Study on the quality and myofibrillar protein structure of chicken breasts during thawing of ultrasound-assisted slightly acidic electrolyzed water (SAEW)

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ABSTRACT

The effects of air thawing (AT), water thawing (WT), slightly acidic electrolyzed water (ET), ultrasound-assisted water thawing (WUT) and ultrasound-assisted slightly acidic electrolyzed water (EUT) on the quality and myofibrillar protein (MP) structure of chicken breasts were investigated. The results showed that WUT and EUT could significantly improve the thawing rate compared with AT, WT, and ET groups. The EUT group not only had lower thawing loss, but also their immobilized and free water contents were similar to fresh sample according to the low-field nuclear magnetic resonance (LF NMR) results. The EUT treatment had no adverse effect on the primary structure of the protein. The secondary and tertiary structures of MP were more stable in the EUT group according to Raman and fluorescence spectra. The muscle fibers microstructure from EUT group was neater and more compact compared with other thawing methods. Therefore, EUT treatment could be considered as a novel potential thawing method in the food industry.

1. Introduction

In recent years, the production and consumption of chicken have increased rapidly due to its advantages of low fat, low calorie, and high protein [1]. According to the National Bureau of Statistics of China, the production growth rate of poultry meat, mostly chicken, has exceeded 25% in the last five years [2]. Poultry meat production is expected to reach 28.61 million tons in 2020. Furthermore, per capita consumption of poultry meat in China has increased from 9.1 kg to 12.7 kg in the last five years. Because of their high nutritional value and water content [3], as well as the presence of microorganisms and enzymes [4], fresh chicken breasts are prone to spoilage at room temperature. Therefore, they need to be frozen to increase their shelf-life for further processing, which is the most widely used method for all types of meat [5].

The thawing process is considered a crucial stage for frozen foods before further processing. The main goal of thawing is to return frozen meat to its fresh, unthawed state and quality [6,7]. Improper thawing can result in undesirable changes of meat quality, such as deterioration in flavor, texture, color, protein degradation and aggregation. The time, temperature and method of the thawing process are the main factors affecting the meat quality change of thawed meat [8]. Currently, there are several studies on the effect of traditional thawing methods on meat quality. These traditional methods are mainly based on water thawing [9] and air thawing [10]. However, these methods may have a negative impact on meat quality to some extents [11]. Therefore, as a novel and efficient thawing method, ultrasonic thawing has been widely used in meat thawing to speed up the thawing process and reduce the damage to meat quality.

Recently, ultrasonic thawing technology has attracted more and more attention due to its advantages of safety and environmental protection, uniform thawing, high efficiency and low cost [12]. Ultrasound can effectively enhance various mass transfer processes [13]. Different from the traditional thawing method from the outside to the inside, both the frozen and unfrozen tissues of food absorb the energy generated by the attenuation of ultrasonic waves and frozen tissue can absorb more energy than thawed tissue, thus significantly improving the thawing
efficiency [14]. At present, ultrasonic thawing has achieved excellent results in the application for meat [3], fruits [14,15] and vegetables [16]. Meanwhile, these studies have demonstrated that ultrasonic thawing can be applied in the food industry. In addition, water is commonly used as the thawing media in ultrasonic thawing. However, Liao et al. [17] found that slightly acidic electrolyzed water (SAEW), as a substitute for traditional thawing media water, can also be used as a new thawing media.

SAEW is made by electrolyzing sodium chloride solution using a single chamber compartment without a membrane [18]. In recent years, SAEW has gained more attention due to its characteristics of antibacterial activity and environmental friendliness. As a new thawing media, its safety is unquestionable, because it can get back to its initial form when organic matter is existed [19]. In addition, as a food additive, SAEW has been applied in the U.S., Japan and Korea [17]. Xuan et al. found that SAEW ice could significantly inhibit myofibrillar protein degradation [20]. Liao et al. found that SAEW which was used as an active thawing media can better retard lipids and protein oxidation [17]. Cichoski et al. observed that the combined treatment of ultrasound and SAEW not only improved the microbial quality of chicken breasts, but also effectively delayed myofibrillar protein (MP) oxidation [21].

