Quality of Cuttlefish as Affected by Different Thawing Methods

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**ABSTRACT**

To investigate the effect of different thawing methods on the quality of frozen cuttlefish, six different thawing methods were used: hydrostatic thawing (HT), flowing water thawing (FWT), saline solution thawing (SWT), ultrasonic water thawing (UWT), microwave thawing (MT), and 4°C refrigerator thawing (RT). In this study, the water retention (thawing loss rate, centrifugal loss rate, and cooking loss), pH value, malondialdehyde content, TVB-N value, and sulfhydryl content were measured to evaluate the quality after thawing. Protein secondary structure was measured using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, protein tertiary structure was obtained by fluorescence spectroscopy and water migration was determined using low-field NMR spectroscopy. The results showed that the microwave thawing time was the shortest, but the water holding capacity after thawing was the worst, and the TVB-N content was the highest at 14.31 mg/100 g. Ultrasonic thawing led to the best water holding capacity, but ultrasound promoted the oxidation of protein and fat. Microscopic observation showed that the muscle fiber bundles in the saline solution thawing samples were compactly arranged and intact, with minimal gaps. The samples thawed in saline solution had the highest hardness and chewiness. The results of comprehensive analysis showed that saline solution thawing and ultrasonic water thawing are more suitable for thawing cuttlefish, and this study provides some theoretical basis for the selection of thawing methods in the actual production of cuttlefish.

**INTRODUCTION**

Cuttlefish belongs to Mollusca, Sepiida.\textsuperscript{[1]} Its meat is rich in nutrients, high-quality protein, polyunsaturated fatty acids and a variety of trace elements required by the human body.\textsuperscript{[2]} Cuttlefish mainly live in shallow waters of tropical and temperate coasts and often move to deep waters in winter. Cuttlefish is one of the four major aquatic products in China, and the other three are *Larimichthys crocea*, *Larimichthys polyactis* and *Trichiurus lepturus*. It is caught in large quantities, and has high market demand.\textsuperscript{[3]}

Cuttlefish are prone to deterioration at room temperature due to the method of catching and handling, the fragile and soft flesh, and the high activity of autolytic enzymes and attached microorganisms.\textsuperscript{[4]} To slow the spoilage of cuttlefish and maintain the maximal nutritional value, freezing and frozen storage are generally used. Freezing can effectively inhibit the growth and reproduction of microorganisms in aquatic products, reduce the activity of enzymes, maintain the good quality of aquatic products, and extend the shelf life of these products.\textsuperscript{[5]} Most of the cuttlefish in the market are also circulated as frozen products. Thawing is a necessary process before processing.
frozen products and plays a vital role in the quality of meat products after frozen storage. However, improper thawing methods will have an irreversible effect on the quality of the fish, especially the loss of moisture during the thawing, the destruction of texture, color and flavor, the oxidation of lipids and proteins, the denaturation of proteins, and other problems. Therefore, it is necessary to choose a suitable thawing method to maintain the quality. The traditional methods include water thawing, air thawing and refrigerator thawing, which are often used to thaw frozen food. In recent years, some new defrosting techniques including ultrasonic thawing, microwave thawing, ohmic thawing, etc., have also been widely used for thawing frozen foods. However, each thawing method has its own shortcomings, which may lead to decreased water holding capacity, lipid oxidation and protein oxidation. Therefore, it is necessary to explore a thawing method suitable for freezing cuttlefish in order to help maintain the quality.

Six thawing methods (hydrostatic thawing, water thawing, saline solution thawing, ultrasonic water thawing, microwave thawing, and 4°C refrigerator thawing) were used to thaw frozen cuttlefish in this experiment. The quality changes of cuttlefish meat including water holding capacity, texture, pH value, TVB-N, protein structure and fat oxidation, under different thawing methods were investigated in order to propose the most suitable method to thaw frozen cuttlefish and to provide theoretical reference for cuttlefish preservation, improving the economic benefits and food quality of cuttlefish.

Materials and Methods

Sample preparation

Fresh cuttlefish is frozen with -55°C ultra-low temperature cold storage and then stored in a -18°C refrigerator. Frozen cuttlefish was purchased from Dongshan Island, Zhangzhou, Fujian, with an average weight of (1100 ± 50) g per unit. It was transported by the cold chain at -18°C for 20 h to the laboratory, and immediately stored in the refrigerator at -18°C.

The frozen cuttlefishes were removed from the refrigerator, divided into 6 groups with 3 each, and thawed according to the different thawing methods in Table 1. 34970A multi-channel thermocouples (Agilent, Santa Clara, CA, USA) were used to measure the temperature change during thawing. The temperature measuring end of the multi-channel thermocouples were inserted from the head of the cuttlefish to the center of the cuttlefish body. The end of thawing is when the center temperature reaches 4°C.

Thawing loss

Thawing loss was determined according to the protocol as described by Honikel et al. The weight of the cuttlefish before thawing was weighed with an analytical balance and the mass M1 was recorded. After thawing, the surface of the cuttlefish was dried with kitchen paper, weighed again and the mass M2 was recorded. And the thawing loss rate was calculated according to the following equation.

\[
\text{Thawingloss/\%} = \frac{M2}{M1} \times 100\%
\]  

(1)

Cooking loss

Cooking loss was extracted in line with the method of Zhang et al. Cuttlefish samples of size 2 cm×2 cm×2 cm were weighed before steaming (M3), heated in a water bath at 85°C for 20 min, then removed and cooled to room temperature, and the water on the surface of the cuttlefish was absorbed with absorbent paper, weighed after steaming (M4) The cooking loss rate of cuttlefish was calculated according to equation (2). Each group was repeated three times and the average value was taken.
Table 1. Six methods of thawing frozen cuttlefish.

| Thawing method                        | Operation method                                                                                                                                 |
|---------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| 4°C refrigerator thawing (RT)         | Place the cuttlefish sample in a plastic tray and place it in a refrigerator at 4°C to thaw.                                                  |
| Hydrostatic thawing (HT)              | Put the cuttlefish sample in a constant temperature water bath at 25 ± 1°C to thaw.                                                             |
| Flow water thawing (FWT)              | Put the cuttlefish under 25 ± 1°C tap water with a flow rate of 25 mL/s to thaw.                                                               |
| Saline solution thawing (SWT)         | Prepare saline solution with a concentration of 3.0%, place the cuttlefish sample in the salt water, and thaw at room temperature (25 ± 1) °C. |
| Ultrasound water thawing (UWT)        | Put the cuttlefish sample into the ultrasonic device, set the power to 200 W, the working frequency to 53 kHz, and the temperature to (25 ± 1) °C to defrost. |
| Microwave thawing (MT)                | Put the cuttlefish sample in the microwave oven and set it to defrost mode.                                                                   |
Centrifugal loss

