The impact of quick-freezing methods on the quality, moisture distribution and microstructure of prepared ground pork during storage duration

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**ABSTRACT**

The aim of present study was to investigate the influences of ultrasound-assisted immersion freezing (UIF), immersion freezing (IF) and air freezing (AF) on the quality, moisture distribution and microstructure properties of the prepared ground pork (PGP) during storage duration (0, 15, 30, 45, 60, 75 and 90 days). UIF treatment significantly reduced the freezing time by 60.32% and 39.02%, respectively, compared to IF and AF (P < 0.05). The experimental results of quality evaluation revealed that the $L^*$ and $b^*$ values, juice loss, cooking loss, TBARS values and carbonyl contents were decreased in the UIF treated samples, while the $a^*$ value, peak temperatures ($T_m$), enthalpy ($\Delta H$) and sulfhydryl contents were significantly higher than those of IF and AF treated samples (P < 0.05). In addition, low-field nuclear magnetic resonance (LF-NMR) and differential scanning calorimetry (DSC) analysis demonstrated that UIF inhibited the mobility of immobilized water and reduced the loss of immobilized and free water, and then a high water holding capacity (WHC) was achieved. Compared to the IF and AF treatments, the UIF treated PGP samples possessed better microstructure. Therefore, UIF could induce the formation of ice crystals with smaller size and more even distribution during freezing process, which contributed to less damage to the muscle tissue and more satisfied product quality.

1. Introduction

In the past few decades, due to increasing working loading and stresses on people with a nine-to-fiver, especially young people whom have less time for preparing and cooking meal, as well as the change of consumption viewpoints, a variety of prepared and ready-to-eat foods have been developed and top sold in the market, such as frozen dumplings, steamed stuffed buns and pies are extremely popular in China, especially for the northern area. In the formulation of the above-mentioned food products, prepared ground pork (PGP) has been considered as an important component and gains increasing attention because of its characteristics of tasty, easy preparation, simple process, and quick frozen process for sale, circulation and storage. However, just like most quick frozen foods, PGP was also prone to be deteriorated in color and taste during the quick-freezing process and storage period, leading to a shortened shelf-life and a huge amount of food resource waste [1–3]. It has also proven that the above-mentioned quality deterioration is mainly related to the size and distribution of ice crystals formed during quick-freezing process [4]. The large and unevenly distributed ice crystals destroyed muscle fibrin and caused the deterioration of quality, water migration, as well as destruction of microstructure [5,6]. Therefore, it is critical to explore a suitable quick-freezing method and develop the optimum operational conditions for PGP.

As far as we know, traditional quick-freezing techniques, such as air blowing, plate contact, fluidized bed freezing, and immersion freezing, have been widely adopted in the food industry. Somehow, large and uneven ice crystals formed inside food during the freezing process may cause damage to food quality [7,8]. Recently, various types of new composite freezing technologies have also emerged and developed [9–11]. Among them, ultra-high pressure and magnetic resonance quick-freezing methods have a high requirement on food materials and equipment [12,13]. Ultrasound-assisted immersion freezing (UIF), a new green freezing technology that combines ultrasonic and immersion freezing, has received more and more focus. The main working mechanism of UIF is discovered to be that mechanical, cavitation and thermal effects are generated by ultrasonic wave, thus to promote the formation of crystal nuclei in the freezing process, inhibit the regressive growth of ice crystals, and then reduce the damage of ice crystals to food products [14–17]. Up till now, UIF has been applied in the quick-freezing process...
of plenty of food products, including agar gel [18,19], frozen dough [20,21], and vegetables, such as mushrooms [4,22], potatoes [23], radishes [24,25], lotus root [7], broccoli [26], cabbage [27]. Besides, meat products are prone to fat oxidation, protein oxidation and denaturation during quick-freezing and frozen storage period, resulting in texture loss and flavor change [1,2,28,29]. Previous studies have shown that UIF improved the quality of meat products during quick-freezing and storage duration, for example, carp [30–33], chicken breast [34,35], pig long muscle [5,36] and so on. However, to the best of our knowledge, there are limited reports on the application of UIF technology on PGP processing.

In this study, the effects of three different types of quick-freezing methods, including UIF, immersion freezing (IF) and air freezing (AF), on the quality, moisture distribution, and microstructure of PGP within a storage period of 90 days were investigated. The results could provide useful information and guidance of properly selecting freezing processing for meat with high quality and cost-effective method.

2. Materials and methods

2.1. Chemicals

All chemicals used throughout the present study were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample

2.2.1. Preparation of ground pork

The fresh boneless pork leg and ingredients were purchased from a local supermarket (Hefei, China). Pork was cleaned with running water, cut into pieces, added with ingredients of 2% salt, 0.6% sugar, 2% cooking wine and 12% ice water, and then minced by a meat grinder (MJ-LZ225, Midea Co., Guangzhou, China). The subsequent treatments were classified into three groups as described in Fig. 1.

