Improvement of gelation properties of myofibrillar proteins from porcine *longissimus dorsi* muscle through microwave combined with air convection thawing treatment

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Abstract: The effects of the microwave combined with air convection thawing (MAT) on the gelling properties of pork myofibrillar proteins (MPs) were further studied and compared with those of fresh meat (FM), and single thawing methods (microwave thawing (MT) and air convection thawing (AT)). Results revealed that the thawing methods, excluding MAT, induced deterioration in the gelling properties of MPs. There was no significant difference (p>0.05) in the water holding capacity (WHC), cooking loss, whiteness, and strength of gel samples subjected to MAT and those of FM samples, demonstrating that the gelling properties were retained after MAT. As well, protein aggregation was limited, since MAT reduced the change in zeta potential and turbidity compared to that observed with MT or AT. The dynamic rheology and scanning electron microscopic results were relatively consistent, revealing that among the different thawing techniques, MAT had the least negative impact on the microstructure of MPs gel, leading to the generation of a more elastic and uniform gel structure than that of the MT or AT gels. Moreover, MAT resulted in higher water retention in the gels than that achieved with MT or AT. These findings indicated that MAT improved the gelling properties of MPs, thereby confirming the suitability of this treatment for use in the meat processing industry.

Keywords: microwave combined with air convection thawing, gelling property, myofibrillar protein, moisture distribution, microstructure

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1 Introduction

In the meat industry, frozen storage plays a vital role in maintaining meat freshness and extending the shelf life of products[1]. Thawing, therefore, is a necessary step before any subsequent processing and cooking. However, during the process of thawing, some undesirable deterioration of muscle quality, such as changes in the texture, color, water retention, and flavor, can occur, partly due to changes in the myofibrillar proteins (MPs)[2]. Therefore, optimization of the thawing methods is necessary for commercial meat manufacturing. Some emerging techniques, such as microwave thawing (MT), are being increasingly employed to shorten the thawing time and improve the meat quality compared to traditional thawing treatments. Nevertheless, the practical application of MT in the meat industry has been limited, due to localized overheating and poor penetration. Accordingly, to address the shortcomings of MT, microwave combined with other thawing methods have attracted considerable attention in recent years. Several studies have shown the positive effect of the combination of microwave treatment with other thawing methods on meat quality. Cao et al.[3] stated that microwave plus magnetic nanoparticles thawing could maintain the quality of red seabream and prevent moisture migration. Cai et al.[4] also reported that when the meat was thawed by microwave combined with ultrasonic thawing, moisture migration, and protein denaturation were inhibited. Furthermore, in our previous study[5], changes in the porcine physicochemical properties caused by microwave-based methods were evaluated, and microwave-combined thawing methods were found to be effective in reducing nonuniform temperature distribution. In particular, microwave combined with air convection thawing (MAT) reduced the thawing time by half compared to AT whilst maintaining meat quality.

Myofibrillar proteins (MPs) are the major components of meat proteins (55%-60%) and enable the meat proteins to form a protein gel[1]. The heat-induced gelation of MPs, which determines the properties of meat products, is a convoluted physico-chemical process[6]. A good protein gel has a compact network structure, which ensures textural stabilization and water binding[7]. The gelling properties of MPs are known to be affected by numerous factors, including pH, ionic strength, temperature, additives, protein concentration, processing parameters, and the storage procedure[8]. To date, many studies have focused on the changes in quality or protein structure caused by single thawing methods[1]; recently, several reports have emphasized the protein gelling properties affected by thawing methods. Li et al.[6] pointed out that the quality of *mirror carp* MPs gels declined with increasing freeze-thaw

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cycles. Wang et al.[7] also reported that different thawing methods resulted in significant differences in the MPs gelling properties of porcine *longissimus dorsi* muscle (p<0.05). Our previous study[9] demonstrated that MAT resulted in less damage to the MPs and maintained a superior MPs quality, with less protein oxidation, less water loss, and a more stable structure, than that achieved with AT or MT. Nevertheless, relatively limited information is available regarding the effects of microwave-combined thawing methods, in particular the effect of MAT treatment, on the gelling properties of MPs.

Thus, this study aimed to further clarify the effect of MAT on the gelling quality of porcine MPs, thereby providing a theoretical basis for employing MAT in the meat industry.

