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Research article

Impact of frozen storage on physicochemical parameters and quality changes in cooked crayfish

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ABSTRACT

Customers are increasingly opting for ready-to-eat and easy-to-prepare food products, such as cooked crayfish. It is highly valued for its unique taste, tender meat, and nutritional properties. Therefore, we conducted an investigation into its quality parameters over an 8-week period at −20 °C. Parameters such as water distribution, oxidation reactions, color, microstructure, texture properties, and physicochemical parameters were examined. The physicochemical results indicated that as the storage time increased, the levels of pH and TVB-N (total volatile basic nitrogen) showed a significant increase, while the water holding capacity decreased significantly ($P < 0.05$). After two months of frozen storage, the carbonyl content and TBARS (thiobarbituric acid reactive substances) increased to 4.15 ± 0.16 nmol/mg protein and 1.6 ± 0.00 mg/kg, respectively. Additionally, the total sulfhydryl content decreased to 4.91 ± 0.10 mol/10^5 g protein, which had an impact on the quality of the crayfish. Electron microscopy revealed that with increasing storage time, the fiber structure gradually deteriorated due to water crystallization, leading to severe damage and breakage of muscle fibers. Interestingly, these changes related to storage affected color and texture parameters, thereby influencing the overall quality of the crayfish.

1. Introduction

Procambarus clarkii, also called red swamp crayfish, has various names such as crawfish (USA), estacoza (Egypt), and Xiao longxia (China) [1]. They are characterized by their dark red color, large claws, long heads, and distinct rows of bright red bumps on the legs.

Freshness is an important component in evaluating the quality of seafood products, alongside qualitative and quantitative qualities and consumer perception [2]. The two main crayfish sales modes are fresh and frozen. The concept of "fresh sale" is based on the ability to maintain and guarantee crayfish quality to ensure freshness. However, due to the time required to transport these products to distant markets, shelf life remains a limiting and critical factor. To maintain the highest possible nutritional value, freezing is an appropriate and preferred storage method for off-season crayfish production or export [3].

Freezing at low temperatures for extended periods is widely used to preserve the quality of fresh food, particularly seafood with

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high protein and moisture content. It increases product quality by reducing the rates of chemical changes and consequently extending the shelf-life of seafood products [4]. Thermal processing is an important step in protecting public health and food safety, and boiling is the most common traditional heat treatment for crayfish. It involves cooking the crayfish to a precise core temperature to denature proteins, allowing muscle separation from the shell. However, heat treatment can cause physical and chemical changes that alter the shelf life and quality parameters of seafood products, depending on the cooking method [5].

Lorentzen et al. [6] investigated how freezing and thawing affected weight loss, microbial growth, and melanosis in mildly cooked snow crab clusters. Dima et al. [7] determined the quality parameters and biochemical composition of refrigerated Patagonian crabmeat at 4 °C. Fan et al. [8] evaluated the cooking process of crayfish to better understand heat-induced quality changes and optimize heat processing. Thermal treatments and freezing are among the most important preservation methods, and crustaceans are frequently processed into frozen or cooked products to extend their shelf life [9]. As the time for home cooking is limited in today's world, consumers are increasingly seeking ready-to-eat products that are easy to prepare, safe, high-quality, and minimally processed [10]. Therefore, the goal of this study is to provide research and scientific information on the effects of combining cooking with freezing on the quality characteristics and shelf life of crayfish stored at −20 °C over an 8-week period. The study will include analysis of water distribution, protein oxidation, color, microstructure, texture properties, and physicochemical parameters.

2. Materials and methods

Fresh crayfish (about 320 pieces) were obtained at Fresh Market (Qingdao, Shandong, China) and subsequently transported to the laboratory in an empty cooler. Chemicals and solution reagents were obtained from Sigma Aldrich (St. Louis, Missouri, USA), and chemical test kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.1. Preparation of samples

2.1.1. Thermal treatment (cooking)

The samples were cooked in boiling water for 5 min at 100 °C, and the muscle was manually separated from the shells with spatulas.

2.1.2. Freezing

Cooked crayfish samples were frozen at −20 °C in transparent polyethylene Ziplock bags for two months, and samples were defrosted every two weeks at 4 °C for further examination.

2.2. Physicochemical properties

2.2.1. Weight changes (WC%)

Each batch was opened to measure crayfish weight changes (WC including f1 and f2) during freezing at −20 °C and thawing at 4 °C for two months. The weight changes caused by freezing (F1) were calculated using the following equation, with the weights of the samples recorded both before and after freezing (zero time):

$$F_1\% = \frac{(wf - wc)}{wc} x100$$

where Wf is the weight of frozen crayfish, and Wc is the weight of cooked and cooled crayfish (before freezing).

Weight changes were measured during storage and thawing (F2) of crayfish as follows: This procedure was carried out every two weeks.

$$F_2\% = \frac{(wt - wf)}{wf} x100$$

where Wt is the weight of thawed crayfish, and Wf is the weight of frozen crayfish before storage.

2.2.2. Total volatile basic nitrogen (TVB-N)

The total volatile basic nitrogen levels in the samples were analyzed using the method suggested by Yanlei et al. [11] using the following equation:

$$TVB - N\ (mg\ /\ 100g\ sample) = \frac{(V_1\ -\ V_2)\ X\ C\ X\ 14}{M\ X\ (V\ /\ V_0)}\ X\ 100$$

where V1 is the volume (mL) of hydrochloric acid standard titration solution used by the test solution, V2 is the volume (mL) used by the reagent blank, C is the concentration (mol/L) of the hydrochloric acid standard titration solution, M is the sample mass (g), V is the volume (mL) of the absorbed filtrate, V0 is the total sample solution volume (mL), and 100 is the conversion coefficient.