Remove 1 cm×1 cm×1 cm of cuttlefish meat and record the weight as M5. Wrap with filter paper and place in a centrifuge tube. Centrifuge at 5000 rpm for 10 min at 4°C. At the end of centrifugation, remove it and weigh it and record the weight of cuttlefish meat M6. The centrifugal loss was calculated as follows.

\[
\text{Centrifugal loss} = \frac{M_5 - M_6}{M_5} \times 100\%
\]

Color measurement

The color measurement was determined according to Tan et al.\(^{[19]}\) The color values L*, a* and b* of the thawed cuttlefish were measured by a colorimeter (CR-400, Konica Minolta, Tokyo, Japan), and whiteboard correction was performed before the measurement of the cuttlefish products. Each group was repeated 3 times and the average value was taken. The whiteness values were then calculated according to equation (4).

\[
W = 100 - \sqrt{(100 - L^*)^2 + a^*^2 + b^*^2}
\]

Instrumental texture analysis

The texture properties of cuttlefish meat was measured according to the method of Li et al.\(^{[21]}\) Thawed cuttlefish meat was removed, cut into 3 cm × 3 cm × 1 cm squares, and measured using a texture analyzer (TMS-Pro, FTC Corporation, US) with a P/6 flat-bottom column probe. The test rate was 3 mm/s, the test rate was 1 mm/s, the deformation rate was 50% and the return distance was 20 mm.\(^{[22]}\)

Microscopic observation

The microstructure of the samples was observed as described by Jiang et al.\(^{[23]}\) The cuttlefish was cut into 4 mm×4 mm×3 mm sections, fixed in formalin solution with a volume fraction of 10% for 24 hours, treated with gradient elution of ethanol solution, and the sections were soaked in formalin solution with a volume fraction of 10% for 24 h and then eluted with a gradient of ethanol solution. Then the following operations were performed sequentially, transparent with xylene solution, embedded in paraffin, and cut into 10 μm thick sections with a slicer. Sections were dried and stained with hematoxylin eosin stain, then observed and photographed with an Eclipse E200 biomicroscope.

MP extraction

Myofibril solution was extracted in line with the method of Li et al.\(^{[24]}\) 2 g of minced cuttlefish was accurately weighed into a 50 mL centrifuge tube, buffered with 20 mL of buffer (20 mmol/L Tris-maleate, 0.05 mol/L KCl, pH = 7.0), homogenized, and the precipitate was taken by centrifugation at 10,000 r/min for 15 min, and the process was repeated twice. The last precipitate was added into 20 mL buffer B (20 mmol/L Tris-maleate, 0.6 mol/L KCl, pH = 7.0), homogenized, extracted at 4°C for 3 h, centrifuged at 10,000 r/min for 15 min, and the supernatant was myofibrillar protein solution.
Lipid oxidation

Weigh 2.0 g of cuttlefish meat and homogenize in a centrifuge tube using 18 mL of physiological saline and centrifuge at 8000 r/min for 15 minutes. The supernatant was taken to measure the amount of malondialdehyde (MDA). The amount of MDA was quantified by thiobarbituric acid reaction method (TBA) using commercial kit A003-1 (Nanjing Jiancheng Institute of Biological Engineering, China).\textsuperscript{[17]} After using the kit, it was measured at 532 nm using an enzyme-labeled instrument (FC, Thermo scien-tific, China).

Determination of sulfhydryl (SH) group content

Myofibrillar protein solution was added to the reagents sequentially according to the method of the kit A063-1 (Nanjing Jiancheng Institute of Biological Engineering, China), mixed well and left for 10 min at room temperature, zeroed with distilled water, and the absorbance of each tube was measured by spectrophotometer (YS6010, 3nh, China) at 412 nm, and the average value was obtained by repeating each group three times.

Fourier infrared spectroscopy

The secondary structure of cuttlefish proteins was studied using a FT-IR spectrometer (Spotlight 400, PerkinElmer Instruments, USA). Referring to the method of Liu et al.,\textsuperscript{[25]} samples were scanned 64 times from 600 to 4000 cm\textsuperscript{-1} with a resolution of 1 cm\textsuperscript{-1}. Spectra were collected at room temperature (20–22°C) using approximately 0.5 g of lyophilized MP placed on the surface of attenuated total reflectance (ATR) crystals. The obtained spectra were deconvoluted and curve-fitted using PeakFit EXE professional software to analyze protein secondary structure changes. Three measurements from duplicate samples were collected to obtain an average of the spectral data.

Intrinsic fluorescence spectra

Intrinsic fluorescence emission spectra of MP samples were determined on an F-7100 fluorescence spectrophotometer (Hitachi Co., Tokyo, Japan), according to the modified method of Zhang et al.\textsuperscript{[26]} The protein solutions were excited at 290 nm (slit width was 5 nm), and the emission spectra were recorded from 300 to 410 nm at a scanning speed of 1200 nm/min. Background spectra under the same conditions were recorded and subtracted from treated samples. All determinations were conducted in triplicate.

Determination of transverse relaxation time (T\textsubscript{2}) of Low-Field nuclear magnetic resonance and proton magnetic resonance imaging

With a slight modification referring to Wang’s method.\textsuperscript{[27]} The thawed cuttlefish meat was made into a sample of 3.0 cm×3.0 cm×1.0 cm and placed in the measurement channel of an LF-NMR analyzer (MesoMR23-060 H.1, Niumag Corporation, Shanghai). A Carr-Purcell-Meiboom-Gill (CPMG) sequence was used with T2 measurements: SW = 100 kHz, RG1 = 20, P1 = 19.00 μs, DRG1 = 3, TD = 400066, PRG = 1, TW = 2000 ms, NS = 4, P2 = 37.00 μs, TE = 0.500. The CPMG exponential decay profile was obtained followed by iterative inversion to obtain the transverse relaxation time T\textsubscript{2} profile. Imaging was performed by the PQ001 benchtop pulsed MRI analyzer, followed by uniform mapping and pseudo-coloring of the proton density map to obtain the MRI map.
Total volatile base nitrogen (TVB-N)

Determination of TVB–N by the method of Yu.[28] Five g of cuttlefish muscle was weighed and an automated Kjeltec nitrogen analyzer (Kjeltec 8400, Foss, Denmark) was used to determine TVB-N values.