2 Materials and methods

2.1 Materials

The *longissimus dorsi* muscles (average weight: 2.8-3.0 kg) were obtained from six-month-old Duroc×Landrace×Yorkshire crossbred pigs (three gilts and three barrows) from Gaojin Food Co., Ltd. (Xinxiang, Henan, China). Chops (length×width×height: 60 mm×50 mm×35 mm) with a weight of (150.0±0.5) g were collected and packaged in polyethylene bags (120 mm×170 mm) with 16 holes (diameter 6 mm) corresponding to the two sides. The groupings and experimental tests were conducted according to the procedures described by Zhu et al.[5]. More specifically, the samples were divided into four groups, one of which was the fresh meat sample (FM). The other samples were stored at −20°C for approximately 5 d and, then thawed using three different methods, i.e., AT, MT, or MAT. AT was performed in an atmosphere of 20°C, and MT was performed in a 100 W in microwave oven (Media Microwave Electronics Co., Ltd., China). The two-stage thawing process of MAT was initially carried out a 100 W in a microwave oven until the central temperature reached −4°C and then operated the AT process. Tempering was complete when the central temperature reached ~2°C, which was monitored in real-time using a thermometer (Testo 160, Testo Instruments International Trading Co., Ltd., Shanghai, China). According to the results of our previous study[5], MT (100 W) involves the shortest thawing time (4 min), followed by MAT (110 min) and AT (20°C, 220 min).

2.2 Preparation of the thermal MPs gel

MPs gel was prepared using the method described by Guo et al.[10] with minor modifications. The chopped muscle (150 g) was mixed with four volumes of 10 mmol/L PBS buffer (pH 7.0) and homogenized. The homogenate was centrifuged at 10 000 × g and 4°C for 15 min to obtain the sediment. Subsequently, the sediment was washed with four volumes of 0.1 mol/L NaCl three times and centrifuged (10 000 × g, 15 min, 4°C) to acquire MPs. The final MPs were obtained and preserved at 4°C in less than 48 h. The MPs were dispersed in phosphate buffer containing 0.6 mol/L NaCl (50 mmol/L, pH 6.0). The concentration was determined using the Biuret method with bovine serum albumin as the standard and the final concentration was 50 mg/mL. Thermal gels were prepared from the four experimental groups by heating each sample (10 g) in a tube in a 75°C water bath for approximately 40 min and storing the samples for 24 h at 4°C.

Water-holding capacity (WHC) and cooking loss of MPs gels

The WHC of the gel samples was determined according to the method described by Wang et al.[7]. The following equation was used by weighing the MPs gels before (W_0) and after (W_1) centrifugation:

$$\mathrm{WHC}(\%) = \frac{W_1}{W_0} \times 100\% \quad (1)$$

The cooking loss of the MPs gels was measured according to the method described by Wang et al [11]. After storing on ice for 24 h, the gel (~3 g) was removed from the beaker and dried. The cooking loss was determined according to the following equation by weighing the MPs gels before (W_2) and after (W_3) cooking:

$$\text{Cooking loss}(\%) = \frac{W_2 - W_3}{W_2} \times 100\% \quad (2)$$

2.3 Whiteness of MPs gels

The whiteness of the gel was measured using a colorimeter (CR 400; Minolta Camera Co., Japan). The L^*, a^*, and b^* values of the samples were recorded. The whiteness values were determined using the following equation:

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (3)$$

2.4 Strength and texture profile analysis (TPA) of MPs gels

Gel strength was evaluated by a TA.XT. plus texture analyzer (Stable micro systems Ltd., UK) equipped with a P/0.5R probe[12]. Each sample was measured at a 1 mm/s test speed with a 3 g trigger force.

The textural properties of the MPs gels, including hardness, springiness, cohesiveness, and chewiness, were also evaluated using the TA.XT. plus texture analyzer equipped with a P36/R probe[13]. The compression strain was 60%, and the pre-test, test, and post-test speeds were 5, 1, and 5 mm/s, respectively, with a trigger force of 5 g.

2.5 Zeta potential tests

The zeta potential was analyzed using a Nano-ZS Zetasizer (Malvern Instruments Ltd., UK)[14]. An APD detector was used. The MPs solutions (1 mL, 0.1 mg/mL) were added to the zeta cell, and the zeta potential (mV) of the MPs was automatically calculated using the Malvern SOP software that was supplied with the instrument.

2.6 Rheological tests

The MPs solutions (50 mg/mL) were poured between two parallel steel plates (thickness 60 mm, clearance 1 mm) and a dynamic rheometer (Thermo fisher scientific Ltd., USA) was used to analyze the dynamic rheology[5]. The samples were held at 20°C for about 10 min and, then heated at 2°C/min till the temperature reached 80°C. Each measurement was performed in an oscillatory mode with a constant frequency of 0.1 Hz. Variations in the storage modulus (G′ values) and loss modulus (G″ values) were recorded.

2.7 Turbidity tests

The turbidity of MPs was measured as reported previously[15]. The MPs solutions, either fresh or thawed, were diluted to 1 mg/mL. Then, 5 mL of the diluted solutions were heated at 30°C, 40°C, 50°C, 60°C, 70°C, or 80°C for 30 min. The absorbance at 600 nm was recorded by a spectrophotometer (TU-1810, Beijing Persee Instruments Co., Ltd., Beijing, China) after cooling to evaluate the changes in the turbidity of MPs.