Although there are some studies available on the application of ultrasound-assisted thawing in meat products, the effects of thawing media on the quality and MP structure of chicken breasts were not previously investigated. Therefore, this research aimed to evaluate the effects of thawing methods on quality and MP structure of chicken breasts. The thawing rate, meat quality, microstructure, MP primary structure, secondary and tertiary structure of chicken breasts under five thawing methods were evaluated.

2. Materials and methods

2.1. Preparation of samples

2.1.1. Materials

Fresh chicken breasts were purchased in a local supermarket (Qingdao, China). The chicken breasts were trimmed into samples of uniform size, shape and weight (3 × 3 × 3 cm³, 64 ± 5 g) after removing fat, fascia and connective tissue, and then divided into 6 groups. One group, served as the control, and the remaining five groups were individually packed in polyethylene bag to prevent water loss during storage, pre-cooled at 4 °C for about 3 h and frozen at −20 °C for 4 weeks.

2.1.2. SAEW preparation

SAEW was made by electrolyzing a mixture of NaCl and HCl solution in a device (Anywhere-320 W, Beijing, China) with a voltage of 220 V and a current of 8.0 A for 20 min at room temperature. The pH and oxidation reduction potential (ORP) of SAEW were determined by a pH meter (FE20, Mettler-Toledo, Mettler Toledo Instruments Co., ltd., China). The available chlorine concentration (ACC) was measured by a digital chlorine test kit (RC-3F, Saitama, Japan). SAEW was used as the thawing media with a pH of 6.25, ORP of 875 mV, and ACC of 35 mg/L.

2.1.3. MP extraction

MP was extracted from chicken breasts following the methods of Lefèvre et al. [22] and Xiao et al. [23] with some modifications. The samples were added to 10 times the volume of 20 mM buffer A (containing 100 mM NaCl, 1 mM EDTA, pH 7.0) and then homogenized at 15,000 rpm for 1 min at 4 °C. Subsequently, the filtered liquid was centrifuged at 6,000 rpm for 15 min at 4 °C (5810R, Eppendorf, Hamburg, Germany) and the supernatant was discarded. After that, the same operation is repeated twice. The precipitate was homogenized and centrifuged at 4 °C after the addition of 4 times the volume of 0.1 M NaCl solution. The above operation was repeated twice. The resulting precipitates were purified MP. Purified MP precipitates were diluted with 25 mM buffer B (containing 0.6 M NaCl, pH 7.0). The concentration of MP was determined using the Biuret method [24].

2.2. Thawing process and curve

The frozen chicken breasts samples were thawed using five different treatments: air thawing (AT, 4 °C); water thawing (WT, 4 °C distilled water); slightly acidic electrolyzed water thawing (ET, 4 °C SAEW); ultrasound-assisted water thawing (WUT) and ultrasound-assisted SAEW thawing (EUT), thawed at applied in a ultrasonic bath (KQ-400DB, Kunshan ultrasonic instrument Co., ltd, China) with a power of 200 W and frequency of 40 kHz using distilled water and SAEW in thawing process, respectively. After removing the polyethylene bags, each sample was placed into a beaker (500 mL) for thawing at 4 °C by adding ice.

During the thawing process, a temperature recorder (NAPU thermocouple, Mod. TR 230X-8, Guangdong, China) was used to continuously record the thawing time and sample temperature until the geometric center temperature of the sample reached 0 °C.

2.3. pH and color

A 10 g sample of chicken breasts was added to 100 mL deionized water and then homogenized (MiniMix 100, Interscience, France) for 5 min. The precipitated meat tissue was filtered off with filter paper. The filtrate was measured with a pH meter (FE20, Mettler-Toledo, Mettler Toledo Instruments Co., ltd., China) at room temperature. The pH electrodes were calibrated in pH 4.01 and pH 7.00 standard buffers at room temperature before measuring pH.

