thawing, which leads to a decrease in sulfhydryl content (Kong *et al.*, 2013). As shown in Figure 2B, after the traditional thawing methods—namely, water immersion, natural air and refrigerator thawing—the protein oxidation had readily occurred, and the sulfhydryl content decreased by 34.12 per cent, 15.84 per

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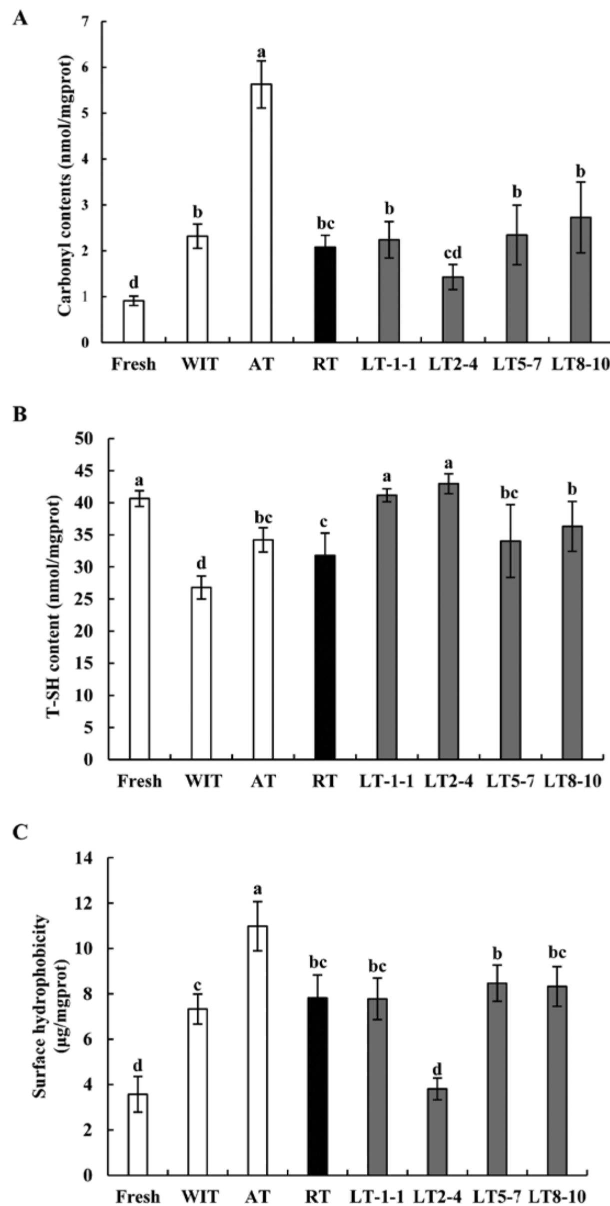

Figure 2. Effects of thawing methods on carbonyl content (A), T-SH content (B) and surface hydrophobicity (C) of myofibrillar protein in *Portunus trituberculatus*. Values followed by different Superscripts indicate significant differences (P<0.05). AT, air thawing; LT, low-temperature combined with high-humidity thawing; RT, refrigerator thawing; T-SH, total sulfhydryl; WIT, represents water immersion thawing.

cent and 21.79 per cent, respectively, compared with that of the fresh control group (P<0.05). After thawing at a low temperature in the range of –1 °C to 4 °C under high humidity, the sulfhydryl content increased by 53.74–60.43 per cent (P<0.05) compared with that of water immersion thawing, 20.34–25.57 per cent (P<0.05) compared with that of natural air thawing, and 29.49–35.13 per cent (P<0.05) compared with that of refrigerator thawing, and there was no significant difference compared with the fresh value (P>0.05). The degree of protein oxidation was lower in this low-temperature, high-humidity group than in the other groups. This result is consistent with the findings in the study of low-temperature high-humidity thawing of butterfish (Cui *et al.*, 2018) and beef (Li *et al.*, 2014a), indicating that compared with traditional thawing methods, low-temperature high-humidity thawing can effectively inhibit the conformational changes of proteins and reduce sulfhydryl exposure and oxidation, which may be closely related to a lower thawing temperature, shorter thawing time and oxygen isolation from the water film. However, under the conditions of high temperature (5–10 °C) and low temperature and high humidity, the sulfhydryl content decreased obviously, which may be due to the relatively high thawing temperature, acceleration of biochemical reactions, enzymatic activity and microbial reproduction, which led to changes in protein conformation and sulfhydryl oxidation.