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

Moisture distribution was analyzed using an NMI20-040V-I analyzer (Shanghai Niumag Analytical Instrument Co., Shanghai, China)[16]. The MPs gel was cut into 15 mm×15 mm×20 mm (length×width×height) pieces and loaded into round glass tubes (diameter 25 mm). The Carr-Purcell-Meiboom-Gill (CPMG) sequence was applied to assay the Transverse relaxation time (T_2) under the following operating conditions: sampling frequency (SW) =100 kHz, the number of scans (NS)=8, half echo time (TE)=0.8 ms, repeat sampling time interval (TW)=5500 ms, echo number

(NECH) = 16 000, and sampling point (TD)=1280 018. Data were obtained using the Multi Exp Inv Analysis software that was supplied with the instrument. Origin Pro 8.0 was used to integrate the peak area (P_2).

2.9 Scanning electron microscopy (SEM)

The microstructures of MPs gel samples were measured using a Quanta 200 FEG scanning electron microscope (FEI Technologies Inc., USA). Briefly, the samples (length×width×height: 3 mm× 3 mm×5 mm) were immobilized for 12 h, followed by dehydration with an ethanol series (50%, 70%, 90%, 95%, and 100%, 15 min each). Subsequently, the samples were dipped in tertiary butyl alcohol and dried in a DZF-6020 vacuum drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd., China). Finally, each sample was sprayed with gold and monitored at 15 kV and 1000× magnification. The quantitative analysis of SEM photographs was carried out using ImageJ V1.52s (NIH, Bethesda, MD, USA) according to the methods described by Zhang et al.[17] with small modifications. In brief, the photographs were converted into grayscale with suitable threshold values and transformed into 8-bit binary images. Then, porosity was expressed as the ratio of porous area to the total area, and the fractal dimension (D_f) was calculated using the box-counting method through a Fracac V3.0 plug-in module of ImageJ software.

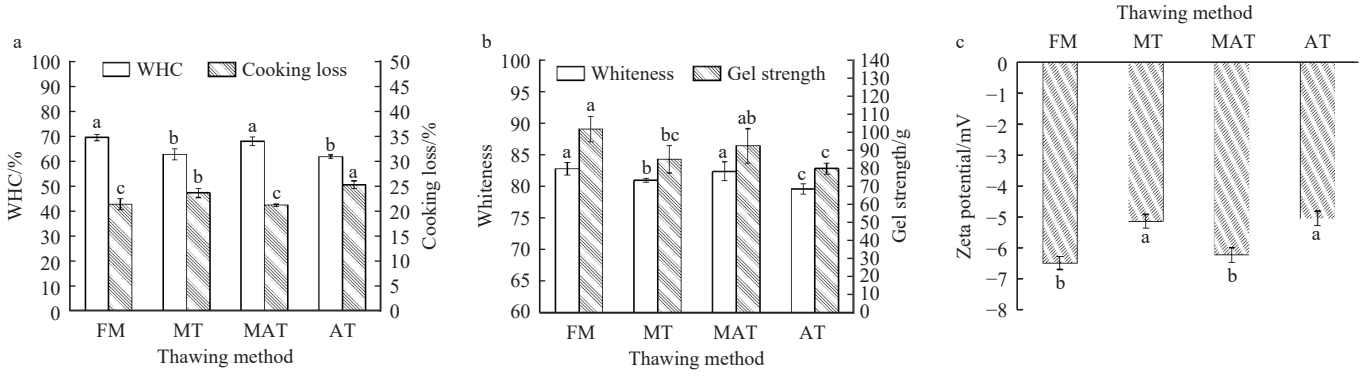
2.10 Statistical analysis

Three independent trials (experimental replicates=3) were performed for the statistical analysis. SPSS version 25.0 (SPSS Inc., SA) was used for the statistical analyses. A $p<0.05$ for Duncan's multiple range test was considered significant. For the turbidity experiments, data were evaluated using a one-way analysis of variance (ANOVA) and the general linear model (GLM) program, with the different thawing methods and temperatures as fixed effects and the replicates as random effects. The LSD method was used to identify significant differences between the means. Besides, ANOVA was applied to analyze the differences among other experimental groups.

3 Results and discussion

3.1 WHC and cooking loss of MPs gels

The changes in WHC and cooking loss of MPs gels obtained using various thawing methods are shown in Figure 1a. The WHC, which is a crucial indicator of the water retention capacity of the protein gel network structure, determines the quality and yield of the final meat product[18]. A denser gel network structure can capture more moisture and thus has a higher WHC[13]. Following MAT, MT, and AT, the WHCs of the heat-induced MPs gels decreased by 2.05%, 9.68%, and 11.02%, respectively. This indicated that the thawing procedures damaged the three-dimensional network, which is supported by the results of SEM. Indeed, Wang et al.[19] pointed out that the WHC of MPs gel might be related to myosin integrity, which plays a crucial role in MPs gelation since the connection among myosin chains can lead to the generation of a continuous gel network in which moisture can be captured. Cao et al.[3] also confirmed the denaturation of myosin after thawing. Furthermore, a decrease in the α-helix content might also account for these observations[20]. The WHC of MPs gels obtained from the MT and AT samples showed a significant decrease ($p<0.05$) compared to that of FM, whereas an insignificant decrease was founded for the MAT samples ($p>0.05$). This might be due to the generation of a poorer three-dimensional network subjected to MT and AT, which is supported by the SEM results. This was attributed to local overheating during MT and long thawing times during AT[3].