2.2.3. pH values

Ten grams of sample were homogenized with 90 mL of deionized water (1:10, w/v) in a Waring blender for 1 min before being cooled at 4 °C for 30 min. The pH of the suspension was measured using a pH meter (FE20, Mettler Toledo Instruments Co., Ltd.,

China).

2.2.4. Water holding capacity (WHC)

The water holding capacity (WHC) was measured in triplicate using the "centrifuge drip" method with a CR21G centrifuge (Hitachi, Tokyo, Japan) [12]. The water-holding capacity was determined by multiplying the ratio of initial and final sample weights before and after centrifugation by 100 % according to this equation.

$$\mathrm{WHC} = \frac{\mathrm{B}}{\mathrm{A}}\ \mathrm{X}\ 100$$

where A and B are the initial and final sample weights before and after centrifugation.

2.2.5. Expressible moisture (EM)

Expressible moisture was assessed following the procedure outlined by Li et al. [13]. A 2 g sample was first enclosed within a two-layer cheesecloth and then placed between four filter papers, with two on the top and two on the bottom. A weight of 35 kg was applied to the top of the sample and held for 5 min. EM was calculated by determining the percentage of weight loss in relation to the original sample weight, as shown in the following equation:

$$\mathrm{EM\%} = \frac{\mathrm{m1} - \mathrm{m2}}{m_1}\ x\ 100$$

where **m1** *and* **m2** *were the weights of the samples before and after pressing.*

The measurements were performed in triplicate.

2.3. Chemical composition

The chemical composition of crayfish was calculated during frozen storage, including moisture, protein, fat, and ash content [14].

2.3.1. Moisture content

The moisture content of crayfish samples was calculated using the direct drying method following China's National Food Safety Standard GB 5009.3-2016.

2.3.2. Crude protein

The crude protein content was calculated using the Kjeldahl method, which used a conversion factor of 6.25.

2.3.3. Fat content

To extract crayfish lipids, the improved Folch extraction method involved homogenizing 9 g of muscle tissue in a 2:1 chloroform: methanol solution (180 mL), followed by a 24-h standing period, addition of a 0.9 % NaCl solution, separation of organic and aqueous phases, evaporation of the lower aqueous phase using a rotary evaporator, and subsequent calculation of fat content.

2.3.4. Ash content

Ash content was calculated by ashing the samples at 550 °C in a muffle oven for 8 h, and all analyses were performed in triplicate, with concentrations reported in g/100 g (w/w).

2.4. Protein and lipid oxidation

2.4.1. Carbonyl content

Carbonyl content was measured following modifications to the protocol by Oliver et al. [15]. Samples were diluted and centrifuged with a 2.0 mg/mL TCA solution at 2000×*g* for 10 min. Sediments were treated with 2 g/L DNPH in 2 mol/L HCl for 1 h in the dark, followed by precipitation with 20 % TCA (w/v) and centrifugation. Sediments were washed with ethanol: ethyl acetate (1:1, v/v) and then treated with guanidine (6 mol/g) and sodium phosphate buffer (20 mmol/L) overnight at 4 °C. Absorbance at 370 nm was measured as nmol/mg of protein by using an absorbance coefficient of 22,000 M^{-1} cm^{-1}.

2.4.2. Total sulfhydryl content

To determine total sulfhydryl content, Ellman's reagent (DNTB) was used with modifications following Ellman's method [16]. The test sample (0.5 mL, MP) was diluted 10 times with Tris-HCl buffer (0.2 M, pH 8.0) containing EDTA (3 mM), SDS (1 %), and urea (8 M). Then, 0.5 mL of 10 mM DTNB in Tris-HCl buffer (pH 8.0) was added, and the mixture was incubated at 40 °C for 30 min. The total sulfhydryl content was measured as moles per 10^5 g of protein, by using an extinction coefficient of 13,600 M^{-1} cm^{-1} and measuring the absorbance at 412 nm.

2.4.3. Thiobarbituric acid reactive substances (TBARS)

The TBARS method, as explained in Ref. [17], was employed to determine lipid oxidation. Muscle homogenate (approximately 1 g)

was mixed with butylated hydroxytoluene (BHT) and trichloroacetic acid (TCA). After filtration, 1.5 mL of the filtrate was transferred to two new tubes and mixed with a thiobarbituric acid (TBA) solution. The resulting samples were kept in the dark at room temperature at 20 °C for 15–20 h, and the TBARS level was measured as the concentration of malondialdehyde (MDA) in μg/kg of the sample.

2.5. Color

A colorimeter (Color Quest XE, Hunter Associates Laboratory Inc., USA) was utilized to determine color changes in crayfish samples during storage, following the method described by Ref. [8]. The color determination was performed using D65 as the illuminant, CIE 1964 as the standard observer, and a 6.3 mm aperture.

2.6. Texture

The texture properties of crayfish were measured using a TMS-TOUCH instrument (Food Technology Corporation, USA) following the method described by Ref. [18]. Each sample was positioned with the red dorsal surface facing upwards, and a P/5 (5 mm diameter) flat-bottomed cylindrical probe was employed to perform a two-cycle compression perpendicular to the muscle fibers. The test parameters used were as follows: testing speed = 2.4 mm/s, strain, target pattern, the interval was set to 5 s, and starting force = 0.5 N. The Texture Expert software recorded all TPA parameters.

2.7. Scanning electron microscopy (SEM) analysis

SEM (JEOLJSM-5800 LV, Tokyo, Japan) was used to examine the changes in crayfish microstructure during frozen storage. The samples were trimmed to a thickness of 2–3 mm and then subjected to vacuum freeze-drying in a lyophilizer (Christ alpha 1–4, Martin Christ, Germany) for 36 h at −44 °C. Subsequently, the freeze-dried samples were sputter-coated with a gold alloy using an auto-fine coater. To obtain high-quality images, the coated samples were placed on a copper net and observed at a magnification of 300x with an accelerating voltage of 20 kV, following the method described by Ref. [19].