2.2.2. Sample processing

PGP samples were frozen using three different quick-freezing methods at $-30 \pm 0.5 \, ^\circ C$. For UIF process, the samples were frozen in a UIF system mainly composed of a constant temperature circulating tank (TMS8005-8R25, Tomos Technology Co., Zhejiang, China) and a ultrasonic probe (JY98-III DN, Xinzhi Biotechnology Co., Ningbo, China) as shown in Fig. 2, with 95% ethanol plus 5% fluoride as a coolant. The power density of ultrasonic equipment was set at 80 W/L with a frequency of 20 kHz. Ultrasound with a 10 s on/15 s off cycle was applied throughout the whole experiments. For IF process, all the operational conditions were the same as those of UIF except for no ultrasound involved the treatment. For AF process, the samples were frozen in a common freezer (BC/BD-300DT, Meiling Co., Hefei, China). For all

![Fig. 1. Flow chart for experiment design.](Image)  
![Fig. 2. Schematic diagram of the ultrasound-assisted immersion freezing system.](Image)
samples, the freezing process was regarded to be completed when the geometric centre temperature reached approximately to \(-18^\circ C\), and then samples were stored in a freezer at \(-20 \pm 0.5^\circ C\). After that, the samples were collected on the days of 15, 30, 45, 60, 75, and 90 for the subsequent analysis and determination. Before analysis, the frozen samples were thawed by placed in a refrigerator until the central temperature reached 4 \(^\circ C\).

2.3. Freezing curve

During the freezing period, the central temperature of samples was recorded from 10 to \(-18^\circ C\) every 30 s with a thermocouple (HY101, Yuanhengtong Technology Co., Shenzhen, China). With freezing time as the abscissa and central temperature as the ordinate, the freezing curves of three different freezing methods were plotted.

2.4. Color

Before determination, the moisture on the surface of the thawed PGP samples was wiped with paper towel. The thawed samples (4 \(^\circ C\)) were evenly laid on a clean cuvette with a diameter of 30 mm, and the values of \(L^*, a^*\) and \(b^*\) were measured using a colorimeter (SC-100, Kangguang Optical Instrument Co., Beijing, China). Each sample was taken at different positions and measured three times in parallel.

2.5. Water holding capacity (WHC)

2.5.1. Thawing loss

The thawing loss of PGP was determined according to the method of Mortensen et al. [37]. Briefly, the samples were weighed before thawing (\(W_0\)), and the frozen samples were thawed until the central temperature reaching 4 \(^\circ C\), dried with paper towel and then weighed (\(W_1\)) immediately. The thawing loss was calculated with the following equation.

\[
\text{Thawingloss(\%) = } \frac{W_0 - W_1}{W_0} \times 100\% 
\]

2.5.2. Centrifugal loss

The centrifugal loss was measured by the method of Huang et al. [28]. The thawed samples were weighed (\(W_1\)) and then placed into a 50 mL centrifugal tube with filter paper inside. After that, the samples were centrifuged in a centrifuge (TG16-WS, Xiangli Scientific Instrument Co., Hunan, China) at 2000 r/min for 15 min and then weighed (\(W_2\)). The calculation of centrifugal loss was performed with the following equation.

\[
\text{Centrifugalloss(\%) = } \frac{W_1 - W_2}{W_1} \times 100\% 
\]

2.5.3. Cooking loss

The cooking loss was evaluated according to the method described by Faridnia et al. [38]. The thawed samples were weighed (\(W_1\)) and then steamed with boiled water (100 \(^\circ C\)) for 15 min. The steamed samples were immediately blotted dry and weighed (\(W_2\)). The cooking loss was calculated by using the equation described as follows.

\[
\text{Cookingloss(\%) = } \frac{W_1 - W_2}{W_1} \times 100\% 
\]

2.6. Texture profile analysis (TPA)

The TPA was analysed according to Li et al. [2] with minor modification. After thawing, PGP samples were steamed with boiled water at 100 \(^\circ C\) for 15 min and then cooled down to 20 \(^\circ C\). The samples (25 \(\times\) 20 \(\times\) 15 mm\(^3\)) were performed with a Texture Analyser (TA-Xtplus, Stable company, USA) and the parameters were setting as follows: P/50 (flat- surface cylindrical probe, 5 cm diameter cylindrical probe, 2 mm/s crosshead speed, and 40\% “Strain” measurement mode.

2.7. pH measurement

The pH value of samples was measured as described by Li et al. [2]. The thawed samples of 10 mg were mixed with 90 mL distilled water and maintained at 4 \(^\circ C\) for 30 min. After that, the suspension was measured by a pH meter (PHS-3C, INESA Scientific Instrument Co., Shanghai, China).