The color of chicken breasts was determined by a colorimeter (CR-400, Konica Minolta Inc., Osaka, Japan) with an 8 mm aperture, 2° observer and illuminant C. The color changes were described using the color space of L*, a*, and b*. The colorimeter was calibrated using a white calibration plate before measurement.

2.4. Water holding capacity (WHC)

2.4.1. Thawing loss

The thawing loss was determined following the method of Wang et al. [25]. Before thawing, the sample weight was quickly measured and expressed as M₀. The sample was thawed until its geometric center temperature reached 0 °C, weighed again and expressed as M₁.

Thawing loss = (M₀ − M₁)/M₀ × 100%

2.4.2. Cooking loss

The cooking loss was determined following the method of Liao et al. [17] with minor modifications. The sample (M₂) was placed in a centrifuge tube in an 80 °C water bath for 30 min. Then, the samples were removed from the centrifuge tubes and cooled to room temperature. After that, the sample weight was recorded as M₃.

Cooking loss = (M₂ − M₃)/M₂ × 100%

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

The water distribution was performed by LF NMR analyzer (NIMI20-040 V-1, Suzhou, China). The LF-NMR analyzer was firstly calibrated by Q-Free Induction Decay (Q-FID) procedure with a standard oil sample. The chicken breasts (1 × 1 × 2 cm³) were placed in 40 mm tubes. The relaxation time (T₂) was measured by the Carr-Purcell-Meiboom-Gill (CPMG) procedure. The image was obtained through inversion, and further analysis was performed to obtain relaxation time and peak area ratios using MultiExp Inv Analysis software (Suzhou Niumai Analytical Instruments Co., ltd.).
samples were cut into cubes (2 × 2 × 1 cm³). The P 50/R probe was used to determine the texture of the sample. The TPA parameters of the sample were automatically calculated by the supporting software.

The shear force was determined on a tenderness analyzer (C-LM3B, Tenovo, Beijing, China). The samples were cut into cuboid (2 × 2 × 5 cm³) along the direction parallel to the muscle fibers.

2.7. MP primary structure

2.7.1. Total sulfhydryl content

The total sulfhydryl content was measured following the method of Benjakul et al. [26] with slight modifications. One mL of MP solution (1 mg/mL) was added to 9 mL of 0.2 M Tris-HCl (containing 1 mM EDTA, 0.6 M KCl, 8 M urea, 2 % SDS, pH 6.8) and mixed well. The above mixture of 4 mL was added with 0.4 mL of 0.1 % 5,5'-dithiobis (2-nitrobenzoic acid). Then, the mixture was kept for 25 min at 40 °C. The absorbance was determined by spectrophotometer (TU-1810, Beijing, China) at 412 nm, and the blank was replaced by 0.6 M KCl solution. Each group was measured in triplicate. The total sulfhydryl content was calculated as follows:

\[ \text{Total sulfhydryl content (nmol/mg)} = A_{412} \times 10^3 / (1.36 \times 10^3) \]

2.7.2. Carbonyl content

The carbonyl content was measured by the method of Sun et al. [27]. Two mL of MP solution (2 mg/mL) was added to 2 mL of 10 mM DNPH at 37 °C for 1 h. After that, the above mixture was centrifuged to obtain precipitate. The precipitate was washed by ethyl acetate-ethanol solution and then added to 5 mL of 6 M guanidine hydrochloride solution and kept at 37 °C until the precipitate was dissolved. The above mixture was centrifuged to get the supernatant. Finally, the absorbance of the supernatant was determined by a spectrophotometer (TU-1810, Beijing, China) at 370 nm. The carbonyl content was calculated as follows:

\[ \text{Carbonyl content (nmol/mgs)} = A_{370} \times 10^6 / (2.2 \times 10^3) \]