2.8. Low-field nuclear magnetic resonance (LF-NMR)

An NMR analyzer (MR25, Niumai Electronic Technology Co., Ltd., Shanghai, China) was used to determine the water distribution in the crayfish samples. According to Ref. [20], "the spin-spin relaxation time (T_2) was determined with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence".

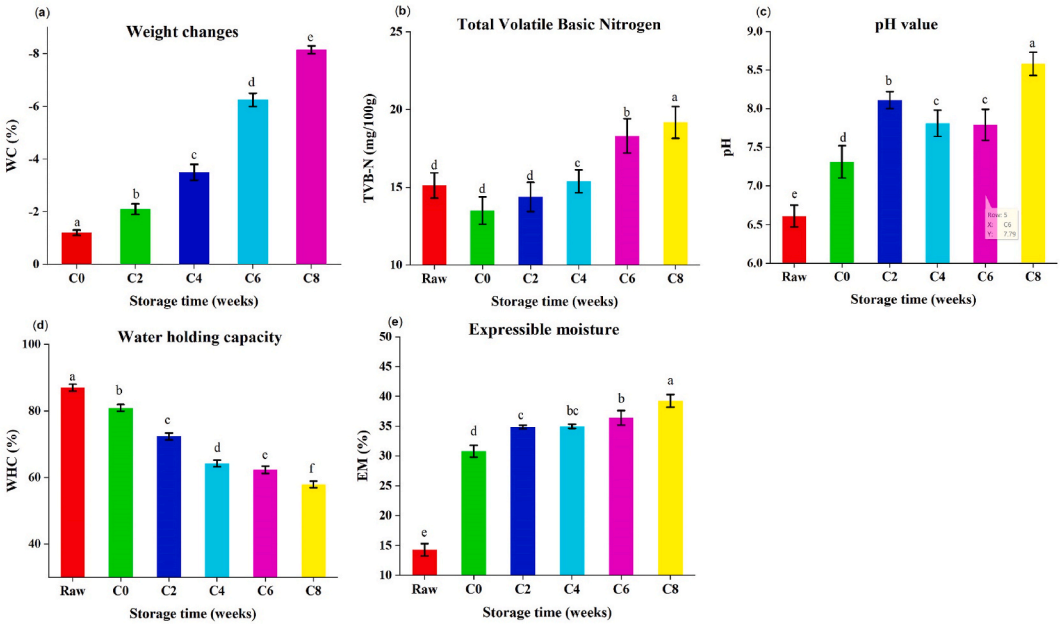

Fig. 1. (a) Weight changes (%), (b) Total Volatile Basic Nitrogen (mg/100g), (c) pH values, (d) Water holding capacity (%), and (e) Expressible moisture (%) of crayfish during two months of storage at −20 °C. C0: cooked (zero time); C2: cooked and frozen (2 weeks); C4: cooked and frozen (4 weeks); C6: cooked and frozen (6 weeks); C8: cooked and frozen (8 weeks). The values shown represent means and standard deviations (n = 3). The small letters indicate a statistical difference (Duncan's multi-range test, $p < 0.05$).

2.9. Statistical analysis

All analyses involved at least three replicates, and the data were expressed as mean ± standard deviation (SD). The Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows, developed by SPSS Inc. in Chicago, IL, USA, was used to analyze the data. A one-way ANOVA with Duncan's multiple range test was applied to compare the results, with a significance level of $P < 0.05$. All graphs were made with Origin 8.1 software, developed by Origin Lab Corporation in Massachusetts, USA.

3. Results and discussion

3.1. Physicochemical properties

3.1.1. Weight changes (WC%)

The percentage of weight change (WC%) in cooked crayfish showed significant differences ($P < 0.05$) during frozen storage at −20 °C. The WC% values immediately after freezing (zero time) were −1.2 ± 0.06 %. Subsequently, these values decreased to −2.1 ± 0.11 %, −3.5 ± 0.17 %, −6.25 ± 0.14 %, and −8.15 ± 0.09 % for the 2nd, 4th, 6th, and 8th weeks of storage, respectively (Fig. 1a). Notably, the sample stored for 8 weeks experienced the most significant weight loss.

The differences in weight change values can be attributed to the effects of storage time and the freezing method on ice crystal formation and growth. The pressure from these generated ice crystals damages the muscle cell membrane, resulting in more weight loss due to faster fluid loss. Additionally, cooking before freezing and protein denaturation during frozen storage are associated with changes in how molecules cross-link, leading to weight changes [21]. Apart from the evident variations in weight loss among the samples, water loss had a significant impact on texture and taste.

3.1.2. Total volatile basic nitrogen (TVB-N)

Crustaceans contain a higher amount of non-protein nitrogen (NPN) compounds, such as trimethylamine oxide (TMAO), compared to fish. TMAO can undergo conversion into TVB-N molecules, which contribute to the development of unwanted off-flavors and odors, making TVB-N a common indicator for assessing the freshness, quality, and shelf life of seafood products [22]. In our study, cooked samples exhibited lower TVB-N values (13.5 ± 0.50 mg/100 g) compared to raw samples (15.12 ± 0.47 mg/100 g) (Fig. 1b). The cooking process may have reduced the TVB-N value of the cooked samples by affecting cell membrane permeability, leading to the leaching of compounds such as non-protein nitrogen (NPN) compounds and free amino acids, resulting in a lower TVB-N value. Furthermore, we observed a significant increase in TVB-N levels in all cooked samples as the storage period increased, reaching a maximum of 19.17 ± 0.59 mg/100 g during the 8th week of storage. These data align with the results of our previous studies [1,2]. The freezing storage affects the internal structure of crayfish, causing protein decomposition and the buildup of nitrogen-containing compounds. Hence, as the duration of frozen storage increases, there is a gradual accumulation of TVB-N [2,23].