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan’s multiple range test were used to determine significant differences between means using SPSS 22.0. Analysis of variance (ANOVA) and Duncan’s multiple range test \((p = .05)\) were performed. And Origin 2021 software was used for plotting.

Results and discussion

Effect of Different Thawing Methods on the Thawing Time of Cuttlefish

The thawing times of cuttlefish with HT, FWT, SWT, UWT, MT and RT were 75 min, 50 min, 60 min, 55 min, 20 min and 874 min, respectively. Figure 1 shows the thawing curves for the six thawing methods. From the figure, it can be seen that the time of MT was the shortest. The dipoles of molecules inside the food rotates continuously under the alternating action during microwave thawing and generates heat through friction, which can greatly increase the thawing speed.[29] However, due to the unique morphological characteristics of cuttlefish, the temperature distribution of the microwave thawing process was not uniform, and there was a local overheating phenomenon. If the radiation continued, the temperature would rise rapidly, resulting in the ripening phenomenon.[30]

Saline solution thawing takes less time than hydrostatic water thawing. Because salt dissolution increases the ion concentration in water, the liquid-phase vapor pressure of water decreases, but the solid-phase vapor pressure of ice remains constant. To reach a state of equal solid-liquid-phase vapor pressure when the ice and water mixture coexist in equilibrium, the ice will melt, so SWT can shorten the thawing time of cuttlefish. In recent years, many studies have demonstrated that ultrasound water thawing can also improve the heat transfer efficiency and shorten the thawing time by producing cavitation bubbles through the ultrasonic action of water.[31,32]

Figure 1. Thawing curve of cuttlefish under different thawing methods.
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Figure 2. Comparison of water retention of cuttlefish by different thawing methods. Thawing loss(A). Water holding capacity(B). Cooking loss(C). The letters “a–c” indicate significant differences (P < .05). Error bars show standard deviation.

**Effect of Different Thawing Methods on the Water Retention of Cuttlefish**

As shown in Figure 2A, among the six thawing methods, the RT group had the highest thawing loss, which was probably due to the long thawing time. There was no significant difference between SWT, UWT and MT in terms of the thawing loss rate (P > .05). The HT group had the least thawing loss. During the thawing process, the frozen cuttlefish meat was in direct contact with water, and some soluble proteins and other substances were washed away by the flowing water as thawing proceeded. Therefore, the thawing loss of the FWT group was larger than that of the HT group. The thawing loss rate of MT was also greater than that of HT, which was due to the unique morphological characteristics of cuttlefish. In the process of thawing and heating, the cuttlefish parts experienced different degrees of heat absorption. The MT thawing rate was faster so that the cuttlefish meat partially cooked, accelerating the loss of water, and the protein damage after MT was greater, after the melting of ice crystals, the water molecules could not be recombined with protein molecules, resulting in a larger thawing loss rate. This is consistent with Oliveira’s study. This result was similar to that of a study by Oliveira et al.\(^{[33]}\)

The water holding capacity refers to the ability of muscle tissue to physically prevent water from leaking out, and the higher the water holding capacity is,\(^{[34]}\) the better the integrity of muscle cell tissue can be maintained after thawing. The results of the effect of different thawing methods on the water holding capacity of cuttlefish are shown in Figure 2B. The lowest water holding capacity of microwave-thawed cuttlefish meat was the lowest, at 75%, probably due to the large amount of heat generated during the thawing process, which disrupted the protein network structure and led to a decreased water holding capacity.\(^{[35]}\) UWT led to the highest water-holding capacity of 90%, followed by brine thawing, where the water-holding capacity of the meat was improved to some extent due to the expansion of the myofilament lattice by the binding of salt ions.\(^{[23]}\) There was no significant difference
in the water holding capacity of cuttlefish after hydrostatic thawing, running water thawing and refrigerator thawing. Regarding the cooking loss rate, ultrasonic-assisted water thawing led to the lowest cooking loss rate of 11.85%, while brine thawing led to the highest cooking loss rate (20.15%).

**Color and texture analysis**

The color appearance of aquatic products is one of the most important factors affecting consumer acceptance of these products. Related studies found that color changes in seafood muscle after thawing were influenced by various factors such as fat oxidation, pigment degradation and protein denaturation.\[^{35}\] The increase in whiteness of fresh cuttlefish after freezing treatment was due to the generation of ice crystals in the cuttlefish meat during freezing, the increase in free water in the muscle tissue after thawing, and the enhancement of reflected light on the surface of the cuttlefish. From Table 2, it can be seen that among the six thawing methods, cuttlefish meat thawed in the refrigerator had the highest whiteness, while that thawed in the microwave had the lowest whiteness value. The carbonyl compounds formed by protein oxidation easily reacted with amino acid compounds to generate dark-colored substances, and the protein oxidation of cuttlefish was serious after microwave thawing. Therefore, microwave thawing of cuttlefish may lead to accelerated protein oxidation due to the increase in temperature, thereby decreasing its whiteness.\[^{36}\]

The texture includes hardness, elasticity, chewiness and stickiness indices. Textural changes in aquatic products reflect the sensory quality of the food to a certain extent and are also important factors affecting mechanical processing in the fish industry. The measurements results are shown in Table 2. The hardness and chewiness of cuttlefish meat after thawing were significantly different (\(P > .05\)), but the elasticity and stickiness were not significantly different (\(P < .05\)). Among these indices, the hardness and chewiness of cuttlefish meat after SWT were the highest. This result may be due to the increased in ionic strength in the solution because of the dissolution of salt in water and the increase in hardness due to polymerization between molecules of the multi-skin chains, resulting in changes in the protein structure and properties.\[^{37}\]