2.8. Secondary structure

The secondary structure of the sample was measured using Raman spectrum (DXR 2Xi, Thermo Fisher Scientific, USA), which was equipped with a 50 × objective and a 532 nm laser to capture a full spectral range of 400–3400 cm⁻¹. The sample was placed on a single concave slide for spectra scanning. Data acquisition of Raman spectra was performed using a 50 μm slit, 10.0 mW laser power, 0.25 s exposure time, and 120 scans. Secondary structure was calculated by amide I spectra of Raman spectrum. The Raman spectrum of the sample was analyzed using Peakfit 4.12 software (San Rafael, CA, USA) to obtain protein secondary structure content. Secondary structure in Raman spectroscopy were shown as: 1615–1637 cm⁻¹ (β-sheet), 1637–1645 cm⁻¹ (random coil), 1646–1664 cm⁻¹ (α-helix), 1664–1680 cm⁻¹ (β-turn), and 1680–1700 cm⁻¹ (β-sheet).

2.9. Tertiary structure

Intrinsic fluorescence spectra of MP solution (0.2 mg/mL) were measured by a spectrophotometer (F-2700, Hitachi, Japan). The determination conditions of fluorescence spectra were as follows: excitation wavelength of 280 nm and emission spectrum of 300 to 400 nm.

2.10. Scanning electron microscopy (SEM)

Chicken breast samples were cut into slices (2 × 2 × 1.5 mm³) and placed in 2.5 % glutaraldehyde solution at 4 °C for overnight fixation. After fixation, the samples were washed three times using phosphate buffer. The samples were eluted using ethanol solutions (50, 70, 80, 90, and 100 %). Subsequently, the samples were put in the freeze-drying machine and coated with gold. Finally, the samples were observed with SEM (S-3400 N, Hitachi, Tokyo, Japan) at 2000 × Magnification.

2.11. Statistical analysis

Data were analyzed by Duncan’s multiple range test using IBM SPSS 23 (IBM Corporation, Armonk NY, USA). \( P \leq 0.05 \) indicated a significant difference in the data. All the experiments were carried out in triplicate. The data were expressed as mean ± standard deviation. All figures were generated using Origin 2018 (Origin Lab Corp., MA, USA).

3. Results and discussion

3.1. Thawing discussion

The thawing process and thawing time of the chicken breasts are shown in Fig. 1A and Fig. 1B, respectively. The AT group had the longest thawing time among the five thawing methods, which reached 859.38
rate, the endothermic phase (≤ 0°C) can generally be grouped into two phases depending on the thawing before and after thawing to the thawing time [28]. The thawing process thawing rate was defined as the ratio of the temperature difference to reduce the thawing time and improve the thawing efficiency. The results showed that WUT and EUT treatments can and EUT groups were reduced by 80.62%, 85.56%, 97.30% and 97.23 %, respectively. The results showed that WUT and EUT treatments can and EUT groups were reduced by 80.62%, 85.56%, 97.30% and 97.23%.

Table 1
Changes in the pH and color under different thawing methods. Control, fresh chicken breasts; AT, air thawing; WT, water thawing; ET, slightly acidic electrolyzed water immersion thawing; WUT, ultrasound-assisted water thawing; EUT, ultrasound-assisted slightly acidic electrolyzed water thawing.

| Thawing methods | pH     | a*     | b*     | ΔE    |
|-----------------|--------|--------|--------|-------|
| Control         | 6.02 ± | 2.24 ± | 10.15 ±| –     |
| AT              | 0.05a  | 2.15 ± | 0.74a  | 0.47a |
| WT              | 0.13a  | 1.68c  | 0.67c  | 1.42a |
| ET              | 0.05a  | 3.59ab | 0.31a  | 1.52c |
| WUT             | 0.09a  | 58.80 ±| 1.94 ± | 40.16 ±|
| EUT             | 0.07a  | 1.40b  | 0.19a  | 0.87a |

The results are mean ± SD. Different letters for the same index indicate significant differences (P ≤ 0.05).

![Fig. 2](image)

The photographs of samples under different thawing methods. Control, fresh chicken breasts; AT, air thawing; WT, water thawing; ET, slightly acidic electrolyzed water immersion thawing; WUT, ultrasound-assisted water thawing; EUT, ultrasound-assisted slightly acidic electrolyzed water thawing.