Note: FM: Fresh meat; MT: Microwave thawing (100 W); MAT: Microwave combined with air convection thawing; AT: Air convection thawing; WHC: Water holding capacity; MPs: Myofibrillar proteins. Different letters indicate significant differences among groups ($p<0.05$).

Figure 1 Changes in the WHC & cooking loss (a), whiteness and gel strength (b), and zeta potential (c) of the MPs gels induced by different thawing methods

Cooking loss is also considered to be an indicator of the quality, yield, and cost of a gel product due to its close relationship with the WHC. However, additional information on the degree of water binding inside the gel structure over time can be obtained by considering the cooking loss rather than the WHC[13]. Compared to the cooking loss observed for the FM sample (21.38%), there was no significant increase in the cooking loss following MAT (21.21%) ($p>0.05$), whereas the corresponding values following MT (23.64%) and AT (25.27%) rose notably ($p<0.05$) (Figure 1a). This result corroborates the WHC results, thereby indicating that the water entrapment efficiency of the gel was reduced during MT and AT. Overall, the MAT treatment resulted in a better WHC of the MPs gel than that achieved with MT or AT treatment alone ($p<0.05$).

3.2 Whiteness and gel strength of MPs gels

As shown in Figure 1b, the whiteness of the samples subjected to MT, MAT, and AT treatments (i.e., 80.96, 82.36, and 79.58, respectively) was reduced compared to that of the FM MPs gel (i.e., 82.76). The whiteness of gels is associated with the levels of denaturation and oxidation of the protein, both of which result in poor color stability[21]. This result, therefore, indicates that the degree of denaturation and oxidation of the MPs increased during thawing, which is consistent with our previous findings[9]. Similarly, Zhang et al.[22] found that the gel whiteness decreased with increasing MPs denaturation. Compared to that of the FM sample,

the whiteness of the MPs gels subjected to MT or AT decreased significantly (p<0.05), whereas the whiteness of the MPs gel subjected to MAT did not (p>0.05). These findings suggest that MAT helps in maintaining the color of MPs gel. This might be attributed to the uniform heating and short thawing time involved in the MAT process, which resulted in less protein oxidation[9]. Moreover, the whiteness of the MPs gel subjected to AT was less than that of the gel subjected to MT (p<0.05), which might be correlated with the reduced cooking loss after MT treatment compared to that after AT treatment (Figure 1a).

Gel strength reflects the level of protein aggregation in the network structure[12,23]. As shown in Figure 1b, compared to the value obtained for the FM sample (101.46 g), the gel strengths of the MPs gels subjected to MT, MAT, and AT were decreased by 16.3%, 9.05%, and 21.4%, respectively. This decrease in MPs gel strength suggests that the thawing procedure disrupts the uniform gel network structure to some extent, due to the dissociation of muscle proteins and subsequent protein denaturation and non-covalent bond fracture[24,25]. Li et al.[6] also observed that the strength and gel-forming ability of MPs gels were affected by the kind of freezing-thawing procedure employed. The strength of the MAT MPs gel was similar to that of the FM gel (p>0.05), whereas the gel strengths of MT and AT gels were lower than the strength of the FM gel (p<0.05). The protein oxidation caused by uneven heating or longer thawing times during the MT or AT processes might be responsible for the observed reduction in gel strength[9]. Smyth et al.[25] also found that protein oxidation may cause a decrease in protein solubility, which is detrimental to gelation and can result in reduced gel strength. Furthermore, the variations in gel strength correlated with the porosity and D_f of the microstructure, such that high gel strength was positively correlated with a high-density and uniform gel microstructure[12]. Overall, MAT can prevent structural deterioration during heat-induced gelation.

3.3 Textural properties

The texture is an important indicator used to assess the quality of a protein gel, and changes in the sample quality can be assessed by simulation of the chewing course of the human mouth. Some common textural parameters, including hardness, springiness, cohesiveness, and chewiness, were therefore used to analyze the textural properties of the MPs gels. As listed in Table 1, the hardness, springiness, cohesiveness, and chewiness decreased to some extent after thawing, which was consistent with the WHC and whiteness findings (Figure 1). The lowest values for hardness, springiness, cohesiveness, and chewiness were obtained for the AT samples, and these were notably lower than those of the FM samples (p<0.05). This could be explained by protein oxidation induced by the longer thawing time of the AT treatment, which might promote the excessive cross-linking of protein molecules, ultimately resulting in the degradation of the gel texture[21,26]. The