2.8. Thiobarbituric acid reactive substances (TBARS)

The TBARS value was determined following the method of Xia et al. [39] with a minor modification. The thawed samples of 2.0 g were weighed accurately and placed in a 50 mL centrifuge tube, and then 3 mL of 7.5\% (w/v) trichloroacetic acid (TBA) solution and 17 mL of 2.5\% trichloroacetic acid-HCl solution were subsequently added. The mixtures were heated in boiled water at 100 \(^\circ C\) for 30 min. After being cooled to 20 \(^\circ C\), a 4 mL aliquot of the suspension was mixed with 4 mL of chloroform, and then vortexing for 1 min, followed by centrifugation with a centrifuge (TG16-WS, Xiangli Scientific Instrument Co., Hunan, China) at 5500 r/min for 20 min. Finally, the absorbance of samples was measured at 532 nm. The TBARS value was expressed as the following.

\[
\text{TBARS(mg MDA/kg)} = \frac{A_{532}}{W_s} \times 9.48 
\]

where \(A_{532}\) is the absorbance of the assay solution at 532 nm, \(W_s\) is the weight of PGP samples (g), and “9.48” is a constant derived from the dilution factor and the molar extinction coefficient (152000 M\(^{-1}\) cm\(^{-1}\)) of the red TBA reaction product.

2.9. Differential scanning calorimetry (DSC)

The changes of protein thermal stability and freezable water content of samples were investigated using a DSC (Q200, TA, Co., USA). The parameters of endothermal transitions were described as follows. The thawed samples of 10 ~ 15 mg were accurately weighed and placed into standard aluminum pans, sealed and then heated from 25 to 100 \(^\circ C\) at a scanning rate of 5 \(^\circ C\)/min under a nitrogen atmosphere. An empty pan was set as the reference. The peak temperatures (\(T_m\)) and enthalpy (\(\Delta H\)) were estimated from the thermogram by using Pyris-12 software (Perkin-Elmer Instruments, USA). There were some differences for the parameters when freezable water content detected. After the thawed samples of 10 ~ 15 mg accurately weighed, added into standard aluminum pans and sealed, the temperature dropped from 0 to \(-40^\circ C\), equilibrated for 3 min and then heated up to 100 \(^\circ C\) at a scanning rate of 5 \(^\circ C\)/min under a nitrogen atmosphere. The changes of \(\Delta H\) on the freezable water were recorded.

2.10. Protein oxidation

2.10.1. Extraction of myofibrillar protein (MP)

The method of MP extraction was described by Xia, et al. [39]. Briefly, the thawed fatless samples of 20–30 g were mixed with 5 volumes of phosphate buffer solution (0.1 M NaCl, 2 mM MgCl, 1 mM EDTA, 10 mM Na\(_2\)HPO\(_4\), pH 7.0). After homogenization, the extraction solution was centrifuged at 5000×g for 15 min. The precipitate was extracted four times with 5 volumes of phosphate buffer solution as described above, washed twice with 5 volumes of NaCl solution (0.1 M), and then re-dissolved with 5 volumes of NaCl (0.1 M). After that, the pH of the mixed solution was adjusted to 6.2, and filtered through 4 layers of gauze. After filtration, the suspension was centrifuged using the same conditions as described above to obtain the MP precipitate and then stored in a 4 \(^\circ C\) refrigerator for further analysis. The protein content of the MP solution was determined using the biuret method with bovine
serum albumin as the standard solution, and MP solution was used within 48 h [31].

2.10.2. Carbonyl content

The carbonyl content of MP was determined by the method reported by Sun et al [31]. The protein solution (1 mL, 2.0 mg/mL) was incubated with 10 mM DNPH for 1 h and shaken every 10 min at 25 °C. Then, 20% trichloroacetic acid was added and mixed for 15 min. For the blank control, an equal volume of 2 mol/L HCl was used instead of DNPH, and the mixtures were centrifuged at 1000 × g for 5 min. Excess DNPH was removed, and the precipitates were washed 3 times with 1 mL of ethanol: ethyl acetate (1:1, v/v). The protein particles were dissolved in 3 mL of guanidine (6 mol/L) at 37 °C and shaken for 15 min. The absorbance of the solution was measured at 370 nm. The carbonyl content of the protein hydrazone was calculated with the absorption coefficient of 22000 M⁻¹ cm⁻¹, and was expressed in nmol/mg.

2.10.3. Total sulfhydryl content

The total sulfhydryl content of MP was determined as described by Zhao et al. [40] with a minor modification. The volume of 8 mL of tris-glycine buffer (10 mM EDTA, 0.2 M Tris-HCl, 8 M urea, pH 8.0) was added to the protein solutions (1 mL, 2 mg/mL). Then the mixed solutions were homogenized for 2 min, and centrifuged at 1000 × g for 15 min. The 4.5 mL of supernatants were mixed with 0.5 mL Ellman’s reagent (0.1 M tris-glycine buffer, 10 mM DTNB, pH 8.0) and kept at 25 °C for 30 min. Finally, the absorbance of supernatants was measured at 412 nm. A blank solution was made by using 4.5 mL of tris-glycine buffer (pH 8) instead of the equivalent volume of supernatant. The total sulfhydryl content of MP was calculated using the extinction coefficient of 13,600 M⁻¹ cm⁻¹.