Effects of the thawing methods on the surface hydrophobicity of myofibrillar protein in from swimming crabs

Surface hydrophobicity is a sensitive index that can be used to determine the changes in the fine structure of proteins and reflects the relative content of hydrophobic amino acids on the protein surface. This parameter is closely related to physicochemical and functional properties, such as water retention of the protein (Chelh *et al.*, 2006; Kong *et al.*, 2013; Zhang *et al.*, 2015). In this experiment, the amount of bromophenol blue that bound was used to reflect the surface hydrophobicity. As shown in Figure 2C, the surface hydrophobicity of all treatment groups increased significantly compared with that of fresh swimming crabs, except for the LT2–4 group and the surface hydrophobicity of natural air thawing was the highest. This is because the protein is oxidized and unfolds during thawing, the molecular conformation changes, the peptide chain breaks or the structure extends, and the hydrophobic groups inside the molecule are exposed, which leads to an increase in surface hydrophobicity (Kong *et al.*, 2013; Jommark *et al.*, 2018; Li *et al.*, 2019). There was no significant difference in the surface hydrophobicity values among the water immersion, refrigerator, LT–1–1, LT5–7 and LT8–10 groups. Notably, the increase in surface hydrophobicity of the LT2–4 group was the smallest and was significantly lower than that of the other treatment groups and close to the fresh value. This result was basically consistent with the change in carbonyl content results.

According to the above protein oxidation indexes, the thawing method with a low temperature ranging from 2 °C to 4 °C and high humidity was the best among the tested conditions, at which the degree of protein oxidation was the lowest and the crab quality was better after thawing. The thawing method of low temperature at –1 °C to 1 °C and high humidity was the second best, but its long thawing time affected thawing efficiency. The protein oxidation degree after thawing at low temperature in the range of 5–10 °C under high humidity was similar to that of refrigerator thawing, while the protein oxidation deterioration after water immersion thawing and natural air thawing was more serious, which was related to the higher thawing temperature.

Effects of the thawing method on the Ca^{2+}-ATPase activity of swimming crabs

Ca^{2+}-ATPase activity reflects the structural integrity of the myosin head, and its activity is a common index to measure the degree of protein denaturation (Benjakul *et al.*, 1997, 2003). The effects of the different thawing methods on Ca^{2+}-ATPase activity are shown in Figure 3. After thawing, the loss of Ca^{2+}-ATPase activity in swimming crabs was relatively high (P<0.05). The Ca^{2+}-ATPase activity in the water immersion, natural air, refrigerator, LT–1–1, LT2–4, LT5–7 and LT8–10 samples decreased by 54.00 per cent, 38.08 per cent, 17.60 per cent, 34.10 per cent, 12.61

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per cent, 29.85 per cent and 41.55 per cent, respectively, compared to that of the control group. This protein was severely denatured in these experimental groups. After thawing at low temperature at 2–4 °C and high humidity (sample LT2–4), the Ca^{2+}-ATPase activity was the highest and the degree of protein denaturation was relatively low, so this condition was considered a suitable thawing method for swimming crabs. As reported, a decrease in Ca^{2+}-ATPase activity after thawing may be attributed to sulfhydryl oxidation, myofibrillar proteolysis, mechanical action of ice crystals, etc. (Jiang *et al.*, 1988; Benjakul and Bauer, 2020). Among the different thawing methods, the LT method helped to reduce sulfhydryl oxidation and myofibrillar proteolysis, as mentioned above, involving comprehensive effects from low temperature, shortened thawing time and oxygen isolation.

Correlation analysis of the quality indexes

The correlation among the quality indexes of the swimming crabs after thawing is shown in Table 2. There was a significant negative correlation between the pH and drip loss of thawing and centrifugation. There was a significant positive correlation between the TBARS value and the drip loss of thawing and centrifugation. The carbonyl group content and surface hydrophobicity were positively correlated with the drip loss due to thawing, cooking and centrifugation. The activities of T-SH and Ca^{2+}-ATPase were significantly negatively correlated with the drip loss of thawing, cooking and centrifugation. These results showed that lipid oxidation and protein oxidation and denaturation had obvious effects on the water retention of crab meat. Lower levels of TBARS, carbonyl groups, and surface hydrophobicity and higher of T-SH and Ca^{2+}-ATPase activities (Figures 1C, 2–3) were important for the best water retention observed in the LT2–4 group (Table 1).

Ca^{2+}-ATPase activity was negatively correlated with the water retention index, TBARS value and surface hydrophobicity but positively correlated with T-SH. This shows that the decrease in muscle water retention capacity, thiol oxidation and exposure of hydrophobic residues are important reasons for the inactivation of Ca^{2+}-ATPase. This conclusion is consistent with the literature (Reza *et al.*, 2009; Cao *et al.*, 2015; Zhang *et al.*, 2018). Under the low-temperature and high-humidity thawing conditions at 2–4 °C, the loss of Ca^{2+}-ATPase activity was relatively low, which may be closely related to the relatively high water retention (Table 1) and sulfhydryl content (Figure 2B) and low TBARS and surface hydrophobicity values (Figure 2C) observed in this group.