Table 1 Changes in the texture of MPs gels induced by different thawing methods

Thawing methods	Hardness/g	Springiness	Cohesiveness	Chewiness/g
FM	446.93±34.25^{a}	0.79±0.06^{a}	0.28±0.02^{a}	98.42±6.99^{a}
MT	271.06±5.15^{c}	0.68±0.03^{ab}	0.22±0.03^{ab}	39.82±5.98^{b}
MAT	359.34±44.27^{b}	0.79±0.06^{a}	0.29±0.06^{a}	80.95±14.26^{a}
AT	260.05±38.07^{c}	0.64±0.08^{b}	0.20±0.03^{b}	34.89±11.6^{b}

Note: FM: fresh meat; MT: microwave thawing (100 W); MAT: microwave combined with air convection thawing; AT: air convection thawing. The values are expressed as means ± SD. Different superscript letters in the same column indicate significant differences between those values according to Duncan's multiple range test (p<0.05).

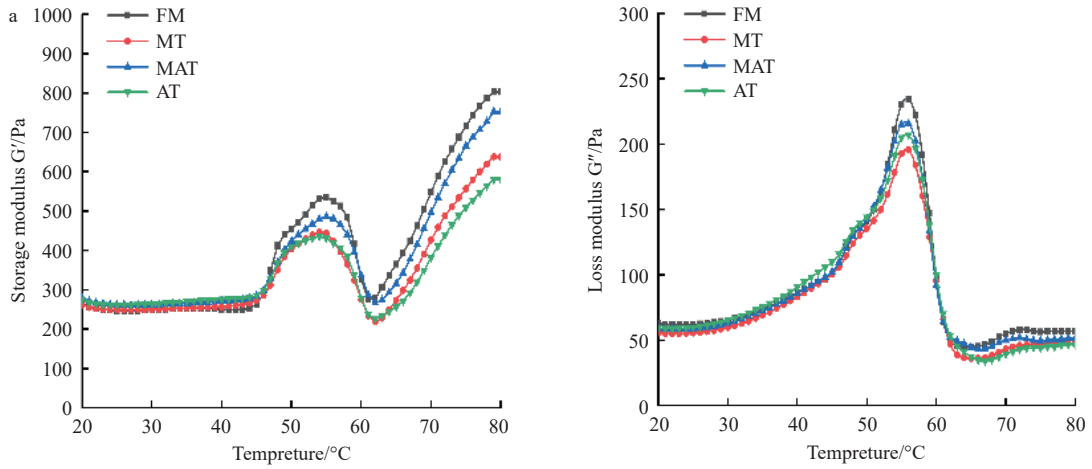
decrease in the abundance of water, the poor WHC, and the excessive water mobility in MPs gels from samples subjected to AT (Figure 1 and Figure 2) observed in the present study might be the additional reasons. However, the MAT samples exhibited significantly higher hardness and chewiness values (p<0.05) than the MT and AT samples, with values close to those of the FM samples (p>0.05). This could be due to the MAT process preventing the destruction of hydrogen and covalent bonds, thereby resulting in the generation of a dense protein gel network[27].

3.4 Surface charge

The zeta potential has been identified as an important indicator of protein aggregation[28]. The zeta potentials of FM and thawed samples were negative (Figure 1c), indicating that negative contributions from the aspartic and glutamic acids were more than positive ones[29]. The decreases in the zeta potentials of AT and MT samples were remarkable (p<0.05) compared to that in the zeta potentials of FM sample, whereas that for the MAT sample was not significant (p>0.05). Zhang et al.[14] reported that a protein with a high zeta potential (positive or negative) is electrically stable, whereas a protein with a low value is easy to coagulate or aggregate. The result agrees with the particle size results reported in our previous study[9], indicating that the thawing process weakened the electrostatic repulsion between MPs particles, resulting in the generation of large protein aggregates. However, the zeta potentials of AT and MT samples were notably less than that of MAT samples (p<0.05). This was attributed to the fact that the MT and AT processes promote MPs unfolding, resulting in the exposure of amino acid residues, which further changes the charge distribution of protein molecules, leading to a lower zeta potential[30]. These results indicate that MAT might disrupt protein aggregation, and strengthen interparticle electrostatic repulsions, thereby inhibiting further aggregation and improving the dispersion stability.