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

The relaxation measurements were carried out according to the method of Zhang et al. [34] with a LF-NMR analyser (NMII20-015V-I, Newmai Electronic Technology Co., Shanghai, China). The magnetic field intensity was set at 0.47 T, and the frequency of corresponding proton resonance was 20 MHz. The thawed samples (4 °C) were placed in NMR tubes with a height of 3 cm. For each sample, 16 scans were performed at 2 s intervals, with a total of 3000 echoes. After the standardization of the original data, Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence and Contin algorithm were used to analyze the transverse relaxation time (T₂) of samples.

2.12. Microstructure

The microstructure of PGP samples was observed as described by Islam et al. [4]. The thawed samples were steamed with boiled water at 100 °C for 10 min, cooled to 20 °C, and then cut into 2 × 5 × 2 mm strips. Firstly, the strips were prefixed in 2.5% glutaraldehyde solution for 24 h and then washed 3 times with phosphate buffered saline (0.1 M, pH 6.8) for 15 min. After that, the strips were dehydrated with a gradient of ethanol solutions (50%, 70%, 80% and 90%, each for 15 min), and then 100% ethanol was used to dehydrate 3 times for 15 min each time. After degressed with chloroform for 1 h, the strips were replaced by 100% ethanol: tert-butanol (1:1) and pure tert-butanol of replacement fluid (15 min each time). Finally, the strips were put in a freezer at −80 °C for 8 h, and then dried in a freeze dryer (FD-1, Boyukang Technology Co., Beijing, China) for 24 h. A cold-field scanning electron microscope (SU8020, Hitachi LTD, Japan) was used to observe the microstructure of PGP samples.

2.13. Statistical analysis

For each group, all samples were tested three times as technical repetition. The experimental results were expressed as the mean value ± standard deviation (SD), and analyzed by one-way analysis of variance (ANOVA) method and a Duncan multiple range test at a 5% significance level, using SPSS 22.0 software (IBM Corporation, Armonk, NY, USA). All graphs were generated by using Origin 9.1 software (Origin Lab, Northampton, Massachusetts, USA).

3. Results and discussion

3.1. Freezing curve

The temperature-time curves of the samples treated by different quick-freezing methods are shown in Fig. 3. The total freezing time of AF, IF, and UIF was determined to be about 63, 41, and 25 min, and their corresponding phase-change time was approximately 16, 12, and 8 min, respectively. The freezing time of IF was significantly shorter compared to AF (P < 0.05), due to the high heat transfer coefficient of liquid. Thus the results suggested that the freezing rate of IF samples was higher than the AF treated samples. A similar result was previously observed in the freezing process of strawberries [41]. Moreover, UIF samples showed the shortest time to pass through the largest ice crystallization zone, and the same result was obtained for the total freezing time, which was closely related to the cavitation and mechanical effects of ultrasound treatment. During the freezing process, many cavitation bubbles produced by ultrasonic waves acted as crystal nuclei to induce ice crystal formation when the bubble threshold reached [16]. In addition, the phenomenon of turbulence was easily produced by cavitation bubbles under ultrasound treatment in the liquid medium. Then the formation of ice nucleation was accelerated and the heat transfer efficiency was enhanced [26,42]. These findings were consistent with the experimental results of UIF on agar gel [19], dough [20], and porcine longissimus muscles [36].

3.2. Color

Color has been considered as one of the most obvious parameters for the evaluation of food quality. The color change would influence and has a negative impact on consumer purchase decision, and is seriously affected by the freezing rate and storage time [43,44]. The L*, a* and b* values of PGP samples treated by three quick-freezing methods were detected and the results are listed in Table 1. When the frozen storage period reached 90 days, UIF samples had the lowest L* and b* values, which were significantly lower than that of AF and IF samples (P < 0.05). Meanwhile, the samples treated with AF had the highest L* and b* values.
values. In terms of the a* value, all samples in AF, IF and UIF groups decreased during frozen storage. However, the a* value of UIF was significantly higher than that of AF and IF samples (P < 0.05). The samples of 0 d represented that the PGP was detected immediately after quick-freezing without frozen storage.