These findings are consistent with previous reports. For instance Ref. [24], reported a significant increase in TVB-N levels in uncooked and sous-vide-cooked lobster tails during storage. Additionally [25], found a continual increase in TVB-N levels in deshelled crayfish samples during storage. Similarly [26], observed an increase in TVB-N levels in mussels with storage time. The TVB-N content of the samples in the 8th week (19.17 ± 0.59 mg/100 g) falls within the commonly accepted TVB-N range. According to Ref. [27], the TVB-N content in freshwater products should be less than 20 mg/100 g. Despite remaining within the acceptable limit, the samples gradually deteriorated lost their acceptability, and became less fresh.

3.1.3. pH values

Crayfish pH is directly linked to its acceptability and is commonly used to assess its freshness. pH values measure the amount of "free hydrogen ions (H+)". High pH levels indicate decomposition or degradation in marine products [28]. The initial pH values of raw crayfish (6.61 ± 0.08) were significantly lower than those of cooked samples (7.31 ± 0.12), as shown in Fig. 1c.

The pH levels of the samples fluctuated significantly throughout the storage period ($P < 0.05$). However, there was no statistically significant relationship between storage time and pH levels. Samples with longer storage periods had higher pH levels. Protein oxidation, which results in the production of volatile nitrogen compounds such as ammonia, dimethylamine (DMA), trimethylamine (TMA), and other alkaline substances, may be responsible for the increase in pH values [2].

3.1.4. Water holding capacity (WHC %)

WHC (Thawing and centrifugal losses) refers to a product's ability to retain some or all of its water. It reflects the water retention rate in myofibrillar protein, which is responsible for the connections between tissues and water. Therefore, WHC can be used to evaluate protein denaturation caused by heat-induced structural changes in seafood products [29].

As depicted in Fig. 1d, there were significant differences ($p < 0.05$) between cooked and raw samples. WHC decreased from 86.97 ± 0.59 % to 80.93 ± 0.58 %, indicating protein aggregation and denaturation due to cooking. When heated, protein properties change, affecting their water-holding capacity [30].

Crayfish WHC significantly decreased ($P < 0.05$) during storage, from 80.93 ± 0.58 % (zero time) to 72.33 ± 0.59 %, 64.23 ± 0.57 %, 62.3 ± 0.63 %, and 57.92 ± 0.57 % at the 2nd, 4th, 6th, and 8th weeks, respectively. Ice crystal formation damages the muscle fibers of crayfish, resulting in a decrease in WHC and increased drip losses, which leads to a deterioration in quality due to protein denaturation.

3.1.5. Expressible moisture (EM %)

Changes in muscle protein structure, fluid diffusion, swelling, and fibril contraction influence the expressible moisture (EM) of the muscle [31]. The EM in raw samples was significantly lower than in cooked samples because the protein was preserved in a fresh state. However, the EM of stored crayfish increased significantly ($P < 0.05$) and peaked in the 8th week, as shown in Fig. 1e. This increase in EM is caused by the exposure and aggregation of hydrophobic groups induced by conformational changes in myofibrillar protein [31], as well as the disruption of muscle cell ultrastructure. As the crystallization level of water molecules increases, the tissues become distorted, reducing the ability of crayfish muscles to retain water, which leads to water diffusing outside the cells [32].

3.2. Proximal composition

The percentages of the major biochemical constituents of crayfish muscles on a wet basis are illustrated in Table 1. Raw crayfish moisture and protein contents of 81.79 ± 0.08 % and 15.3 ± 0.20 % were higher than cooked samples of 80.64 ± 0.04 % and 14.39 ± 0.05 %. This is because cooked crayfish have a more porous structure, allowing more protein to leach out. On the contrary, cooked crayfish had slightly higher fat and ash content than raw crayfish. Fat values were 2.30 ± 0.07 % and 1.44 ± 0.08 %, and ash values were 1.82 ± 0.07 % and 1.4 ± 0.05 %, respectively. This increase might be related to protein loss.

These findings were observed by Ref. [7] in cooked Patagonian crabmeat (*Ovalipes trimaculatus*) [33], in cooked crayfish, and [34] in cooked southern king crabmeat (*Lithodes santolla*). They explained that changes in protein-lipid complexes in muscle caused by protein thermal denaturation during the cooking process release bound lipids as free lipids, making them easier to extract.

Generally, there were significant differences in chemical composition ($p < 0.05$) between raw and cooked samples after two months of frozen storage. This research revealed that crayfish have beneficial nutritional properties, including high protein and low lipid content. However, the approximate composition values were generally lower than those reported by Ref. [35], who attributed this increase to the crayfish being fed diets with varying protein levels for eight weeks.

3.3. Protein and lipid oxidation

3.3.1. Carbonyl content

Carbonyl and total sulfhydryl groups are the most indicative and influential indicators of protein oxidation due to their significant influence on protein properties [36]. Cooked samples contained nearly twice as much carbonyl as raw samples, indicating that cooking increased the carbonyl concentration of myofibrillar proteins [37]. Cooking lowers antioxidant defense in the muscles [38], causing protein structure modification by breaking electrostatic or hydrogen bonds and promoting free radical formation, such as oxygen free radicals, which combine with amino acid side chain groups (NH or NH2) and thus oxidize myofibrillar proteins, increasing carbonyl content [39].

After two months of −20 °C storage, the carbonyl content increased significantly ($p < 0.05$) from 0.79 ± 0.12 to 4.15 ± 0.16 nmol/mg protein (Fig. 2a). This increase is attributed to freezing causing muscle structure damage and protein degradation, leading to the release of pro-oxidizing substances and, consequently, an increase in oxidant products. This suggests a higher rate and degree of protein oxidation, particularly after 8 weeks of storage.