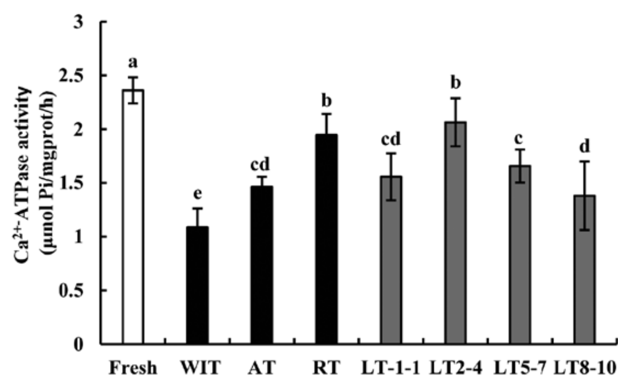

Figure 3. Effects of thawing methods on the Ca^{2+}-ATPase activity of *Portunus trituberculatus*. Values followed by different Superscripts indicate significant differences ($P<0.05$). AT, air thawing; LT, low-temperature combined with high-humidity thawing; RT, refrigerator thawing; WIT, represents water immersion thawing.

Conclusion

Different thawing methods have different effects on the quality of swimming crabs after thawing. The thawing time of the crabs after water immersion was the shortest, followed by thawing at low temperature and high humidity. Compared with the traditional air thawing method, high humidity significantly shortened the thawing time of swimming crabs and effectively increased the thawing rate. The swimming crabs thawed under low-temperature (2–4 °C and 5–7 °C) and high-humidity conditions had better water retention than those samples that under other conditions. The drip loss of the thawed juices in these two groups was significantly lower than those of the water immersion and natural air thawing groups, while that after centrifugal thawing was significantly lower than all three traditional thawing conditions. Thawing at a low temperature and high humidity helped to maintain the freshness of swimming crabs and slow lipid oxidation. After high-humidity thawing at –1–1 °C and 2–4 °C, the pH and TBARS values were significantly lower than those after traditional thawing and close to the value of the fresh sample. The freshness and lipid oxidation degree of high-humidity thawing at 5–7 °C and 8–10 °C were similar to those of refrigerator thawing. Thawing at a low temperature and high humidity can reduce the oxidation and denaturation of myofibrillar protein. After high-humidity thawing at 2–4 °C, the degree of protein oxidation was significantly lower than that of traditional thawing, and there was no significant difference in the carbonyl and T-SH contents and surface hydrophobicity between thawed swimming crabs and fresh

Table 2. Correlation analysis of various quality parameters for thawed *Portunus trituberculatus*

Quality parameter	Thawing loss	Cooking loss	Centrifugal loss	pH	TBARS	Carbonyl content	T-SH	Surface hydrophobicity	Ca^{2+}-ATPase activity
Thawing loss	1.000								
Cooking loss	0.564**	1.000							
Centrifugal loss	0.770**	0.552**	1.000						
pH	–0.697**	–0.167	–0.368*	1.000					
TBARS	0.746**	0.336	0.514**	–0.791**	1.000				
carbonyl content	0.840**	0.315*	0.615**	–0.851**	0.776**	1.000			
T-SH	–0.412**	–0.565**	–0.465**	0.260	–0.572**	–0.307*	1.000		
Surface hydrophobicity	0.759**	0.360*	0.609**	–0.743**	0.763**	0.782**	–0.411**	1.000	
Ca^{2+}-ATPase activity	–0.483**	–0.686**	–0.441**	0.016	–0.416*	–0.108	0.459**	–0.395**	1.000

*Significant correlation ($P<0.05$); **extremely significant correlation ($P<0.01$). TBARS, thiobarbituric acid reacting substances; T-SH, total sulfhydryl.

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swimming crab, while the Ca^{2+}-ATPase activity was significantly higher than that after water immersion and natural air thawing. There was no significant difference in the degree of protein oxidation between the other high-humidity thawing groups and refrigerator thawing groups.

The time required for water immersion thawing was short, but the quality was seriously reduced. The cost of air thawing is low, but it is time-consuming and results in poor-quality thawed products. Although the quality of crab meat is good after refrigerator thawing, this method takes too long and affects the thawing efficiency. Generally, thawing at a low temperature with high humidity gives an efficient thawing rate and can better maintain the muscle quality and myofibrillar protein functional properties of swimming crabs. Among the groups, low-temperature high-humidity thawing at 2–4 °C was the best and the most suitable thawing method for swimming crabs. The quality of the crab meat subjected to the other low-temperature, high-humidity thawing conditions was similar to that of meat subjected to refrigerator thawing, and the thawing rate was significantly improved. However, the effective mechanism of low-temperature high-humidity thawing on swimming crabs and other aquatic products needs to be further studied.

Author Contributions

Jiangang Ling and Xiaoting Xuan contributed to the conception of the study, performed the data analyses and wrote the manuscript; Zihan Xu and Yan Cui performed the experiment and helped perform the analysis with constructive discussions; Tian Ding and Xudong Lin contributed significantly to analysis and manuscript preparation; Donghong Liu contributed to the conception of the study.

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Conflict of Interest

The authors declare no conflict of interest.

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