3.5 Rheological properties

The G′ and G″, which represent the elastic and viscous contributions of protein gels subjected to variations in temperature, are important functional properties that affect meat product quality and stability[27]. Differences in the G′ (Figure 2a) and G″ values (Figure 2b) were apparent for MPs gels thawed by different methods. More specifically, the G′ values of MPs gels exposed to heating exhibited four different phases with oscillation due to the denaturation and gelation of proteins. Initially, the G′ decreased slightly between 20°C-44°C due to the weakening of bonds with this initial temperature increase. Subsequently, the G′ increased markedly above 45°C and peaked at almost 55°C, suggesting that partial unfolding of myosin occurred, resulting from the generation of the initial network at a denaturation temperature of myosin[2]. In the third phase, G′ decreased sharply between 56°C-61°C, indicating that the protein network had been disrupted. Guo et al.[31] reported that such variations could be attributed to the denaturation of myosin tails. Subsequently, G′ steadily increased once again upon heating up to 80°C, which is consistent with the results obtained by Zhou et al.[32], and indicates that a firm and irreversible MPs gel was formed. This behavior might be due to the generation of additional bonds in the open gel structure. The final G′ values of the MPs gels subjected to MT, MAT, and AT decreased by 20.4%, 6.1%, and 27.7%, respectively, compared to the G′ value of the FM sample (802.5 Pa) at 80°C (Figure 2a), due to protein destruction during thawing, and the resulting lower elasticity. However, the G′ of the MAT sample was higher than that of the MT or AT samples, and its peak shape was better than those of the MT and AT samples, thereby indicating that the MAT sample formed a more elastic gel



a. Changes in the storage modulus b. Changes in the loss modulus

Figure 2 Changes in the storage modulus (G′, a) and loss modulus (G″, b) of the MPs gels induced by different thawing methods

during the heating stage.

As shown in Figure 2b, the trend in G″ was generally in line with that of G′. G″ reached its first peak as the temperature reached approximately 55°C, and then decreased dramatically between 55°C and 60°C. Subsequently, G″ remained relatively constant until 80°C. G′ was higher than G″ during the heating procedure for gel generation, which suggested that elasticity was more dominant than viscosity. Li et al.[12] also obtained similar results. These observations indicate that MAT might effectively reduce the level of protein denaturation and help in maintaining a superior protein gel structure compared to MT or AT treatment.

3.6 Turbidity

Turbidity is another vital indicator of the level of protein aggregation during heating[33]. As listed in Table 2, the turbidity of all samples increased significantly (p<0.05) with an increase in temperature from 30°C to 80°C. This is similar to the results obtained by Wang et al.[7] and Chen et al.[34], who demonstrated that the turbidity of porcine and chicken MPs increased during heating. The protein unfolding that occurs with increasing temperature and the larger proteins that subsequently form through the polymerization of monomeric proteins might, therefore, account for the increase in turbidity[35]. Moreover, the turbidity value increased significantly within the temperature range of 40°C-60°C (p<0.05). This might be because that MPs began to form a three-dimensional network structure in this temperature range, as confirmed by the results of dynamic rheology measurements (Figure 2). Compared to that of FM sample, the turbidity of MT and AT samples increased notably (p<0.05) at the same temperature; however, in contrast, the change in the turbidity of MAT samples was insignificant (p>0.05), except at 70°C. This result was in line with the zeta potential results (Figure 1c), indicating that the use of an inappropriate thawing method could induce the formation of protein aggregates, which hinder light transmission[7]. The lower turbidity of MAT sample could be explained by a smaller average particle size, as discovered in our previous study[9], ultimately leading to an increase in the specific surface area available for light scattering.

Table 2 Changes in the turbidity of MPs gels induced by different thawing methods

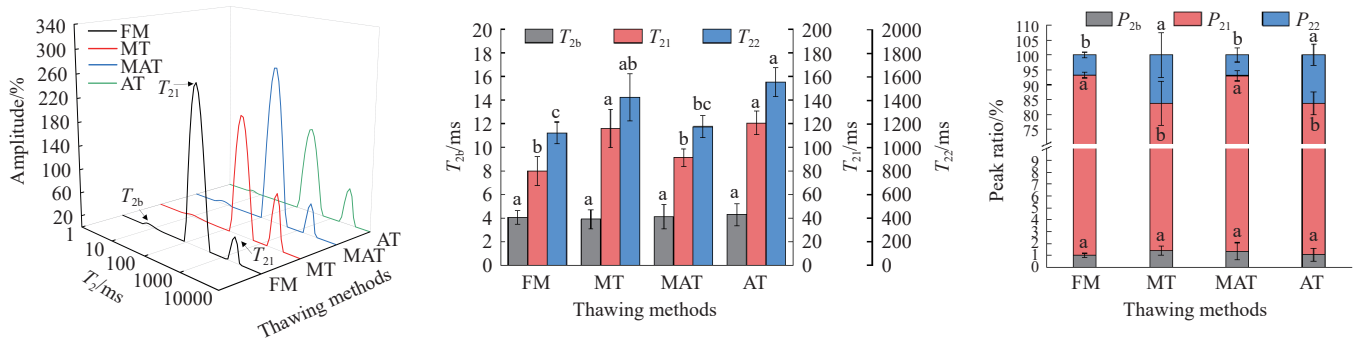
Turbidity	Temperature/°C					
	30	40	50	60	70	80
FM	0.131±0.009^{Fb}	0.159±0.009^{Ec}	0.352±0.009^{Dc}	0.532±0.034^{Cc}	0.580±0.006^{Bd}	0.667±0.018^{Ac}
MT	0.166±0.017^{Fa}	0.216±0.008^{Eb}	0.424±0.028^{Db}	0.581±0.032^{Cb}	0.655±0.015^{Bb}	0.769±0.046^{Ab}
MAT	0.136±0.006^{Fb}	0.164±0.008^{Ec}	0.387±0.013^{Dbc}	0.552±0.012^{Cbc}	0.595±0.004^{Bc}	0.703±0.021^{Ac}
AT	0.180±0.020^{Ea}	0.238±0.012^{Da}	0.488±0.050^{Ca}	0.646±0.034^{Ba}	0.675±0.015^{Ba}	0.819±0.014^{Aa}