**Transverse relaxation time (\(T_2\)) by LF-NMR spectroscopy**

Low-field nuclear magnetic resonance (LF-NMR) spectroscopy utilizes the principle of energy exchange between nuclei at a fixed magnetic distance (\(^1\)H) to analyze the sample composition.\[^{38}\] The moisture content of the sample in different states is determined by measuring the decay pattern of transverse relaxation time (\(T_2\)) of \(^1\)H protons in meat products, expressed as the peak area corresponding to the curve. Figure 3 shows the transverse relaxation time \(T_2\) profiles of cuttlefish in different thawing groups. Three peaks appeared in each group, and the types of water in cuttlefish flesh were divided into three forms representing bound water, nonfluidizable water and free water according to their active state, where the peaks with relaxation times from 0 ms to 10 ms (\(T_{21}\)) represented a portion of water bound to proteins and other macromolecules represented by bound water. Peaks with relaxation times of 10 ms to 200 ms (\(T_{22}\)) represented a portion of water present between the myogenic fibers and the membrane, indicating nonfluidizable water, and peaks with relaxation times of 200 ms to 3000 ms (\(T_{23}\)) represented a portion of water outside the myogenic fibers or outside the cell. Compared with that of fresh samples, there was no significant difference in the \(T_{21}\) of thawed cuttlefish meat, and no significant difference in the fluidity of surface-bound water. The \(T_{22}\) showed a significant downward trend, indicating that the fluidity of intracellular water was decreasing. The change trends of \(T_{23}\) and \(T_{22}\) were the same, indicating that the free water mobility also decreased. The \(T_{22}\) of thawed cuttlefish meat showed a significant decreasing trend, indicating that the intracellular water mobility was weakening. Among the thawing treatments, RT had a greater impact on water distribution mobility than that the other two methods. The reason may be that the time of RT was
Table 2. Comparison of whiteness values and texture of different thawing methods.

|          | FS          | HT          | FWT         | SWT         | UWT         | MT          | RT          |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| W        | 90.81 ± 0.67<sup>d</sup> | 91.32 ± 0.14<sup>c</sup> | 91.76 ± 0.09<sup>b</sup> | 91.81 ± 0.04<sup>b</sup> | 92.14 ± 0.15<sup>a</sup> | 91.08 ± 0.18<sup>cd</sup> | 92.27 ± 0.07<sup>a</sup> |
| Hardness (g) | 36838.24 ± 1055.30<sup>b</sup> | 20702.15 ± 347.92<sup>c</sup> | 24706.43 ± 842.47<sup>c</sup> | 37066.90 ± 951.88<sup>a</sup> | 24167.92 ± 694.37<sup>c</sup> | 25520.80 ± 950.73<sup>c</sup> | 25147.14 ± 892.67<sup>c</sup> |
| Chewiness | 20721.55 ± 551.70<sup>b</sup> | 12100.13 ± 113.50<sup>c</sup> | 18096.29 ± 350.25<sup>b</sup> | 30604.04 ± 926.01<sup>a</sup> | 11967.50 ± 588.17<sup>c</sup> | 17197.14 ± 321.97<sup>b</sup> | 17289.30 ± 486.59<sup>b</sup> |
| Springiness | 0.90 ± 0.04<sup>a</sup> | 0.81 ± 0.11<sup>a</sup> | 0.78 ± 0.06<sup>a</sup> | 0.89 ± 0.05<sup>a</sup> | 0.87 ± 0.11<sup>a</sup> | 0.92 ± 0.03<sup>a</sup> | 0.91 ± 0.03<sup>a</sup> |
| Cohesiveness | 0.73 ± 0.05<sup>a</sup> | 0.71 ± 0.07<sup>a</sup> | 0.66 ± 0.13<sup>a</sup> | 0.58 ± 0.09<sup>a</sup> | 0.75 ± 0.09<sup>a</sup> | 0.75 ± 0.05<sup>a</sup> | 0.73 ± 0.10<sup>a</sup> |

The letters “a–d” indicate significant differences ($P < 0.05$).
relatively long. Moreover, the moisture fluidity reduction of MT was the most obvious. The reason was due to the particularity of microwave processing. During thawing, the sample would receive a certain amount of heat and vibration, reducing the fluidity of free water.

**Figure 3D** shows that $P_{21}$, $P_{22}$ and $P_{23}$ represent the content of bound water, immobile water and free water, respectively. After thawing, the combined water content tended to increase slightly, but the increased content was not very high, which meant that the thawing method did not change the combined water content very much. It can be seen from the $P_{22}$ that, compared with that of fresh
samples, the moisture content of samples after the six thawing methods showed a significant reduction trend. In particular, the moisture content after MT was reduced the most. This outcome was also caused by the special thawing principle of microwaves, which led to fiber breakage and damage, and the amount of water was reduced relatively more. The relative content of free water ($T_{23}$) in cuttlefish thawed in the RT group was the highest ($P < .05$), indicating that this thawing method caused the water myofibrillar protein in the cuttlefish to easily migrate out of the tissue, leading to caused more serious structural damage. This effect was due to the conversion of immobilized water to free water.[39] Compared with the other five thawing methods, the SWT approach led to lowest free water content. The water in the myofibril tissue thawed by saline solution was consistent with the previous results of water retention.

The weighted images of the $T_2$ proton density in the cuttlefish meat samples thawed by different thawing methods are shown in Figure 4. When the proton density was high, the pseudocolor image was displayed in red, and when the proton density was low, the image was displayed in blue. The sample thawed by saline solution was more similar to the fresh sample, and the yellow part of the sample after microwave thawing was more pronounced than that of other samples, and the corners turned to blue, indicating that the degradation and damage of the muscle fiber microstructure was more serious.[40]

**TVB-N**

The TVB-N value is an important indicator to measure the degree of protein decomposition in the processing and storage of animal foods and is an important indicator to evaluate the freshness of meat. This metric is often used to evaluate the shelf life and storage quality of aquatic products. Spoilage usually causes the TVB-N value to increase.[41] The change in TVB-N is related to spoilage bacteria and endogenous enzyme activity, where this enzyme activity causes the production of ammonia, monoethylamine, dimethylamine and trimethylamine, which aggravates fish meat deterioration in terms of odor.[42–44]

The higher the TVB-N value is, the higher the degree of protein degradation. It can be seen from Figure 5A shows that the TVB-N values of cuttlefish after freezing and thawing were significantly higher than the those of the fresh cuttlefish ($P < .05$). In particular, the MT group had the highest TVB-N value (14.50 mg N/100 g), indicating that microwave thawing damaged the protein more seriously.
The next highest value was in the RT group (13.85 mg N/100 g), probably because the thawing time in this RT group was the longest, and the nitrogen content increased under the action of endogenous enzymes and spoilage microorganisms in the cuttlefish meat.\[^{45}\] Furthermore, the TVB-N value of the cuttlefish meat after UWT was the most similar to that of the fresh sample.