In general, the L* value is greatly affected by the freezing rate. The a* value of UIF was significantly higher than both AF and IF samples (P < 0.05). The possible reason was that UIF inhibited water migration and reduced the damage of ice crystals to muscle fibers, thus the juice loss rate and muscle protein oxidation of PGP samples were reduced. In addition, the impact of quick-freezing methods and storage duration on the a* and b* values of different meat ingredients has been evaluated, such as carp [32] and pork tenderloin [36,48]. The ice crystals had a great impact on protein oxidation and fat oxidation, which were closely related to a* and b* values. The decrease in a* value was mainly due to the myoglobin oxidation. Ice crystals would destroy muscle cells of PGP samples, resulting in the release of a large amount of oxidase, which accounted for myoglobin to be oxidized. Moreover, low temperature reduced the activity of methemoglobin reductase, leading to a large accumulation of methemoglobin, and then b* value significantly rose (P < 0.05) [3]. The damage degree of UIF samples by ice crystals was less than that of AF and IF groups, thus the a* value was significantly higher (P < 0.05). The results were similar to the findings of Pinheiro et al. [49], who found that frozen storage caused adverse changes in the color of meat. Furthermore, lipid oxidation can also promote the oxidation of myoglobin and cause poor color. However, UIF could restrain the formation and accumulation of myoglobin caused by lipid oxidation, thus improved the color quality of PGP during freezing process, which was consistent with the results reported by Li et al. [2].

3.3. WHC

The WHC of frozen food is usually evaluated by thawing loss, centrifugal loss, and cooking loss, in which the sum of thawing loss and centrifugal loss is regarded as the juice loss. The WHC is mainly related to the ability of high-yield tuna meat to retain its own water [50]. As shown in Table 1, from 0 day to the 90th day, the thawing loss of UIF, IF and AF samples increased by 2.45%, 3.70% and 4.42%, respectively. The
changes of hardness, chewiness and springiness, the texture of UIF and AF samples. Thus, the above data indicated that the WHC of UIF between AF and IF samples (P < 0.05), and the lowest value was found in the UIF samples. Besides, the cooking loss of UIF, IF and AF samples increased by 4.61%, 4.38% and 5.06%, respectively. There was no significant difference on the cooking loss among UIF, IF and AF samples. Thus, the above data indicated that the WHC of UIF samples was better than that of AF and IF. The decrease in WHC during frozen storage may because the growth of ice crystals could squeeze adjacent muscle fibers and damage those tightly arranged muscle tissue [51]. The results indicated that the UIF treatment could maintain the WHC of samples due to reduced muscle fiber tissue of PGP caused by ice crystals [36,39]. Other reactions such as fat oxidation may accelerate protein oxidation and protein degeneration, thus would lead to a decrease in WHC. However, our following results of TBARS revealed that UIF could decrease the degree of fat oxidation, thus UIF retained the WHC of PGP samples.

3.4. TPA

The changes in hardness, chewiness, and springiness were used as indexes to evaluate texture characteristics of PGP samples. From Table 1, it could be seen that the hardness and chewiness slightly increased at the initial stage of frozen storage from 0 to 15 days (P > 0.05), and then showed a downward trend with the prolonged frozen storage time from 15 to 90 days (P < 0.05). The springiness decreased gradually during the entire storage (P > 0.05). Based on the changes of data, the hardness, chewiness, and springiness values of the UIF samples were higher than those of IF and AF samples, but no significant difference was found among UIF, IF and AF samples (P > 0.05). The increase of hardness and chewiness may be attributed to the sublimation of ice crystals and dehydration of gelatinous structure within 15 days. Then with the growing of the ice crystals, concentration of solute and fat oxidation would occur and triggered the deterioration of muscle fiber protein and gelatin structure, which caused a decline in texture properties [52,53]. In addition, protein degradation and the changes in the protein structure may weaken protein hydration ability and the muscle fibre structure, also leading to the decline of texture [2]. From the changes of hardness, chewiness and springiness, the texture of UIF samples was better than that of AF and IF, owing to the smaller size and even distribution of ice crystal formed in UIF samples which reduced the damage to myofibrin.

3.5. pH

pH value is normally used as an important index to reflect the food quality. As shown in Table 1, the pH values of UIF, IF and AF increased from 5.81, 5.81 and 5.88 to 6.09, 6.20 and 6.45 after stored for 90 days, respectively. During storage time from 0 to 90 days, the pH values of three groups were increased, and the UIF samples were lower compared to IF and AF samples. At 90 days, no significant difference was observed between AF and IF samples (P > 0.05), but there was a significant difference between UIF and AF samples (P < 0.05).

The pH rise could be attributed to the degradation of the skeleton protein and the release of amino acids induced by cathepsin. Similar results of pH changes in beef and pork were reported [3,54]. In addition, the accumulation of alkaline substances caused by the actions of autolytic enzymes and microorganisms, for example ammonia and trimethylamine also increased the pH values [36,55]. The size of ice crystals formed in UIF process was smaller compared to AF and IF, which reduced the damage to muscle fibrin and the oxidative decomposition of protein, so the pH values of UIF samples were lower than those of IF and AF samples.