Note: FM: fresh meat; MT: microwave thawing (100 W); MAT: microwave combined with air convection thawing; AT: air convection thawing. The values are expressed as means ± SD. Values with different lowercase letters (a-d) in the same column differ significantly among the four groups at the same temperature (p<0.05). The means in the same row with different uppercase letters (A–F) differ significantly among the temperature points within a group (p<0.05).

3.7 Moisture distribution

The T_2 and P_2 of MPs gels were analyzed by LF-NMR to reveal the water distribution and mobility in the gels[19]. As shown inFigure 3a, the three peaks corresponded to three different types of water molecules: bound water (T_{2b}, 0-10 ms), immobilized water (T_{21}, 100-200 ms), and free water (T_{22}, 1000-2000 ms). As shown inFigure 3b, none of the thawing treatments had any significant effect (p>0.05) on the T_{2b} values of MPs gels compared to that on the T_{2b} values of FM sample. These results indicate that the thawing procedure did not influence the fluidity of bound water, which agrees with the moisture migration and distribution results reported in our previous study[9]. Mcdonnell et al.[36] have also suggested that bound water has a strong resistance to heating or freezing due to its tight bonding with muscle proteins. Compared to those of FMsample, the T_{21} and T_{22} values of all other samples, except the MAT sample, were significantly increased (p<0.05). These increased values of T_{21} and T_{22} demonstrated that the fluidity of moisture in the MPs gel was increased due to the generation of a poor three-dimensional MPs gel network following MT and AT, which is supported by SEM results (Figure 4).

Figure 3c shows the changes in the peak area ratios of the three water forms in MPs gel samples (i.e., P_{2b}, P_{21}, and P_{22}). There were no significant differences in the P_{2b} values among the various samples (p>0.05), which is in line with the results obtained for T_{2b} (Figure 3b). The P_{21} values for the MT and AT samples were significantly lower (p<0.05), whereas the P_{22} values were markedly higher (p<0.05) than the corresponding values for FM sample. These results indicate that partially immobilized water molecules



a. Moisture distribution b. T_2 c. P_2

Note: Different letters indicate significant differences among groups (p<0.05).

Figure 3 Changes in the moisture distribution, relaxation time (T_2), and peak ratio (P_2) of the MPs gels induced by different thawing methods

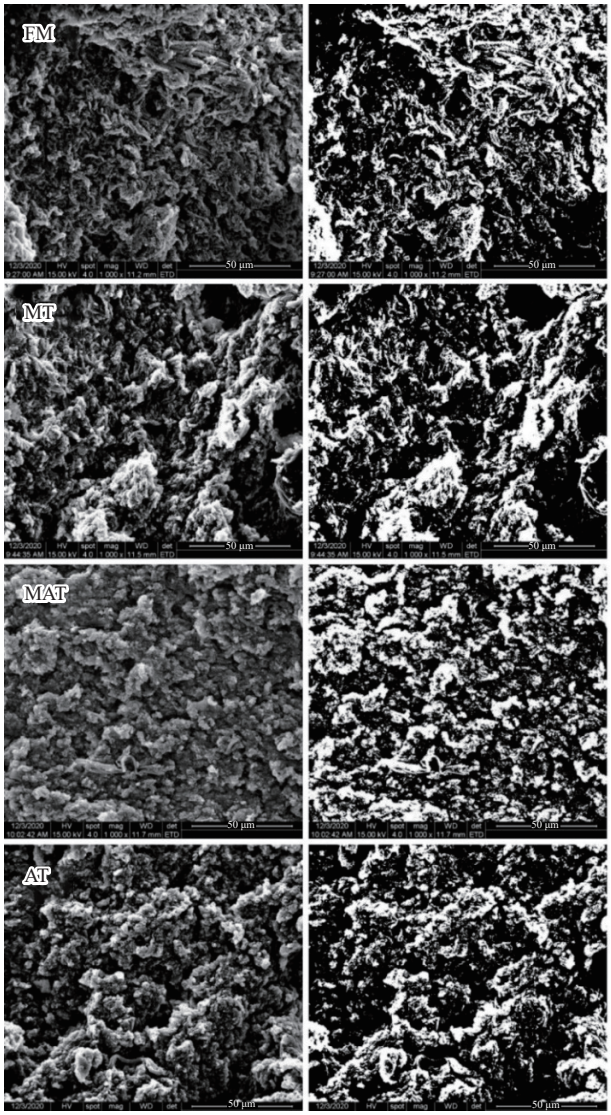


Figure 4 Scanning electron micrographs (left) of MPs gels subjected to different thawing methods (scale bar=50 μm), and their corresponding binarized images (right)

