Influence of ultrasound-assisted tumbling on NaCl transport and the quality of pork

Ruyu Zhang, Jian Zhang, Lei Zhou, Lin Wang, Wangang Zhang *

Key Laboratory of Meat Products Processing, Ministry of Agriculture, Jiangsu Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

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ABSTRACT

The present study aimed to investigate the impact of ultrasound-assisted tumbling (UAT; 20 kHz, 100, 300, 500 and 700 W) with different treatment time (30, 60, 90 and 120 min) on the diffusion and distribution of NaCl as well as the change of pork texture properties during curing. Results showed that in comparison with the single tumbling (ST), the NaCl content and the NaCl diffusion coefficient were increased along with UAT treatment (P < 0.05). The scanning electron microscopy and the energy dispersive X-ray analysis showed that UAT treatment changed the microstructure of pork which may facilitate the NaCl dispersion homogeneously. In addition, the moderate UAT treatment of 300 W with 60 min could significantly improve the tumbling yield, water-holding capacity and textural properties of pork compared with the ST treatment (P < 0.05). Meanwhile, in comparison with the ST group, protein extraction was considerably increased after UAT (300 and 500 W) treated for 120 min (P < 0.05). Our study demonstrated that UAT treatment could effectively promote the penetration and distribution of NaCl and improve pork meat quality via facilitating the extraction of meat protein.

1. Introduction

Curing has been used for preserving food for many years. Among curing ingredients, NaCl is the most important one that could inhibit microbial growth and improve the flavor, texture and other quality attributes of food [1]. The traditional curing approaches, including dry-curing and wet-curing, have been proved to consume too much time in the practical process [2]. Thus, the industry has sought novel process methods for accelerating the curing efficiency of food products [3]. Among which tumbling has been revealed to have positive effects on the curing of meat and meat products [4,5]. When meat samples are subjected to tumbling, they could be beaten by the tumbler paddles and rubbed through the meat-meat and meat-apparatus. These mechanical effects could disrupt meat cells and facilitate brine distribution into meat [6]. Besides, Gao et al. [5] revealed that tumbling could extract myofibrillar proteins and form protein exudate covered on the meat surface. Consequently, the protein exudate could play the role of “glue” to bind meat pieces, thus improving the sliceability, texture, tenderness, and cohesion of meat and meat products [7]. However, Li et al. [8] pointed that the present single tumbling (ST) is time-consuming and potentially causes uneven distribution of NaCl. Even though the ST could enhance the meat production efficiency compared with the traditional curing method, it could still take 10–16 h when used interval ST [9].

High-intensity ultrasound (US, 20–100 kHz, 10–1000 W/cm²) has been considered as an innovative and green technology applied in mass transport [10], and US could well shorten the curing time and improve the meat quality [11,12]. Kang et al. [13] used US with different ultrasonic intensities (2.39, 6.23, 11.32, 20.96 W/cm²) and observed that US could significantly increase the final NaCl content compared with static immerse curing. Inguglia et al. [14] found that in chicken breast marination, US treatment (25, 45, 130 kHz) for 6 h could reach a similar NaCl concentration as the control treatment for 16 h (without US). The effects of US could be attributed to the following mechanisms: (1) when US travels through a liquid medium, it causes microturbulence and microstirring in brine; (2) the microjets which result from ultrasonic cavitation could cause erosion on food surface and disruption in meat cell; (3) the sinusoidal pressure of ultrasonic wave could cause meat matrix compression and expansion (“sponge effect”) and thus generate microchannel [15,16]. All the above effects could diminish the external and internal resistance of meat during the curing process, thus accelerating brine penetration into the meat and enhancing mass transfer. However, no published study has investigated the effects of ultrasound-
assisted tumbling (UAT) on meat curing. Thereby, our work aimed to research the impact of UAT treatment during curing on the diffusion and distribution of NaCl and the quality of pork.

2. Materials and methods

2.1. Meat sample and brine preparation

Pork *mesoglutaeus* was obtained from Sushi Meat Processing Company (Huai’an, Jiangsu province, China) after 24 h of slaughtering. The pH was detected using Consort C831 pH-meter (Consort N.V., Turnhout, Belgium) paralleling to the direction of muscle fiber and the pH of meat ranged from 5.6 to 5.8 was selected for the experiment. After all the visible fat and connective tissue were trimmed, the meat samples were cut into cuboids (100 × 40 × 40 mm³) and vacuum packaged in a plastic bag. The packaged meat samples were kept at −20 °C and thawed completely at 4 °C before the experiment. The brine used for tumbling contained 0.34% (w/v) sodium tripolyphosphate, 0.17% (w/v) sodium pyrophosphate, 0.34% (w/v) sodium hexametaphosphate and 8.6% (w/v) NaCl. The prepared brine was cooled at 4 °C before the experiment. The brine was used for tumbling after 24 h of slaughtering. The brine was used for tumbling after 24 h of slaughtering. The percentage of explained variance (% VAR) was used to evaluate the fitness of the model by equation (2)

\[
\% \text{VAR} = 1 - \frac{S_{\text{est}}^2}{S_{\text{sam}}^2}
\]  

where \(S_{\text{est}}^2\) and \(S_{\text{sam}}^2\) are the variance of the estimation and sample, respectively.

2.5. Scanning electron microscopy (SEM) with energy dispersive X-ray analysis (SEM-EDX)

The microstructure of meat samples was observed by SEM with an accelerating voltage of 3 kV (SU8010, Hitachi, Tokyo, Japan). The slices of meat samples (1 × 3 × 3 mm³) were cut from 1 cm below the meat surface and fixed in 2.5% (v/v) glutaraldehyde acid. Then the slices were operated by a series of post-fixation, washing and dehydration according to Wang et al. [20]. As for the SEM-EDX spectrometric analysis, the slice samples were fixed by oven drying instead of being immersed in 2.5% (v/v) glutaraldehyde acid to prevent NaCl dissolving. Then the X-ray detector (X-Max 80, Oxford, UK) was used for observing the distribution of Na⁺ and Cl⁻ inside the meat samples.