**Lipid oxidation**

MDA is a secondary lipid oxidation product that can be used to determine the degree of oxidative rancidity of fat in cuttlefish. The larger the value is, the greater the degree of fat oxidation. The MDA content in cuttlefish after freezing and thawing was higher than that in the fresh sample. UWT led to the highest MDA content, followed by MT, which may be due to the oxidation of muscle fat caused by the increase in the medium temperature during the thawing process. Cai et al.\[^{45}\] found that fat oxidation intermediates such as peroxides can react with proteins to form complexes and further promote their oxidation. However, the microwave thawing time (20 min) was shorter than the ultrasonic thawing time (55 min), so the degree of lipid oxidation was smaller. Benjakul\[^{46}\] proposed that the ice crystals formed during the freeze-thawing process destroyed muscle tissue cells; this process not only denatured and inactivated some important antioxidant enzymes but also caused the release of fat oxidation promoters such as Fe\[^{2+}\], which accelerated the oxidation of fat.

**Total sulfhydryl content (T-SH)**

The sulfhydryl group is considered to be the most functional and reactive functional group in the proteins. It has the function of stabilizing the spatial structure of myofibril proteins. This moiety is easily oxidized to form disulfide bonds at low temperature or under heating. The sulfhydryl group is
Figure 6. Effect of different thawing methods on ATR-FTIR spectra (A), secondary structure content (B), second-derivative fitted curve of the Amide I band (C) of myofibrillar protein in cuttlefish. The letters “a–c” indicate significant differences ($P < .05$).
closely related to the denaturation and polymerization of the proteins. Some studies suggested that during low-temperature storage, protein changed, especially in the head area, and this reaction occurred rapidly in the first 5 days. These changes may lead to the exposure of active sulfhydryl groups, which thereby undergo oxidation to form disulfide bonds, resulting in a decrease in the content of total sulfhydryl groups and an increase in the disulfide bonds content.\cite{47} The effect of the thawing method on the total sulfhydryl content of cuttlefish is shown in Figure 5C. Compared with that of fresh cuttlefish samples (0.428 mmol/g prot), the total sulfhydryl content in cuttlefish meat thawed by freezing and then thawing was significantly lower ($p < .05$), and the total sulfhydryl content in cuttlefish thawed by saline solution was significantly higher than that of the other thawing methods (0.3325 mmol/g prot). The total sulfhydryl content of cuttlefish thawed by UWT and MT was significantly lower than that of other thawing methods (0.1374 and 0.1595 mmol/g prot, respectively).

**ATR-FTIR spectroscopy**

The extracted cuttlefish myofibril protein was scanned at 550 ~ 4000 cm$^{-1}$. Generally, the absorption peak in the 1600 ~ 1700 cm$^{-1}$ band due to the tensile vibration of C = O is called the amide I band. These characteristic absorption peaks are mainly caused by the vibrations of the secondary
structure of peptides and proteins. For the amide I band, the second derivative and deconvolution technique were used for peak separation, as shown in Figure 6A. The secondary structure of the protein was quantitatively analyzed by curve fitting. Figure 6B shows the relative content of MP secondary structure in cuttlefish with different thawing methods. Compared with that of fresh samples, after freezing and thawing, the α-helix content in the myofibril protein in the cuttlefish was significantly reduced, the irregular curl content was significantly increased, and ordered structures such as protein α-helixes were transformed into random coils due to freezing and thawing. Hydrogen bonding between the carbonyl groups and the amino groups was the main force that maintained the stability of the α-helical structure. During freezing, the increased ice crystals content caused extrusion and mechanical perforations of the protein, and thawing destroyed the internal hydrogen bond networks of the molecules. The decrease in the α-helix content indicated that the hydrophobic groups originally in the MP molecules of cuttlefish protein were exposed, and the hydrophobicity of the protein surface was increased. After microwave thawing, the cuttlefish protein α-helix content was the lowest, and the content of irregular curls was the highest. This result may be due to rotation of the dipole inside the protein in the microwave field, causing the protein molecules to unfold. The hydrophobic groups on the protein surface were exposed, which reduced the hydration capacity of the protein and decreased the water holding capacity of the raw meat. This observation was consistent with Li’s research. The α-helix content of cuttlefish thawed in the refrigerator decreased significantly, suggesting that long-term low-temperature thawing may disrupt the α-helix structure, and the α-helix content after SWT and UWT was most similar to that of fresh samples, consistent with the previous results on the water retention capacity and TVB-N reaction.

Intrinsic fluorescence intensity (IFI)

The tertiary structure of a protein refers to a compact three-dimensional structure formed by the interaction of side-chain groups on the basis of the secondary structure of the protein polypeptide chain. Its structural stability depends mainly on the hydrophobic force between the side chains of amino acid residues. At present, tryptophan is often used as an endogenous fluorescent probe to study the changes in protein tertiary structure. Protein endogenous tryptophan fluorescence is very sensitive to the polarity of the surrounding microenvironment. When the protein is in the folded state, tryptophan residues are mainly located in the hydrophobic environments, such as the protein core. At this time, the excited tryptophan has a relatively high fluorescence intensity. When the protein is partially or completely unfolded, tryptophan residues will be more exposed on the surface of the protein molecule, causing the fluorescence intensity of the excited tryptophan to decreases. Therefore, the fluorescence properties of endogenous tryptophan are often used to reflect changes in the tertiary structure of proteins.

As shown in Figure 7, the maximum emission wavelength of tryptophan for fresh samples was located near 336 nm. Compared with that of the fresh sample, FS, the λmax of all other samples shifted to slightly longer wavelengths (redshifted) after freezing and thawing; the λmax of the myofibrillar protein of cuttlefish decreased slightly (blueshifted), and indicating that the environment in which the tryptophan side chain group was located was less polarized, and the λmax values of the HT, FWT, SWT, UWT, and RT groups were not significantly changed. With the emission of maximum fluorescence of the MT group, the wavelength of the MVT group was significantly blueshifted, but its fluorescence intensity was lower than that of the other groups, probably because the structure of some proteins was severely disrupted during thawing, and secondary folding occurred. In addition, the fluorescence intensity increased in all thawing groups, except microwave thawing, which was consistent with the results of Zhang et al. This finding may be due to the local structural changes of proteins caused by the freeze-thaw process and protein-protein binding, resulting in an overall higher fluorescence intensity in the thawed group. In particular, the fluorescence intensity of SWT was the closest to that of the fresh sample.
Microscopic observation