![Fig. 3](image)

Fig. 3. The thawing loss and cooking loss under different thawing methods. Control, fresh chicken breasts; AT, air thawing; WT, water thawing; ET, slightly acidic electrolyzed water immersion thawing; WUT, ultrasound-assisted water thawing; EUT, ultrasound-assisted slightly acidic electrolyzed water thawing. Different letters for the same index indicate significant differences (P ≤ 0.05).

Table 2
Changes in the texture (hardness, springiness, chewiness, and resilience) and shear force under different thawing methods. Control, fresh chicken breasts; AT, air thawing; WT, water thawing; ET, slightly acidic electrolyzed water immersion thawing; WUT, ultrasound-assisted water thawing; EUT, ultrasound-assisted slightly acidic electrolyzed water thawing.

| Thawing methods | Hardness/g | Springiness | Chewiness | Resilience | Shear Force/ N |
|-----------------|------------|-------------|-----------|------------|--------------|
| Control         | 8799.46 ±  | 0.95 ±      | 2926.91 ±  | 0.58 ±     | 15.08 ±      |
| AT              | 827.39b    | 0.01a       | 565.32d    | 0.02a      | ± 0.67a      |
| WT              | 11831.96 ± | 0.75 ±      | 4199.45 ±  | 0.36 ±     | 36.88 ±      |
| ET              | ± 1083.48a | 0.02d      | 914.85f    | 0.02f      | ± 1.92a      |
| WUT             | 10926.36 ± | 0.69 ±      | 6356.17 ±  | 0.47 ±     | 21.00 ±      |
| EUT             | ± 974.04a  | 0.03a       | 126.93b    | 0.06b      | ± 0.78b      |
| WUT             | 11054.84 ± | 0.72 ±      | 3972.55 ±  | 0.51 ±     | 19.25 ±      |
| EUT             | ± 1663.43a | 0.02c       | 563.81f    | 0.01f      | ± 1.69f      |

The results are mean ± SD. Different letters for the same index indicate significant differences (P ≤ 0.05).

thermal conductivity of ice is larger than water [29]. On the other hand, the temperature difference between the samples and the thawing media is larger, thus the heat transfer rate is faster [30]. However, the thawing curve tends to flatten out and the thawing rate is low during the phase transition stage. Because the phase change process needs to absorb more heat [31]. In addition, the thermal conductivity of the sample decreases as the ice crystals are transformed into water. Application of ultrasonic thawing to improve thawing efficiency can be explained as follows: the ultrasound energy is mainly attenuated by the frozen tissue and transferred from mechanical energy to thermal energy, thus speed up the thawing process [4]. Ultrasonic waves are more attenuated in the freezing zone than in the thawing zone. The heat generated by the attenuation of ultrasonic waves is mainly applied to the boundary of the thawing/freezing zone, thereby significantly improving thawing efficiency [14]. In addition, Kiani et al. [32] found that the micro-jet and cavitation effect generated by ultrasonic waves during the media propagation can also increase the heat transfer rate, thus accelerating the thawing process. In conclusion, the EUT and WUT thawing rates
were relatively faster compared to the WT and ET groups, suggesting that it is mainly ultrasound that plays an important role in the thawing process. In combination with ultrasound, both water and slightly acidic potential water have less effect on the thawing rate.

3.2. pH and color

The pH value is an important indicator which can affect the meat color, cooking loss, and shear force. Due to glycolysis, the acid substances produced in the muscle may cause a decrease in pH during the thawing process [33]. In contrast, nitrogenous substances are broken down into alkaline substances, causing an increase in the pH [34]. However, the pH of all groups was not significantly different (P > 0.05), which indicated that the treatments had no negative impact on the pH of chicken breasts.