3.6. TBARS

Fat oxidation caused the quality deterioration, nutrients loss, and toxic substance formation [56,57]. In general, fat oxidation can be evaluated by TBARS values, and the main secondary products are malondialdehyde and a variety of off-flavor carbonyl compounds [58]. As shown in Fig. 4, the TBARS values of AF, IF, and UIF samples were 0.167, 0.146, and 0.115 mg MDA/kg at 0 days, and no significant difference in the TBARS values was observed (P > 0.05). However, the TBARS values of all samples increased with prolonging storage time from 0 to 90 days. The results revealed that the fat oxidation became more serious. Furthermore, the TBARS values rapidly increased to 0.518, 0.400, and 0.331 mg MDA/kg at 90 days for the AF, IF, and UIF samples, respectively (P < 0.05). The lowest TBARS value was found in UIF samples within 90 days. From the changes of TBARS values, it was easily concluded that the fat oxidation rate of UIF samples was lower than that of AF and IF samples.

The increase of TBARS values of PGP samples could be explained from the following aspects. Firstly, the damage of ice crystals to cell structure of samples caused pro-oxidants in organelles released, consequently leading to the acceleration of fat oxidation and the increase in malondialdehyde [39,59]. In addition, the samples inevitably contacted with air during the process, and then the oxygen in the air promoted the oxidation. Meanwhile, previous study reported that non-heme iron could promote fat oxidation, so the non-heme iron in muscle acted as a catalyst for fat oxidation and further enhanced the oxidation reaction [60]. Besides, the water in the muscles was replaced by air after sublimation, hence enlarged the contact area between fat and oxygen, and then increased the degree of fat oxidation. Moreover, the fatty acids in the muscles were transferred from the inside to the surface with the pressure caused by ice crystals and easily reacted with oxygen in the air. Comparing with AF and IF samples, the fat oxidation degree of UIF samples was significantly lower (P < 0.05) due to the fast freezing rate of UIF. During the quick-freezing process, UIF induced the formation of ice crystals with small size and uniform distribution that reduced the damage of ice crystals to the cell structure and inhibited the release of pro-oxidants in the cells. Furthermore, UIF could inhibit protein oxidation, thus the degree of fat oxidation caused by protein oxidation would decrease. However, the final TBARS values obtained from PGP samples treated by three quick-freezing methods in present study were within the flavor threshold (>1.0 MDA mg/kg), a value above which an undesirable rancid smell and taste of meat products would produce [3].

Fig. 4. The changes in TBARS value of PGP samples treated by different quick-freezing methods.
3.7. Protein

3.7.1. Protein denaturation

The stability of protein structure was reflected by $T_m$ and $\Delta H$, in which $T_m$ represented the temperature of protein denaturation, and $\Delta H$ was corresponding to the quantity of necessary energy to induce protein denaturation [61]. During thermal analysis of muscle protein, three absorption peaks were found from 25 to 100 ºC, representing myosin denaturation ($T_{m1}$), myosin tail and myosin denaturation ($T_{m2}$), and actin denaturation ($T_{m3}$) [62]. The DSC results of PGP samples are illustrated in Fig. 5. The $T_m$ and $\Delta H$ of samples decreased with the extension of storage time from 0 to 90 days, indicating that the thermal stability of the protein gradually decreased. It was obvious that the $T_m$ of UIF samples was higher than that of AF and IF. After 60 days, there was a significant difference between UIF and AF samples ($P < 0.05$), but no significant difference of $T_m$ between UIF and IF was observed within 90 days ($P > 0.05$). In addition, within 30 days of frozen storage, there was no significant difference in $\Delta H$ among the three groups ($P > 0.05$). After 30 days, the $\Delta H$ of UIF samples was significantly higher than that of AF and IF samples ($P < 0.05$). It could be explained that the formation of ice crystals increased the ionic strength of cells and new covalent bonds formed due to protein aggregation, which gave rise to conformational changes and the increase of particle size, thus induced protein denaturation [2,63]. The $T_m$ and $\Delta H$ of AF samples decreased rapidly, manifesting that the protein structure was damaged seriously by the ice crystals. Moreover, the $T_m$ and $\Delta H$ of UIF samples were higher than those of AF and IF, suggesting that UIF could retain the protein stability. This may be related to the cavitation bubbles and micro-current effects produced by ultrasonic waves. Then, ice crystals of UIF samples were smaller and evenly more distributed, and thus reduced the damage of ice crystals to the structure of muscle fibrin and effectively maintained the thermal stability of proteins [9].