The freezing process of cuttlefish was destructive to the integrity of the cells from the generation to the growth of ice crystals. Different cuttlefish thawing methods resulted in different ice crystal melting rates, different degrees of mechanical damage to the muscle tissue of cuttlefish, bending and fracture of myogenic fibers, and different degrees of increase in the spaces between muscle bundles. The microstructure deteriorated to different degrees. The structural changes of cuttlefish meat under different thawing methods are shown in Figure 8. As can be seen from the figure, the muscle fiber...
structure of fresh cuttlefish was very neatly arranged, basically without gaps and holes, many muscle fibers were grouped into fiber bundles, and no fiber breakage occurred. The microstructure of muscle fibers was the densest after saline solution thawing, which was consistent with the results of previous studies on the water holding capacity; moreover the cuttlefish had higher water holding capacity and less drip loss after saline solution thawing, which could better maintain the original tissue structure morphology of the muscle. After microwave thawing, the cuttlefish muscle fibers had the largest gaps and distortion because microwave thawing was different from the other five thawing methods, as MT heated the cuttlefish meat directly at the highest temperature and thawed it rapidly, causing a large amount of protein damage. The cuttlefish meat thawed in the refrigerator also had large muscle fiber gaps, probably due to the longer thawing time in the refrigerator and the oxidation of proteins under the action of microorganisms and enzymes, which damaged the integrity of the muscle tissue and diminished the original denseness of the fish tissue. Compared with that after water thawing, the muscle gap of cuttlefish thawed by hydrostatic water was smaller and the muscle bundles were more tightly bound; the reason was that the rate of thawing by still water was more uniform, and when thawing by water, the ice crystals in direct contact with the water flow melted first and thawed more rapidly, and because the air pressure of liquid water vapor was greater than the water vapor pressure of ice crystals, the liquid phase water melted by ice crystals gathered at the tissue fiber gap at low pressure, which increased the tissue gap of cuttlefish. The tissue gap of cuttlefish muscle and cuttlefish flesh became loose. After SWT, the muscle structure of cuttlefish was more complete compared with that after other thawing methods. Although UWT could increase the thawing rate, melt the ice crystals more rapidly, shorten the thawing time, and protect the integrity of the myogenic fibers more completely, the cavitation effect of ultrasonic waves and the local heat generated caused some damage to the samples and partially distorted the myogenic fibers.

Conclusion

This study showed that the thawing method of cuttlefish affected the thawing losses, water distribution, protein oxidation and microstructure. The UWT group had the best water retention capacity, with a TVB-N content closer to that of fresh samples. The hardness and chewiness in the SWT group were significantly higher than those with the other thawing methods. NMR analysis showed that the SWT group samples had less conversion of intracellular water to free water. FTIR and intrinsic fluorescence analysis showed that the SWT samples were more similar to the fresh samples, and the SWT samples under light microscopy showed minimal gaps between myogenic fibers and regular and smooth arrangement of myogenic fiber bundles. While MT- and RT-treated cuttlefish showed the highest degree of proteolysis and the highest TVB-N content, these two methods were not appropriate for thawing cuttlefish. In conclusion, SWT and UWT are more likely to maintain good fillet quality. These treatments can be used as effective methods for thawing frozen cuttlefish.

Disclosure statement

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References

[1] Sikorski, Z. E.; Kolodziejska, I. The Composition and Properties of Squid Meat. *Food Chem.* 1986, 20(3), 213–224. DOI: 10.1016/0032-0889(86)90174-3.

[2] Almansa, E., Domingues, P.; Sykes, A.; Tejera, N.; Lorenzo, A., and Andrade, J. P., et al. The Effects of Feeding with Shrimp or Fish Fry on Growth and Mantle Lipid Composition of Juvenile and Adult Cuttlefish (Sepia Officinalis). *Aquaculture*. 2006, 256(1–4), 403–413.

[3] Vaz-Pires, P.; Seixas, P. Development of New Quality Index Method (QIM) Schemes for Cuttlefish (Sepia Officinalis) and Broadtail Shortfin Squid (Illex Cœndetii). *Food Control*. 2006, 17(12), 942–949. DOI: 10.1016/j.foodcont.2005.07.004.

[4] Mohan, C. O., et al. Effect of Chitosan Edible Coating on the Quality of Double Filleted Indian Oil Sardine (Sardinella Longiceps) during Chilled Storage. *Food Hydrocolloids*. 2012, 26(1), 167–174.

[5] Thanonkaew, A., Benjakul, S.; Visessanguan, W., and Decker, E. A., et al. The Effect of Antioxidants on the Quality Changes of Cuttlefish (Sepia Pharaonis) Muscle during Frozen Storage. *LWT - Food Sci. Technol.* 2008, 41(1), 161–169.

[6] Xu, B., et al. Effect of Different thawing Methods on the Efficiency and Quality Attributes of Frozen Red Radish. *J. Sci. Food Agric.* 101, 3237–3245 2020.

[7] Cai, L.; Zhang, W.; Cao, A., and Cao, M., et al. Effects of Different Thawing Methods on the Quality of Largemouth Bass (Micropterus Salmoides). *LWT*. 2020, 108908. DOI: 10.1016/j.lwt.2019.108908.

[8] Ersoy, B.; Aksan, E.; Ozeren, A. The Effect of Thawing Methods on the Quality of Eels (Anguilla Anguilla). *Food Chem.* 2008, 111(2), 377–380.

[9] Leygonie, C.; Britz, T. J.; Hoffman, L. C. Meat Quality Comparison between Fresh and Frozen/thawed Ostrich M. Iliofibularius. *Meat Sci.* 2012, 91(3), 364–368. DOI: 10.1016/j.meatsci.2012.02.020.

[10] Boonsumrej, S.; Chaiwanichsiri, S.; Tantratian, P.; Ober, T.; Takai, R., et al. Effects of Freezing and thawing on the quality Changes of Tiger Shrimp (Penaeus Monodon) Frozen by Air-blast and Cryogenic Freezing. *J. Food Eng.* 2007, 80(1), 292–299.

[11] Syed Ziauddin, K.; Mahendrarak, N. S.; Rao, D. N.; Amla, B. L., et al. Effect of Freezing, thawing and Frozen storage on Physico-chemical and Sensory Characteristics of Buffalo Meat. *Meat Sci.* 1993, 35(3), 331–340.

[12] Li, X.; Sun, P.; Jia, J.-Z.; Cai, L.-Y.; Li, J.-R.; Lv, Y.-F., et al. Effect of Low Frequency Ultrasound Thawing Method on the Quality Characteristics of Peru Squid (Dosidicus Gigas). *Food Sci. Technol. Int.* 2019, No. 2, 171–181. DOI: 10.1177/1082013218809556.