3.3.2. Total sulfhydryl content

Sulfhydryl is a highly active component in myofibrillar proteins that preserves the protein's tertiary structure. It belongs to weak secondary bonds that easily transform into intermolecular S–S bonds when exposed to hydroxyl radicals, reducing sulfhydryl groups. It is one of the most commonly used indicators of protein oxidation [36]. The total sulfhydryl groups in myofibrillar proteins were estimated (Fig. 2b). The total sulfhydryl group level in the raw samples was 10.75 ± 0.05 mol/10^5 g protein. However, it significantly decreased ($p < 0.05$) to 7.32 ± 0.06 mol/10^5 g protein after cooking. These changes in the SH group are caused by protein oxidation and aggregation during cooking, resulting in a decrease in SH groups [37].

The T-SH content of stored crayfish significantly decreased ($P < 0.05$) compared to raw samples, and this decrease continued with time extension during frozen storage despite the increase in carbonyl content. This change is linked to the oxidation of sulfhydryl within protein molecules and amino acid chains to form disulfide bonds, decreasing sulfhydryl content [40].

Table 1
Proximate composition of crayfish during two months of storage at 20 °C.

Treatment	Week	Moisture %	Protein %	Fat %	Ash %
Raw	Zero	81.79 ± 0.08^{a}	15.3 ± 0.20^{a}	1.44 ± 0.08^{e}	1.4 ± 0.05^{e}
Cooked	Zero	80.64 ± 0.04^{b}	14.39 ± 0.05^{b}	2.30 ± 0.07^{a}	1.82 ± 0.07^{d}
	2	79.35 ± 0.03^{c}	14.27 ± 0.02^{c}	2.25 ± 0.05^{ab}	1.89 ± 0.01^{cd}
	4	78.55 ± 0.04^{d}	14.22 ± 0.04^{cd}	2.17 ± 0.02^{bc}	1.93 ± 0.02^{bc}
	6	78.12 ± 0.02^{e}	14.16 ± 0.04^{d}	2.09 ± 0.01^{cd}	1.97 ± 0.01^{b}
	8	78.08 ± 0.06^{e}	14.03 ± 0.01^{e}	2.05 ± 0.03^{d}	2.11 ± 0.05^{a}

"Values shown represent means and standard deviations (n = 3). The small letters indicate a statistical difference (Duncan's multi-range test, $p < 0.05$)."

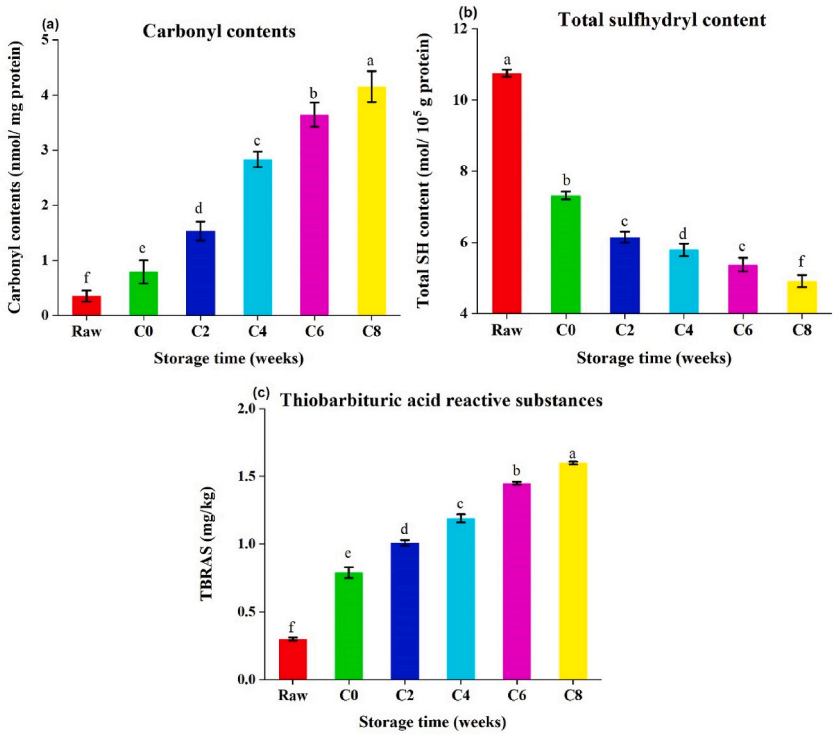

Fig. 2. Carbonyl contents (nmol/mg protein), Total sulfhydryl content (mol/10^5 g protein), and Thiobarbituric acid reactive substances (mg/kg) of crayfish during two months of storage at −20 °C. C0: cooked (zero time); C2: cooked and frozen (2 weeks); C4: cooked and frozen (4 weeks); C6: cooked and frozen (6 weeks); C8: cooked and frozen (8 weeks). Values shown represent means and standard deviations (n = 3). The small letters indicate a statistical difference (Duncan's multi-range test, $p < 0.05$).

3.3.3. *Thiobarbituric acid reactive substances (TBARS)*

The lipid oxidation of frozen crayfish was assessed using TBARS, primarily used to identify secondary reaction products like aldehydes (e.g., malonaldehyde) [41]. The TBARS values in raw crayfish were 0.30 ± 0.00 mg MDA kg^{-1}. They increased to 0.79 ± 0.02 mg MDA kg^{-1} after cooking due to increased lipid peroxidation caused by higher temperatures or during storage [42]. The TBARS value of stored crayfish increased from 0.79 ± 0.02 to 1.01 ± 0.01, 1.19 ± 0.02, 1.45 ± 0.00, and 1.6 ± 0.00 mg MDA kg^{-1}, respectively, after being frozen for 2 months ($P < 0.05$) (Fig. 2c).

These results suggest that oxidative product formation becomes more visible as storage time increases. Freezing significantly impacts ice formation and growth, leading to the loss of muscle cell integrity. Consequently, pro-oxidants such as free radicals and oxidized lipids are released, accelerating the oxidative lipid reaction and increasing malondialdehyde levels [43].