Color has an important influence on the appearance and acceptability of frozen products. During freezing and thawing, protein denaturation, pigment degradation, and lipid oxidation all can lead to color changes in meat. Yi et al. [35] found that lipid oxidation is the main reason for the increase in the $b^*$ value of meat. The color changes under different thawing methods are shown in Table 1. The $L^*$ of chicken breasts was reduced under different thawing methods, and the $L^*$ from AT and WUT groups had significant differences (P ≤ 0.05). Therefore, AT and WUT treatments may cause damage to the quality of chicken breasts. The change of $a^*$ may be related to denaturation of myoglobin and loss of pigment. The main reason for the change in $b^*$ is the formation of yellow pigment due to lipid oxidation [35]. Guo et al. [36]...
also found that the increase in $b^*$ value may be related to lipid oxidation. The total color difference ($\Delta E$) between the samples is mainly caused by the $L^*$, $a^*$ and $b^*$ values during thawing process. The $\Delta E$ was the largest in the AT group, which was significantly different from the sample of other thawing groups ($P \leq 0.05$). In addition, the color difference of the samples can be visually represented by photographs (Fig. 2). We found that the samples surface of WT and ET groups was paler than WUT and EUT groups. Because the thawing rate of WT and ET groups was slow. The samples were soaked in the thawing medium for a long time. It can be concluded that the thawing methods did not change the color of the samples, except for the AT group. The $L^*$, $a^*$, and $b^*$ between the EUT group and control group did not differ significantly ($P > 0.05$), indicating that EUT treatment did not cause the change in the color of chicken breasts.

3.3. Water holding capacity (WHC)

The WHC changes under different thawing methods are shown in Fig. 3. Ice crystals can damage the muscle tissue, thus leading to a decreased WHC of the muscle. The thawing loss from AT group was 4.29 %, which was the highest among all groups. Because the muscle fiber structure of the AT group samples was severely damaged during the thawing process. However, WUT and EUT groups had the lowest thawing loss among all treatments. Because ultrasound treatment improves the structural characteristics of myosin and increases the water holding capacity of muscle proteins [37]. In addition, EUT and WUT can accelerate the thawing rate and reduce the degradation of muscle fibers, thus improving the water holding capacity. The thawing loss from the EUT and WUT groups was not significantly different ($P > 0.05$), indicating that the thawing media did not affect the thawing loss of chicken breasts. In addition, we believe that there is an important association between thawing loss and thawing time of the sample. This can be explained by the fact that the longer the thawing time, the more severely the muscle structure would be damaged due to protein oxidation and degradation, resulting in hindered reabsorption of water [30].

Cooking loss includes the loss of a large portion of water and some nutrients after heating. Thawed food may have better quality due to lower cooking loss. AT group had the lowest cooking loss of 39.63 % among all thawing methods. However, the cooking loss was not significantly different among the WT, ET, WUT and EUT groups ($P > 0.05$).
This may be explained by the AT group had the largest thawing loss. Because the disruption of muscle protein structure can lead to an increase in thawing loss due to the loss of free water, which led to a reduction in cooking loss [38].

3.4. Texture profile analysis (TPA) and shear force

Textural characteristics are one of the main sensory indicators of meat products. As seen in Table 2, the thawing methods had an important effect on the variation of the texture of chicken breasts. The hardness between EUT group and control group was not significantly different (P > 0.05). In combination with previous studies, the residual sodium chloride and other components of SAEW are beneficial in maintaining the hardness of thawed meat products [39]. After thawing, compared with control group, the springiness from all thawed groups was significantly lower (P ≤ 0.05), but the springiness between EUT group and control group was the closest. The result may be explained as follows: on the one hand, the ice crystals can damage the muscle tissue structure of the chicken breasts. On the other hand, water reabsorption of the sample was affected during thawing to some extents [30]. In terms of chewiness, all thawed groups had significantly higher (P ≤ 0.05). The deterioration of meat texture was associated with internal factors of water loss and protein degradation [34] and external factors of thawing time and thawing temperature.

Shear force is negatively correlated with meat tenderness. According to Table 2, the shear force from AT, WT, ET, and WUT groups was significantly increased than control group (P ≤ 0.05). We believe that thawing loss is the main reason for the increased cutting force. However, the shear force did not differ significantly between the EUT and control groups. (P > 0.05), which indicated that EUT can maintain the tenderness of chicken breasts.