[13] Hsieh, C.; Lai, C.-H.; Ho, W.-J.; Huang, S.-C.; Ko, W.-C., et al. Effect of thawing and Cold Storage on Frozen Chicken Thigh Meat Quality by High-voltage Electrostatic field(Article). *J. Food Sci.* 2010, No. 4, M193–M197.

[14] Kim, T.-H.; Choi, J.-H.; Choi, Y.-S.; Kim, H.-Y.; Kim, S.-Y.; Kim, H.-W.; Kim, C.-J. Physicochemical Properties of Thawed Chicken Breast as Affected by Microwave Power Levels. *Food Sci. Biotechnol.* 2011, No. 4, 971–977. DOI: 10.1007/s10068-011-0134-2.

[15] Icier, F.; Izzetoglu, G. T., Bozkurt, H., and Ober, A., et al. Effects of Ohmic Thawing on Histological and Textural Properties of Beef Cuts. *J. Food Eng.* 2010, No. 3, 360–365. DOI: 10.1016/j.jfoodeng.2010.03.018.

[16] Honikel, K. O. Reference Methods for the Assessment of Physical Characteristics of Meat. *Meat Sci.* 1998, 49(4), 447–457. DOI: 10.1016/S0309-1740(98)00034-5.

[17] Zhang, X.; Gao, T.; Song, L.; Zhang, L.; Jiang, Y.; Li, J.-L.; Gao, F., and Zhou, G.-H., et al. Effects of Different Thawing Methods on the Quality of Chicken Breast. *Int. J. Food Sci. Technol.* 2017, 52(9), 2097–2105.

[18] Tan, M.; Ye, J.; Chu, Y., and Xie, J., et al. The Effects of Ice Crystal on Water Properties and Protein Stability of Large Yellow Croaker (Pseudoscaena Crocea). *Int. J. Refrig.* 2021, 130, 242–252. DOI: 10.1016/j.ijrefrig.2021.05.040.

[19] Tan, M.; Li, P.; Yu, W.; Wang, J.; Xie, J., et al. Effects of Glazing with Preservatives on the Quality Changes of Squid during Frozen Storage. *Appl. Sci.* 2019, 9(18), 3847.

[20] Hsieh, C. W.; Lai, C.-H.; Ho, W.-J.; Huang, S.-C.; Ko, W.-C., et al. Effect of Thawing and Cold Storage on Frozen Chicken Thigh Meat Quality by High-voltage Electrostatic Field. *J. Food Sci.* 2010, 75(4), M193–7.

[21] Li, P.; Chen, Z.; Tan, M.; Mei, J.; Xie, J., et al. Evaluation of Weakly Acidic Electrolyzed Water and Modified Atmosphere Packaging on the Quality and Stability of Frozen Puffer Fish (Takifugu Obscursus) during Cold Storage. *J. Food Saf.* 2020, 40(3). doi:10.1111/jfs.12773

[22] de Huidobro, F. R.; Miguel, E.; Blázquez, B.; Onega, E., et al. A Comparison between Two Methods (Warner-bratzler and Texture Profile Analysis) for Testing either Raw Meat or Cooked Meat. *Meat Sci.* 2005, 69(3), 527–536.
[23] Jiang, Q., et al. Changes in Quality Properties and Tissue Histology of Lightly Salted Tuna Meat Subjected to Multiple Freeze-thaw Cycles. Food Chem. 2019, 293, 178–186. DOI: 10.1016/j.foodchem.2019.04.091.

[24] Li, P.; Mei, J.; Xie, J. Chitosan-sodium Alginate Bioactive Coatings Containing ε-polylysine Combined with CO2 Modified Atmosphere Packaging Inhibit Myofibril Oxidation and Degradation of Farmed Pufferfish (Takifugu Obscurs) during Cold Storage. LWT. 2021, 140, 110652. DOI: 10.1016/j.lwt.2020.110652.

[25] Liu, W., et al. Elucidating Antibacterial Activity and Mechanism of Daphnetin against Pseudomonas Fluorescens and Shewanella Putrefaciens. J. Food Qual. 2020, 2020, 1–10.

[26] Zhang, M., et al. Moisture Migration, Microstructure Damage and Protein Structure Changes in Porcine Longissimus Muscle as Influenced by Multiple Freeze-thaw Cycles. Meat Sci. 2017, 133, 10–18. DOI: 10.1016/j.meatsci.2017.05.019.

[27] Wang, J. F.; Yu, W. H.; Xie, J. Effect of Glazing with Different Materials on the Quality of Tuna during Frozen Storage. Foods. 2020, 9(2), 16.

[28] Yu, D.; Regenstein, J. M.; Zang, J.; Jiang, Q.; Xia, W.; Xu, Y., et al. Inhibition of Microbial Spoilage of Grass Carp (Ctenopharyngodon Idelus) Fillets with a Chitosan-based Coating during Refrigerated Storage. Int. J. Food Microbiol. 2018, 285, 61–68. DOI: 10.1016/j.ijfoodmicro.2018.07.010.

[29] Orsat, V.; Raghavan, G. S. V.; Krishnaswamy, K. S. - Microwave Technology for Food Processing: An Overview of Current and Future Applications. In The Microwave Processing of Foods, (Second ed.; Regier, M., Knoerzer, K., Schubert, H., Eds.; Woodhead Publishing, 2017; pp 100–116.

[30] Chizoba Ekezie, F.—G.; Sun, D.—W.; Han, Z.; Cheng, J.—H., et al. Microwave-assisted Food Processing Technologies for Enhancing Product Quality and Product Safety: A Review of Recent Developments. Trends Food Sci. Technol. 2017, 67, 58–69. DOI: 10.1016/j.tifs.2017.05.014.

[31] Li, X. X.; Sun, P.; Ma, Y.; Cai, L.; Li, J.—R., et al. Effect of Ultrasonic Thawing on the Water-holding Capacity, Physicochemical Properties and Structure of Frozen Tuna (Thunnus Tonggol) Myofibrillar Proteins. J. Sci. Food Agric. 2019, 99(11), 5083–5091.

[32] Sun, Q.; Kong, B.; Liu, S.; Zheng, O.; Zhang, C., et al. Ultrasound-assisted Thawing Accelerates the Thawing of Common Carp (Cyprinus Carpio) and Improves Its Muscle Quality. LWT. 2021, 141, 111080. DOI: 10.1016/j.lwt.2021.111080.