The findings are consistent with [44], who observed similar changes in signal crayfish (*Pacifastacus leniusculus*). It should also be noted that there is a link between TBARS levels and protein carbonylation because lipid and protein oxidations occur during frozen storage through a free radical chain reaction. They are undeniably interconnected and influenced by each other during frozen storage [45].

3.4. *Color*

Table 2 illustrates how cooking and freezing affect crayfish color parameters such as lightness (L*), yellowness (b*), and redness

Table 2
Color of crayfish during two months of storage at −20 °C.

Treatment	Week	L*	b*	a*
Raw	Zero	54.26 ± 1.01^{e}	8.09 ± 0.90^{d}	12.64 ± 1.01^{b}
Cooked	Zero	65.86 ± 0.99^{d}	15.96 ± 1.01^{c}	17.64 ± 0.59^{a}
	2	78.15 ± 0.84^{a}	18.94 ± 1.01^{b}	11.37 ± 1.0^{b}
	4	72.64 ± 0.67^{b}	23.23 ± 0.20^{a}	8.17 ± 0.59^{c}
	6	69.88 ± 0.35^{c}	20.32 ± 1.02^{b}	4.26 ± 0.79^{d}
	8	68.49 ± 0.27^{c}	19.58 ± 0.25^{b}	3.74 ± 0.32^{d}

Values shown represent means and standard deviations (n = 3). The small letters indicate a statistical difference (Duncan's multi-range test, $p < 0.05$).

(a*). After cooking, the color of crayfish muscle undergoes a significant change, transitioning from a transparent appearance to white or light yellow. This alteration in color is a clear indication of the influence of temperature, specifically the effect it has on protein denaturation. The process of denaturation disrupts the natural structure of the proteins, resulting in modifications to their conformation and arrangement. Consequently, the proteins' ability to reflect light is altered, leading to a change in color.

The raw sample L*, b*, and a* values were 54.26 ± 0.58, 8.09 ± 0.57, and 12.64 ± 0.58, respectively. These values significantly increased ($P < 0.05$) after cooking to 65.86 ± 0.57, 15.96 ± 0.58, and 17.64 ± 0.34, respectively. These findings are consistent with [8], who indicated that b*, L*, and ΔE of the crayfish tail rose with increasing time for boiling at around the 60s and approximately 50s for microwave heating. However, the* fluctuated slightly, indicating that protein denaturation is the primary cause of color changes in crayfish tails during heating.

Frozen storage had a significant ($P < 0.05$) impact on cooked crayfish color (L*, b*, a*), as the L* value (lightness) of crayfish increased from 65.86 ± 0.57 before storage to 68.75 ± 0.53 at the end of storage ($P < 0.05$). The L* value is linked to the microstructure because the fluid in the fibers may partially move to the outside after thawing, where it reflects light and enhances the lightness of the samples. The b* value (yellowness) changes similarly to the L* value because protein oxidation increases the b* and L* values. The presence of large quantities of moisture in the samples during frozen storage may cause increased light reflection, increasing the b* value. Due to protein oxidation during storage, the a* value (redness) decreased significantly ($P < 0.05$) from 17.64 ± 0.34 before storage to 11.37 ± 0.58, 8.17 ± 0.34, 4.26 ± 0.46, and 3.74 ± 0.02 at the 2nd, 4th, 6th, and 8th weeks, respectively.

Overall, the a* value decreased significantly as the frozen storage period increased ($P < 0.05$). In contrast, the L* and b* values increased significantly, attributed to water distribution and protein denaturation, which affect product quality, despite no statistically significant color change between samples at the 6th and 8th weeks.

3.5. Texture profile analysis (TPA)

Textural properties are essential qualities of muscle products in the eyes of consumers, playing an important role in consumer acceptance [46] as texture is vulnerable to structural changes caused by protein denaturation [47].

TPA parameters of cooked crayfish samples stored at −20 °C for two months are displayed in Table 3. The hardness, cohesiveness, springiness, gumminess, and chewiness of samples all increased ($P < 0.05$) from 3.78 ± 0.14, 3.1 ± 0.12, 0.52 ± 0.02, 1.9 ± 0.10, and 5.89 ± 0.90 to 3.85 ± 0.10, 0.69 ± 0.02, 4.21 ± 0.50, and 16.20 ± 1.10, respectively, compared to raw samples. It is worth noting that [8] found that heating increases crayfish tails' internal hardness, springiness, surface hardness, and compactness. During heating, the cross-linking and denaturation of proteins (particularly sarcoplasmic and connective tissue proteins, and myosin) may increase compactness, springiness, and hardness [48].

The hardness of cooked crayfish decreased significantly ($P < 0.05$) after two months of storage, from 6.1 ± 0.20 N to 2.15 ± 0.13 N (−20 °C), because textural changes are strongly tied to variations in water phases and content during storage, as water is the main indicator influencing crayfish texture properties. As a result, ice crystal growth and formation during frozen storage may damage cell structures and muscle tissue, causing myofibrils to break apart and thus increasing water loss and water migration after thawing, reducing muscle hardness.

Furthermore, the springiness values of samples during storage decreased significantly ($P < 0.05$) in the 8th week to 3 ± 0.02 mm compared to samples before storage, which were 3.85 ± 0.10 mm. This could be attributed to protein changes and degradation, which increased the damage to the crayfish structure during storage and decreased sample elasticity. On the other hand, cohesiveness decreased significantly ($P < 0.05$) to 0.6 ± 0.01 at the end of storage due to textural changes in the crayfish muscle that occur in two steps (cooking and storage), as ice crystals destroy muscle tissue and cause structural damage during storage. Nevertheless, the difference in muscle cohesiveness value was non-significant ($P > 0.05$) during the 2nd week, nor between the 6th and 8th weeks of storage.