3.7.2. Protein oxidation

The changes of carbonyl and sulphydryl contents are shown in Fig. 6. The carbonyl content of AF, IF, and UIF samples was 1.02, 0.87, and 0.83 nmol/mg protein at 0 days, and significantly increased to 2.53, 2.04, and 1.52 nmol/mg protein at 90 days, respectively ($P < 0.05$). The change trend of sulphydryl content was opposite to that of carbonyl contents in the treated samples. The sulphydryl content of AF, IF, and UIF samples was 104.36, 104.96, and 107.14 nmol/mg protein at 0 days, and significantly decreased to 90.51, 96.44, and 99.53 nmol/mg protein at 90 days, respectively ($P < 0.05$). The results demonstrated that the formation of carbonyls in PGP samples was correlated with both freezing method and storage duration, which was consistent with previous research [2]. From the changes of carbonyl and sulphydryl contents, it can be concluded that the AF samples had the highest protein oxidation level, followed by IF and UIF groups. Such observation may attributed to that protein is attacked by reactive oxygen groups (ROS), which leads to the formation of carbonyl compounds and the decrease of sulphydryl contents.
content. Protein oxidation was affected by many factors. Firstly, samples inevitably contacted with air. Then, the ice crystals of PGP destroyed muscle fiber cells and caused the release of free radicals, heme, oxidase and other pro-oxidative factors [64,65]. Furthermore, fat oxidation can also promote protein oxidation. Lipid-derived peroxy radicals were potential initiators for the formation of carbonyl groups [65]. In addition, the malondialdehyde formed caused by fat oxidation, which was one of the substrates for the formation of carbonyl groups [2]. Quick-freezing conditions, storage temperature and time, all of them would affect the oxidation reaction of myofibril protein [2,66]. However, UIF reduced the damage of muscle fibre, because the ice crystals formed in PGP samples were small and evenly distributed. Moreover, UIF could inhibit fat oxidation, thus further restrained protein oxidation of PGP samples.

3.8. Freezable water content

As the experimental results listed in Table 1, the ΔH of AF, IF, and UIF samples increased by 20.70%, 19.79%, and 18.25%, respectively, from 0 days to 90 days. The above-mentioned changes of ΔH may be due to the increase of freezable water content that caused by water migration, which was consistent with our following results of LF-NMR. The freezable water content of the AF samples was more than that of IF and UIF groups. During the AF process, large ice crystals with uneven distribution and recrystallization of ice crystals caused the damaged muscle fiber tissue [5,31], and as a result of the increase of the freezable water content in the PGP samples. The ΔH changes of UIF samples were less than those of AF and IF samples, indicating that the least increment of freezable water was obtained in UIF group. The above results were mainly related to the inhibition of ice crystal recrystallization and water migration, which was caused by ultrasonic cavitation and mechanical effects [16].

Fig. 6. The changes in carbonyl (A) and sulfhydryl contents (B) of PGP samples treated by different quick-freezing methods.

Fig. 7. The changes in T2 relaxation time and A2 corresponding peak area of PGP samples treated by different quick-freezing methods (A: AF treatment; B: IF treatment; C: UIF treatment; D: T2b relaxation time; E: T21 relaxation time; F: T22 relaxation time; G: A2b corresponding peak area; H: A21 corresponding peak area; I: A22 corresponding peak area).
3.9. Low-field nuclear magnetic resonance (LF-NMR)

The quick-freezing methods not only affect the freezable water content, but also change the water distribution and state [67]. There are three different states of water in food, in which T$_{2b}$ (0–10 ms) corresponds to the bound water closely combined with macromolecules, T$_{21}$ (10–100 ms) represents the immobilized water trapped within the myofibrillar protein network, and T$_{22}$ (100–1000 ms) refers to free water existing in the space amongst the fibre bundles [68–71].

As shown in Fig. 7 (A, B and C), the T$_2$ relaxation time corresponding to the three peaks of PGP treated by three different quick-freezing methods shifted to the right in different degrees, which demonstrated that muscle tissue capillaries were damaged in different levels [72]. To be specific, the largest right shift of T$_2$ relaxation time was found in AF samples, while the least one was in UIF samples. Fig. 7 (D and G) presented the T$_{2b}$ and A$_{2b}$ changes within 90 days. During frozen storage for 30 days, no significant difference was observed among three quick-freezing methods (P > 0.05). The T$_{2b}$ relaxation time of UIF samples increased the slowest with increasing storage time from 30 to 90 days, and was significantly shorter compared to the samples treated by AF and IF under the same storage conditions (P < 0.05). Furthermore, there was no significant difference in A$_{2b}$ among the three groups within 90 days of frozen storage (P > 0.05). From Fig. 7 (E and H), the results showed that T$_{21}$ relaxation time of samples shifted to the right in different degrees. However, T$_{21}$ relaxation time of UIF samples was significantly shorter than that of AF and IF samples (P < 0.05). According to A$_{21}$ changes, the UIF samples had the smallest changes. As depicted in Fig. 7 (F and I), the T$_{22}$ relaxation time and A$_{22}$ increased throughout the storage period, revealing a continuous conversion of immobilized water into free water, which was closely related to the destruction of muscle fibers [5].