The results in Table 2 indicated that the texture and tenderness of the EUT group were closer to the control group. The reasons can be explained as follows: ultrasonic power may improve the tenderness of meat [40]. In addition, the residual components in the SAEW were effective in maintaining the tenderness of meat.

3.5. LF-NMR

The relaxation time (T_2) under different thawing methods is shown in Fig. 4A. T_{21} stands for bound water in samples. The bound water was not easily affected by heating or freeze-thaw process. T_{22} stands for immobilized water intracellularly, which is located within the MP structure. T_{23} stands for free water that is easily lost outside the cell [41]. According to Fig. 4B, T_{21} and T_{22} relaxation times were not significantly different for all groups (P > 0.05). Because bound water is tightly bonded to muscle proteins, which are not affected by mechanical stress and microstructural changes during freeze-thawing [38]. The T_{23} relaxation time of AT group was significantly lower (P ≤ 0.05). This reason may be related to thawing time and thawing loss of AT group. However, Li et al. [42] found that the higher T_2 relaxation time indicated higher mobility of water and a lower water holding capacity.

Fig. 7. The MP intrinsic fluorescence under different thawing methods. Control, fresh chicken breasts; AT, air thawing; WT, water thawing; ET, slightly acidic electrolyzed water immersion thawing; WUT, ultrasound-assisted water thawing; EUT, ultrasound-assisted slightly acidic electrolyzed water thawing. Different letters for the same index indicate significant differences (P ≤ 0.05).

Fig. 8. The muscle fibers microstructure under different thawing methods (magnification: 2000 ×). Control, fresh chicken breasts; AT, air thawing; WT, water thawing; ET, slightly acidic electrolyzed water immersion thawing; WUT, ultrasound-assisted water thawing; EUT, ultrasound-assisted slightly acidic electrolyzed water thawing.
P_{21}, P_{22}, and P_{23} represent the proportions of peak areas of T_{21}, T_{22}, and T_{23}, respectively. According to Fig. 4C, the bound water content did not differ significantly between all thawed groups and control group (P > 0.05). The P_{22} of WT group was significantly lower (P ≤ 0.05) and the P_{23} was significantly higher (P ≤ 0.05), indicating that more immobilized water was transferred to the outside of the cells and the thawing loss was severe in the WT group. P_{23} between the EUT group and control group did not differ significantly (P > 0.05). Therefore, the results indicated that ultrasound-assisted thawing could maintain more immobilized water and had better water holding capacity.

3.6. Protein primary structure

The change in the total sulphhydril content under different thawing methods is shown in Fig. 5. The sulphhydril groups are easily converted to disulfide bonds during muscle storage and processing [43]. Although the total sulphhydril content of the thawed groups was lower, the difference was not significant between control group and thawed groups (P > 0.05). This suggests that all thawing methods can have a certain effect on the structure of the proteins. The sulphhydril groups hidden in the internal regions of the protein are exposed to the surface and oxidized to disulfide bonds [44].

The change in carbonyl content under different thawing methods is shown in Fig. 5. Carbonylation is one of the most significant chemical modifications of protein oxidation. The carbonyl content from AT, WT, ET, and WUT groups was significantly higher (P ≤ 0.05). However, the difference between control group and EUT group was not significant (P > 0.05). The result indicated that EUT treatments could better delay the protein oxidation of chicken breasts during the thawing process. The SAEW components (HCl, Cl_{2}, and HClO) can maintain the stability of the protein. Combined with the changes in carbonyl groups and total sulphhydril groups, EUT treatment could better delay the oxidation and degradation of chicken breasts proteins.