[33] Oliveira, M. R.; Guibert, G.; Roman, S. S.; Kempka, A. P.; Prestes, R. C., et al. Meat Quality of Chicken Breast Subjected to Different Thawing Methods. Revista Brasileira de Ciência Avícola. 2015, 17(2), 165–171.

[34] Wang, S.; Zhang, L.; Li, J.; Cong, J.; Gao, F.; Zhou, G., et al. Effects of Dietary Marigold Extract Supplementation on Growth Performance, Pigmentation, Antioxidant Capacity and Meat Quality in Broiler Chickens. Asian-australas. J. Anim. Sci. 2017, 30(1), 71–77.

[35] Tironi, V.; de Lamballerie, M.; Le-Bail, A. Quality Changes during the Frozen Storage of Sea Bass (Dicentrarchus Labrax) Muscle after Pressure Shift Freezing and Pressure Assisted Thawing. Innovative Food Sci. Emerg. Technol. 2010, 11(4), 565–573. DOI: 10.1016/j.ifset.2010.05.001.

[36] Thanonkaew, A.; Benjakul, S.; Visessanguan, W.; Decker, E. A., et al. The Effect of Metal Ions on Lipid Oxidation, Colour and Physicochemical Properties of Cuttlefish (Sepia Pharaonis) Subjected to Multiple Freeze–thaw Cycles. Food Chem. 2006, 95(4), 591–599.

[37] A. F. R. Effect of Alcohols and Neutral Salt on the Thermal Stability of Soluble and Precipitated Acid-soluble Collagen. J Biochem J. 1973, 2(131).

[38] Pearce, K. L.; Rosenvold, K.; Andersen, H. J.; Hopkins, D. L., et al. Water Distribution and Mobility in Meat during the Conversion of Muscle to Meat and Ageing and the Impacts on Fresh Meat Quality Attributes — A Review. Meat Sci. 2011, 89(2), 111–124.

[39] Lan, W., et al. Effect of the Number of Freeze-thaw Cycles Number on the Quality of Pacific White Shrimp (Litopenaeus Vannamei): An Emphasis on Moisture Migration and Microstructure by LF-NMR and SEM. Aquac. Fish. 2020, 5(4), 193–200.

[40] Liu, W.; Mei, J.; Xie, J. Effect of Locust Bean Gum-sodium Alginate Coatings Incorporated with Daphnetin Emulsions on the Quality of Scophthalmus Maximus at Refrigerated Condition. Int. J. Biol. Macromol. 2021, 170, 129–139. DOI: 10.1016/j.jbiomac.2020.12.089.

[41] Botta, J. R.; Lauder, J. T.; Jewer, M. A. Effect of Methodology on Total Volatile Basic Nitrogen (TVB-N) Determination as an Index of Quality of Fresh Atlantic Cod (Gadus Morhua). J. Food Sci. 1984, 49(3), 734–736. DOI: 10.1111/j.1365-2621.1984.tb13197.x.

[42] Fuentes, A., et al. Influence of Sodium Replacement and Packaging on Quality and Shelf Life of Smoked Sea Bass (Dicentrarchus Labrax L.). LWT - Food Sci. Technol. 2011, 44(4), 917–923.

[43] Kykkidou, S.; Giatrakou, V.; Papavergou, A.; Kontominas, M. G.; Savvidais, I. N., et al. Effect of Thyme Essential Oil and Packaging Treatments on Fresh Mediterranean Swordfish Fillets during Storage at 4°C. Food Chem. 2009, 115(1), 169–175.

[44] Goulas, A. E.; Kontominas, M. G. Combined Effect of Light Salting, Modified Atmosphere Packaging and Oregano Essential Oil on the Shelf-life of Sea Bream (Sparus Aurata): Biochemical and Sensory Attributes. Food Chem. 2007, 100(1), 287–296. DOI: 10.1016/j.foodchem.2005.09.045.
[45] Cai, L.; Wan, J.; Li, X.; Li, J., et al. Effects of Different Thawing Methods on Physicochemical Properties and Structure of Largemouth Bass (Micropterus Salmoides). *J. Food Sci.* 2020, 85(3), 582–591.

[46] Benjakul, S.; Bauer, F. Biochemical and Physicochemical Changes in Catfish (Silurus Glanis Linne) Muscle as Influenced by Different Freeze–thaw Cycles. *Food Chem.* 2001, 72(2), 207–217. DOI: 10.1016/S0308-8146(00)00222-3.

[47] Liu, Q.; Chen, Q.; Kong, B.; Han, J.; He, X., et al. The Influence of Superchilling and Cryoprotectants on Protein Oxidation and Structural Changes in the Myofibrillar Proteins of Common Carp (Cyprinus Carpio) Surimi. *LWT - Food Sci. Technol.* 2014, 57(2), 603–611.

[48] Cao, Y.; Xiong, Y. L. Chlorogenic Acid-mediated Gel Formation of Oxidatively Stressed Myofibrillar Protein. *Food Chem.* 2015, 180, 235–243. DOI: 10.1016/j.foodchem.2015.02.036.

[49] Li, F.; Wang, B.; Liu, Q.; Chen, Q.; Zhang, H.; Xia, X.; Kong, B., et al. Changes in Myofibrillar Protein Gel Quality of Porcine Longissimus Muscle Induced by Its Stuctural Modification under Different Thawing Methods. *Meat Sci.* 2019, 147, 108–115. DOI: 10.1016/j.meatsci.2018.09.003.

[50] Xia, W.; Ma, L.; Chen, X.; Li, X.; Zhang, Y., et al. Physicochemical and Structural Properties of Composite Gels Prepared with Myofibrillar Protein and Lecithin at Various Ionic Strengths. *Food Hydrocolloids.* 2018, 82, 135–143. DOI: 10.1016/j.foodhyd.2018.03.044.

[51] Diao, X.; Guan, H.; Zhao, X.; Diao, X.; Kong, B., et al. Physicochemical and Structural Properties of Composite Gels Prepared with Myofibrillar Protein and Lard Diacylglycerols. *Meat Sci.* 2016, 121, 333–341. DOI: 10.1016/j.meatsci.2016.07.002.

[52] Iwasaki, T.; Yamamoto, K. Changes in Rabbit Skeletal Myosin and Its Subfragments under High Hydrostatic Pressure. *Int. J. Biol. Macromol.* 2003, 33(4), 215–220. DOI: 10.1016/j.ijbiomac.2003.08.005.