Moreover, texture profile analysis revealed that the gumminess and chewiness values of the samples declined considerably with increasing storage time. Our findings showed that as storage time at −20 °C increased, the crayfish softened and degraded due to ice crystal formation [49] and changes in tissue structure integrity, both of which have a direct impact on quality and thus affect texture properties.

These results align with previously published literature on frozen crayfish muscle, where hardness, elasticity, and chewiness decreased as frozen storage time increased [23]. In contrast [50], showed that the springiness and hardness increased at week 4 due to protein aggregation caused by low-temperature freezing [51] and then decreased as storage time increased throughout the storage

Table 3
Texture profile of crayfish during two months of storage at −20 °C.

Treatment	Week	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (mj)
Raw	Zero	3.78 ± 0.14^{c}	3.1 ± 0.12^{d}	0.52 ± 0.02^{c}	1.9 ± 0.10^{c}	5.89 ± 0.90^{c}
Cooked	Zero	6.1 ± 0.20^{a}	3.85 ± 0.10^{a}	0.69 ± 0.02^{a}	4.21 ± 0.50^{a}	16.20 ± 1.10^{a}
	2	5.95 ± 0.10^{a}	3.63 ± 0.07^{b}	0.68 ± 0.03^{a}	4.04 ± 0.30^{a}	14.66 ± 1.06^{a}
	4	4.55 ± 0.17^{b}	3.44 ± 0.08^{c}	0.65 ± 0.05^{ab}	2.96 ± 0.60^{b}	10.18 ± 0.99^{b}
	6	3.04 ± 0.15^{d}	3.14 ± 0.05^{d}	0.62 ± 0.04^{b}	1.88 ± 0.80^{c}	5.90 ± 0.62^{c}
	8	2.15 ± 0.13^{e}	3 ± 0.02^{d}	0.6 ± 0.01^{b}	1.29 ± 0.40^{c}	3.87 ± 0.78^{d}

Values shown represent means and standard deviations (n = 3). The small letters indicate a statistical difference (Duncan's multi-range test, $p < 0.05$).

period.

3.6. Microstructure

SEM profiles were evaluated in our study(Fig. 3) because microstructural integrity is a key determinant of the quality of frozen foods. Based on the SEM images obtained before (Fig. 3a) and after after (Fig. 3b) cooking, we noted that cooking has a noticeable effect on muscle structures. The cooked sample of unfrozen crayfish exhibited well-organized, closely connected, and intact muscle fibers. It was also homogeneous, with a clear, well-defined structure, a fine uniform mesh, and a bundle-like cell structure.

Samples stored in the 2nd week (Fig. 3c) maintained a relatively uniform and dense morphology. However, the structure and muscle fibers were slightly damaged, loosened, and chaotic compared to the raw samples. This can be attributed to the small ice crystals produced during this time, which were not enough to damage the cell structures significantly. In the 4th-week samples (Fig. 3d), the muscle fibers were noticeably broken, resulting in a fuzzier structure and larger pores compared to the 2nd-week and raw samples. At the 6th and 8th weeks, the samples exhibited severe muscle fiber dysregulation, leading to further disruption of the protein matrix, shrinkage, and destruction of the muscle fiber structure (Fig. 3e &f).

The size and distribution of ice crystals can be influenced by the freezing rate in intracellular and extracellular regions, as well as the structural integrity of the frozen meat [52]. Freezing causes an uneven and irregular distribution of ice crystals inside and outside of cells, leading to significant damage to cells, tissues, and muscle fibers. Furthermore, as the storage time increases, gap formation increases, which facilitates the formation of dripping channels and increases drip loss after thawing, thereby reducing nutritional value and quality. Thus, the microstructure of frozen products is closely linked to the preservation of acceptable texture, color, and nutritional value.

In summary, the muscle fibers of the freeze-stored samples exhibited varying degrees of damage, such as being discontinuous and having more irregular pores. These findings can primarily be attributed to the crystallization of more water with increased storage time at low temperatures, resulting in severe damage and breakage of muscle fibers.

3.7. Moisture mobility and distribution

Three peaks were found to represent different water phases in muscle tissue: bound water (1 ms $< T_{2b} <$ 10 ms) within highly crystallized structures and macromolecules, immobilized water (10 ms $< T_{21} <$ 100 ms) within extra-myofibrillar and tissue protein structures, and free water (100 ms $< T_{22} <$ 1000 ms) between muscle cells and myofibrils [53]. The proton freedom degree determines the T_2 value, which varies with the physicochemical characteristics of the material. Fig. 4 demonstrates the variation in peak area and relaxation time distribution of the three different water phases.

The heads of the T_{2b}, T_{21}, and T_{22} curves indicate the population of each water component (Fig. 4a). The proportions of T_{2b}, T_{21}, and T_{22} approximate the proportions of each water component to the overall water content and the amounts of bound, free, and immobilized water, expressed as P_2 (Fig. 4b). The interaction degree can be indicated by the relaxation time (T_{21}) between the