might transform into free water during the MT and AT processes, which is substantiated by WHC results (Figure 1a). This finding could be associated with the partial destruction of protein tertiary structure caused by uneven heating during the MT process and long thawing times during the AT process, which was stated in our previous study[9]. Han et al.[37] noted that the denaturation of myosin possibly alters the lattice spacing of MPs, resulting in the shift of some water molecules from the T_{21} to the T_{22} domain. Thus, together with the WHC results, these data demonstrate that the MAT technique can reduce the damage to MPs gel structure to facilitate the retention of greater amounts of water by the gel.

3.8 Microstructures of MPs gels

The SEM and corresponding binarized images of the MPs gel subjected to different thawing methods are presented in Figure 4. Table 3 shows the porosity and D_f, which are used to quantitatively evaluate the impact of thawing on the microstructure of MPs gels, calculated using SEM photographs. Porosity reflects the tightness of the structure, whereas D_f quantifies the complexity, irregularity, and spatial state of the network[38]. The MPs gel of FM sample exhibited a homogeneous and compact structure with the highest D_f value (p<0.05) and the lowest porosity (p<0.05). However, the gel microstructure changed to various extents after thawing. More specifically, the MPs gels of MT and AT samples displayed loose and disordered gel networks, with significantly increased porosity compared to that in the FM sample (p<0.05), whereas the porosity and D_f of the MAT sample were closer to those of the FM sample (p>0.05). Thus, the MPs gel of MAT sample had a smoother and denser microstructure than the MPs gels of MT and AT samples. These results are consistent with those of the gel strength, zeta potential, turbidity, and moisture mobility (Figure 1b, Figure 1c,Table 2, and Figure 3, respectively), which are attributed to the protein aggregation and cross-linking induced by non-uniform thawing or length thawing times during the MT and AT processes[39]. Likewise, Li et al.[12] also reported that the microstructure of MPs gel subjected to MT was disrupted, which promoted the loss of water from the structure. In addition, the smaller average particle size of the MAT sample reported in our previous study[9] might be responsible for the homogeneous and compact structure of MAT MPs gel. Zhang et al.[29] also reported that small particle diameters favored the formation of

Table 3 Changes in the D_f and porosity of MPs gels induced by different thawing methods

Thawing methods	D_f	Porosity/%
FM	2.737±0.021^{a}	32.646±0.361^{b}
MT	2.714±0.002^{ab}	36.840±0.931^{a}
MAT	2.742±0.036^{a}	33.769±0.656^{b}
AT	2.668±0.028^{b}	36.923±0.479^{a}

Note: FM: Fresh meat; MT: Microwave thawing (100 W); MAT: microwave combined with air convection thawing; AT: air convection thawing. The values are expressed as means ± SD. Different superscript letters in the same column indicate significant differences between those values according to Duncan's multiple range test (p<0.05).

regularspatialarrangements of proteins in such MPs gels. Therefore, MAT might help in maintaining the structural stability of MPs gel, which is favorable for water storage and improves gel strength.

4 Conclusions

The results of this study showed that, compared to microwave thawing (MT) and air convection thawing (AT) treatments, microwave combined with air convection thawing (MAT) resulted in a superior gel quality. A better water holding capacity (WHC), lower cooking loss, higher whiteness, and higher strength of the myofibrillar proteins (MPs) gel were obtained using MAT than those obtained using AT or MT, indicating that MAT causes less deterioration of such properties during the heat-induced gelation of MPs. MAT limited the formation of additional protein aggregates, evidenced by no significant difference in the turbidity or zeta potential of MPs gels obtained from FM samples or following MAT treatment (p>0.05). Furthermore, the texture data, rheological analyses, and scanning electron microscopy (SEM) results were relatively consistent, suggesting that MAT caused the least damage to the microstructure of MPs gel among the different thawing methods, thereby restraining the decrease in texture. No significant difference (p>0.05) was observed in the immobilized and free water contents of MAT and FM samples, which is in agreement with the WHC results, demonstrating that MAT did not cause notable damage to the MPs gel structure, thus enabling the gel to retain high volumes of water. In conclusion, MAT treatment allowed the gelling quality of MPs from porcine *longissimus dorsi* muscle to be maintained better than did AT or MT, hence, it can be potentially used in the meat industry. Nevertheless, the changes in proteomics induced by MAT require further study in order to clarify the improvement in the conformation and gelling properties of MPs following MAT.

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