During freezing and frozen storage, the destruction of muscle fiber tissue was mainly correlated with the large size and uneven distribution of ice crystals. It was difficult for the damaged myofibrils to re-absorb melted water from the extracellular space, resulted in partial conversion of immobilized water to free water [67]. A similar observation has been reported by Sanchez-Alonso et al. [73], that is, T$_{21}$ of cod muscle significantly increased with the prolongation of frozen storage time (P < 0.05). In addition, the formation of ice crystals increased the concentration of solutes in cells, and the gradient difference was another important factor affecting water migration. However, the cavitation effect, micro jets, and mass transfer produced by UIF could shorten freezing time, and inhibit the re-crystallization of ice crystals and water migration. Then, smaller ice crystals with uniform distribution formed in UIF samples reduced the damage of ice crystals to muscle fibers. The results were consistent with the above mentioned decrease of WHC during storage.

3.10. Microstructure

The meat grain matrix is mainly composed of myofibril protein, sarcoplasmic protein and fat, which is filled between the meat grains. The gel matrix consists of myofibril protein and sarcoplasmic protein, without fat [1,28]. The changes in the meat grain matrix and the gel matrix in PGP samples can be investigated by microstructure, which is mainly correlated with the integrity of myofibrils, the existence of ice crystals, as well as the structure of myofibrils. Meanwhile, myofibril structure can be affected by ice crystals, thus the size and distribution of ice crystals have an impact on the microstructure. As shown in Fig. 8 (A), the microstructure of fresh samples (without quick-frozen) presented a delicate and tight tissue state. The microstructure of AF, IF, and UIF samples at 45 days are shown in Fig. 8 (B, C and D). The network structure of PGP samples in AF and IF groups became loose, and the meat grain matrix appeared to be broken. Moreover, some holes with different sizes appeared in both AF and IF treated PGP samples. In contrast, the microstructure of the UIF samples was slightly loose, but there was no fragmentation and holes. Fig. 8 (E, F and G) demonstrate the microstructure changes of PGP at 90 days, many obviously large holes appeared in the AF and IF groups, and the structure was looser and rougher compared to storage for 45 days. However, rough, uneven, and small holes also appeared in the network structure of UIF group, but compared to AF and IF samples, the results suggested that UIF can significantly reduce the damage of ice crystals to PGP microstructure (P < 0.05).

The microstructure changes of PGP during the frozen storage period were mainly caused by the existence of ice crystals and the changes of proteins structure. The protein network structure was destroyed by ice crystals, and caused the network structure of the meat matrix became loose. With the extension of frozen storage time from 45 days to 90 days, the size of ice crystals was becoming big, and resulted in seriously damaged the protein structure and muscle tissue. Therefore, the network structure became more rough and uneven. Besides, the denaturation and oxidation of proteins led to the contraction of myofibrils, and then the space of meat matrix was enlarged. As well, the fragmentation of myofibrils took place in the gel matrix. There are two reasons for the presence of holes in the microstructure. One is the muscle cells and proteins were damaged by ice cry crystals. On the other hand, a gap left caused by ice crystals. Comparing the microstructure of PGP treated by different quick-freezing methods for 45 and 90 days, PGP samples treated by UIF had better microstructure. The reason was connected with the small and even distribution ice crystals formed in UIF group, thus reduced the impact on protein and cell. Moreover, UIF induced the nucleation of ice crystallization.
crystals, increased the number of nuclei, and inhibited the growth of ice crystals [16,18]. The microjet generated by cavitation bubbles was the main driving force to promote ice nucleation. Therefore, UIF played a significant role in maintaining the microstructure of PGP samples.

4. Conclusion
The present study was to reveal the effects of quick-freezing methods on the quality, moisture distribution and microstructure of PGP samples during storage duration. The UIF treatment dramatically improved freezing rate, and maintained the color, WHC and pH of PGP samples, compared to AF and IF processes. The degree of protein denaturation, protein and fat oxidation of samples treated by UIF was significantly lower than that of IF and AF samples (P < 0.05). However, no significant difference was found on the texture properties (P > 0.05). Combining the results of DSC and LF-NMR, the UIF inhibited the growth of ice crystals, and then promoted the water distribution and state. According to microstructure changes of PGP samples stored for 45 and 90 days, the UIF could reduce the damage of ice crystals to the protein network structure. All the above-mentioned results suggested that UIF treatment could efficiently inhibit the deterioration of quality, moisture distribution and microstructure of PGP samples. In brief, UIF was an effective way to reduce the deterioration of PGP samples during frozen storage.

CRediT authorship contribution statement
Zeyu Wu: Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. Wanru Ma: Investigation, Methodology, Formal analysis, Writing - original draft. Zhaoxian Jian: Investigation, Methodology, Validation. Qingsong Liu: Investigation, Methodology. Ailing Hui: Validation, Resources. Wencheng Zhang: Data curation, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest
The authors 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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