3.7. Secondary structure

Fig. 6A and Fig. 6B show Raman spectra at 400–3400 cm\(^{-1}\) and second structure content of protein, respectively. Fig. 6C shows the curve obtained by baseline correction, deconvolution, second-order derivation and iterative fitting of the Raman spectra in the range of 1600–1700 cm\(^{-1}\). The \(\alpha\)-helix content from AT, WT, ET, and WUT groups was significantly decreased (P ≤ 0.05). This is because the protein structure is disrupted during thawing, resulting in the exposure of hydrophobic groups. Jia et al. [45] found that the stability of the \(\alpha\)-helix structure in proteins depends mainly on the hydrogen bonds. Therefore, this phenomenon may also be due to the disruption of hydrogen bonds within the protein [42]. The \(\beta\)-sheet content from EUT group was significantly lower. This is due to protein aggregation caused by hydrophobic interactions [38]. In addition, the content of the \(\beta\)-turn and random coil between EUT group and control group did not differ significantly (P > 0.05), indicating that EUT treatment did not disrupt the secondary structure of the proteins. In brief, the EUT group had more stable protein structures compared with other thawing methods.

3.8. Tertiary structure

The intrinsic fluorescence spectra of MP under different thawing methods is shown in Fig. 7. Intrinsic fluorescence is mainly generated from tryptophan residues (Trp) and tyrosine residues (Tyr) in proteins. The fluorescence intensities from AT, WT, ET, WUT, and EUT groups were higher (P ≤ 0.05), but the fluorescence intensities from ET and EUT groups were closer to the control group. The main reason is that the MP structure was disrupted during the thawing process, resulting in the exposure of Trp and Tyr residues. The maximum fluorescence emission peak (\(\lambda_{\text{max}}\)) indicates the extent of conformational changes. The \(\lambda_{\text{max}}\) from AT, WT, ET, WUT, and EUT groups was blue-shifted, indicating that the microenvironment of Trp and Tyr residues was altered in the thawed groups. However, the \(\lambda_{\text{max}}\) from the ET and EUT groups was closest to the control group, probably because the hypochlorite ion in SAEW is an electron-absorbing group [17], which maintained the micro-environment of amino acid residues in the groups to some extent. The results indicate that EUT and ET treatments had less influence on protein tertiary structure.

3.9. Scanning electron microscopy (SEM)

The microstructure of the longitudinal muscle tissues under different thawing methods is shown in Fig. 8. The muscle fibers from the control group had a smooth surface and a tight structure. In the AT group, the muscle fibers were cross-linked and bent, and the overall structure of the muscle surface was disorganized and rough. Compared with the other thawing methods, the muscle structure from AT group was most severely damaged. The reason may be related to the longer time required for the thawing process in the AT group. This might be due to the longer time of AT group during thawing. The muscle fiber’s surface from WT and ET groups was rough and the ordered structure was disrupted. In addition, the muscle fibers from the WT and ET groups showed significant fractures and increased gaps between fibers. This may be related to the formation of large ice crystals, which led to the destruction of muscle tissue [46]. Jiang et al. [47] found that the gaps between muscle fibers were related to WHC. This is consistent with the result of thawing loss in our study. Among all thawed groups, the muscle fibers structure from WUT and EUT groups showed the least change compared with the control group. However, we found that the muscle fibers from the EUT group had a neater structure, more dense arrangement and smoother surface compared with the WUT group.

4. Conclusions

In this study, the effects of different thawing methods on the quality and protein structure of chicken breasts were evaluated. We found that WUT and EUT not only increased the thawing rate but also effectively reduced the thawing loss of chicken breasts compared with other thawing methods. According to LFNMR, the immobilized water and free water contents from WUT and EUT groups were closest to the control group. The EUT treatment could maintain the color and pH of the samples and effectively inhibit lipid oxidation. As for the TPA, shear force, and SEM, the EUT group showed the least change in quality compared with other thawing methods. In addition, ET and EUT treatments did not negatively affect the primary structure of the proteins. According to Raman and fluorescence spectra, the secondary and tertiary structures of MP were more stable in the EUT group. Compared with other thawing treatments, EUT treatment not only could better maintain the quality of chicken breasts but also effectively reduced the protein structure changes during the thawing process. In conclusion, EUT has a positive impact on the quality and protein structure of thawed chicken breast. As a promising thawing method, EUT can provide a theoretical basis for the subsequent processing and utilization of chicken breast in food industry.

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.

Data availability

Data will be made available on request.
Acknowledgments

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