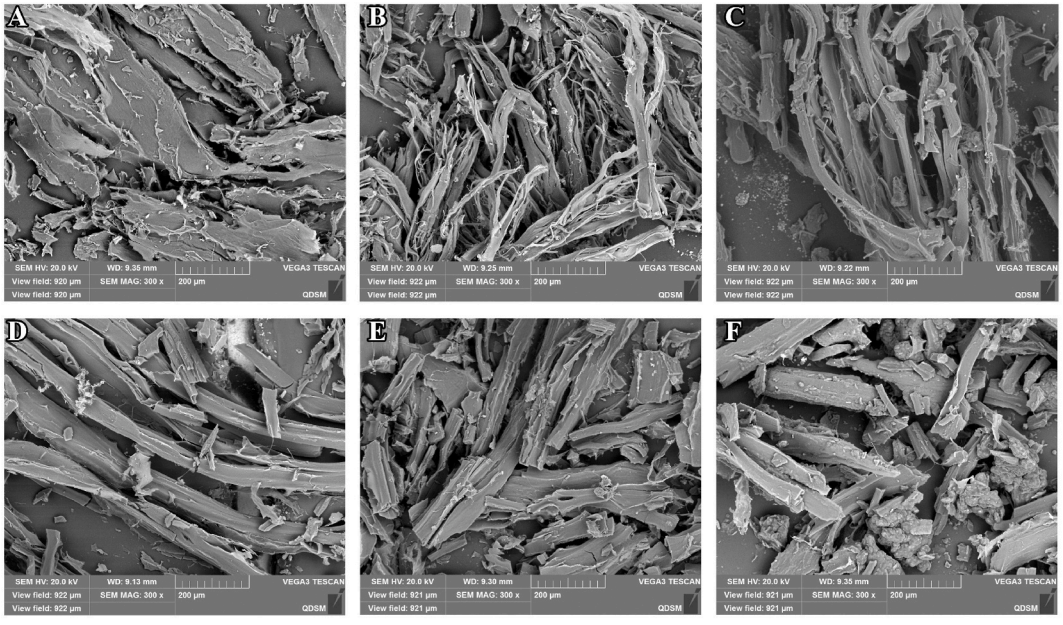

Fig. 3. Representative SEM images of myofibers of crayfish during two months of frozen storage. a: Raw samples; b: cooked (zero time); c: cooked and frozen (2 weeks); d: cooked and frozen (4 weeks); e: cooked and frozen (6 weeks); f: cooked and frozen (8 weeks).

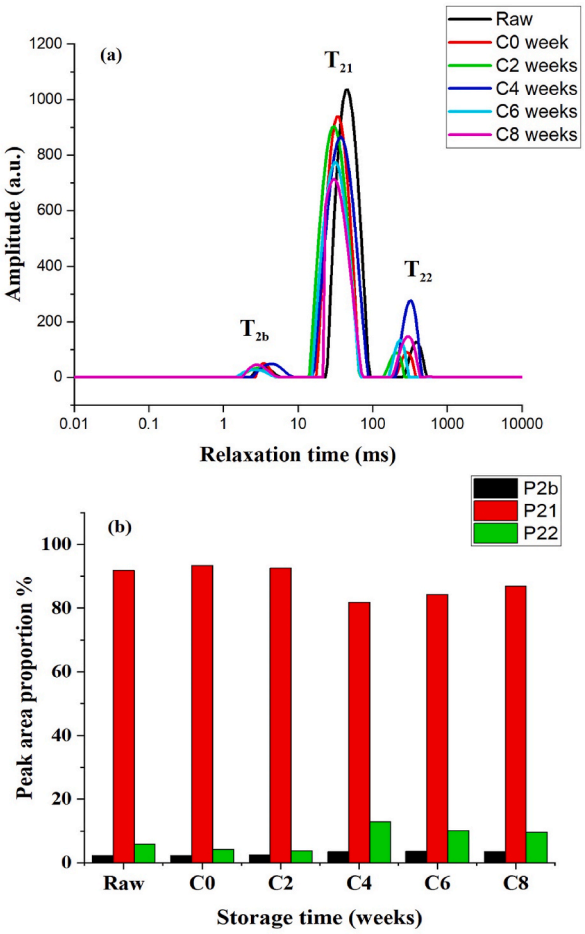

Fig. 4. The influence of cooking and freezing storage on the transverse relaxation time distribution curves (T_2) (a) and peak ratio of T_{2b}, T_{21}, and T_{22} (b) in crayfish.

structures of highly regulated proteins and water molecules. Cooked crayfish exhibited a faster relaxation time (T_{21}) and less immobilized water, resulting in a lower proportion (P_{21}) than raw samples. This is caused by heat-induced protein denaturation and shrinking, which reduces the spacing of the myofibril structure.

Freezing significantly affects changes in water distribution caused by protein denaturation during storage. The location and size of ice crystals formed during freezing influence moisture mobility in stored crayfish. Longer relaxation times indicate that water is more mobile and that water-protein bonds are loose, and the T_{22} increases in muscle are viewed as an increase in inter-myofibrillar spacing. Although storage has little effect on the bound water content, the total moisture weight is reduced due to the decrease in water holding capacity (WHC) caused by freezing and thawing techniques (Fig. 1 d). In general, P_{21} decreases while P_{22} increases because myofibrillar protein denaturation reduces its ability to bind water. Therefore, the water migration rate is faster than the rate of ice crystal formation during freezing, causing changes in water distribution.

4. Conclusion

Overall, this research provides valuable information on the negative impact of freezing on the quality of cooked crayfish. This impact is directly related to physicochemical changes caused by ice crystal formation and growth at −20 °C. In addition to these changes, mechanical damage is exacerbated, and the cellular microstructure is destroyed, which has a negative effect on the quality and texture of crayfish. As a result, future trends should emphasize the use of other effective freezing processes or methods. This may include the addition of cryoprotectant agents in combination with proper packaging to avoid or minimize these negative impacts. By implementing such measures, it is possible to extend the shelf life and maintain the quality of cooked crayfish while stored.

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Ethics approval

This article does not contain any study with human participants or animals performed by any of the authors.

Data availability

The data supporting the study's findings are provided in the publication and its supplementary materials.

CRediT authorship contribution statement

Taher Abdelnaby: Methodology, Investigation, Data curation, Conceptualization. **Tingyu Feng:** Investigation. **Zhang Tiantian:** Data curation. **Xiaoming Jiang:** Investigation. **Wang Yuming:** Formal analysis. **Zhaojie Li:** Validation, Software, Resources, Project administration. **Changhu Xue:** Writing – review & editing, Writing – original draft, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interestsProf. Xue Changhu reports financial support was provided by Shandong Provincial Department of Science and Technology. Prof. Xue changhu reports a relationship with Shandong Provincial Department of Science and Technology that includes:. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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