2.6. Tumbling yield

The tumbling yield was calculated according to Steen et al. [7] using the following equation (3):

\[
\text{Tumbling yield} = \frac{w_1 - w_0}{w_0} \times 100\% 
\]  

where \(w_0\) and \(w_1\) refer to the weight of meat samples before and after tumbling, respectively.

2.7. Cooking loss

The cooking loss of meat samples was determined following the methods of Inguglia et al. [14,21] with some changes. Briefly, after meat samples were cut off 1 cm strip, the new cuboid was cut into 5 × 3 × 2 cm³ and cooked at 90 °C until the center temperature reached 72 °C. The cooking loss was calculated by the percentage of weight loss in relation to the initial weight before cooking.

2.8. Protein concentration in the brine

The methods of protein determination were followed by McDonnell et al. [22] with a slight change. After tumbling, the brine was collected and centrifuged at 10,000×g for 10 min at 4 °C to precipitate meat crumbs (Beckman Avanti J-E, Beckman Coulter, Fullerton, CA, USA). The protein content of the supernatant was measured by the Biiuret method with the BSA as the standard protein [23].

2.9. Texture profile analysis (TPA)

The samples after the cooking loss analysis were cut into cubes (1 × 1 × 1 cm³) for TPA (TA XT Plus, Stable Micro Systems, Surrey, UK) according to Zou et al. [24]. The cylindrical probe was selected as P50, the compression rate was modified to 75%, and the test, pre-test, and post-test speed was 1 mm/s, 5 mm/s, and 5 mm/s, respectively. Five samples of each treatment replicate were determined for hardness, springiness, cohesiveness, and chewiness.

2.10. Statistical analysis

The software of SAS 9.2 was used. The main effects of US power,
3. Results and discussion

3.1. NaCl content

The NaCl content of meat is the vital index to assess the degree of meat brining, and the influence of different treatments on NaCl content is presented in Table 1. The US power and treatment time obviously influenced the NaCl content (P < 0.05), while their interaction had no significant impact on NaCl content (P > 0.05). Notably, at the treatment time of 30 min, the NaCl content of UAT groups (300, 500 and 700 W) was apparently higher than that of ST groups (P < 0.05), reaching the highest value at UAT-700 W group. Similarly, at the treatment times of 60, 90 and 120 min, a considerable enhancement in NaCl content was observed after UAT treatment (500 and 700 W). These results indicate that UAT treatment could accelerate the diffusion of NaCl into meat compared with ST treatment. Thus UAT treatment had the potential to shorten curing time. The superiority of UAT treatment might be due to the ultrasonic cavitation, which produces the microjets attacking myofibrils and thus generating microchannels in the meat boundary, which is conducive for NaCl to penetrate into the meat [25]. Our results are consistent with previous studies which have proved the positive effects of the US on meat brining. Mcdonnell et al. [11] used the US (4.2, 11 and 19 W cm\(^{-2}\)) for pork brining and found that US-assisted brining groups significantly gained more NaCl content than control groups.

3.2. NaCl diffusion

NaCl diffusion coefficient could reflect the intrinsic dynamics of NaCl transfer. As shown in Table 2, all %VAR of the treatments was more than 95% suggesting great fitness for the experimental NaCl kinetics and NaCl transfer model. During curing, the physical properties of meat tissue could be changed [26]. Thus, the pseudo-binary system (solute-tissue) is used in determining the NaCl diffusion coefficient in meat tissue based on Fick's second law [26]. In this case, meat samples are regarded as a slab that could keep a stable shape during curing without any shrinkage, the mass transport is unidirectional, and the NaCl diffusion coefficient is a constant value [13]. Many researchers have modified Fick's second law for different immerse curing conditions [27,28]. However, to the best of our acknowledge, only one study from Siró et al. [19] used equation (1) to evaluate the NaCl diffusion coefficient when meat samples were treated by the ST treatment. Nevertheless, it is unclear whether equation (1) could describe the NaCl diffusion coefficient in UAT treatment. In this study, the high %VAR indicated that the equation (1) was well adequate to describe the NaCl diffusion coefficient in UAT treatment. In terms of the NaCl diffusion coefficient, the UAT treatment owned the higher value than that of the ST treatment. Moreover, the NaCl diffusion coefficient was notably increased with the increase of US power (P < 0.05) and reached the highest value in the UAT-700W group. The enhancement of the NaCl diffusion coefficient is consistent with the result of NaCl content. When the cavitation bubbles asymmetric collapse near the meat surface, it could generate the microjets and possess instantaneously high speed (100 m/s), thus destroying meat tissue [15]. Besides, the turbulence and agitation produced by ultrasonic waves could further enhance the NaCl transfer.

3.3. SEM micrographs and SEM-EDX analysis

As shown in Fig. 1, the muscle fibers of untreated meat were intact and compact, which was the typical microstructure of pork meat. Compared with untreated meat, meat samples subjected to ST treatment showed that the connective tissue fibers were slightly disrupted and dispelled. However, the gap between fibers was still small. As for the UAT-300 W group, the muscle fibers were significantly damaged. With the destruction of the perimysium structure, some obvious microfissures might be related to protein denaturation. In addition, the connective tissue fibers were slightly disrupted and the muscle fibers were significantly damaged. The SEM-EDX mapping images showed the distribution of NaCl of different tumbling treatment groups (Fig. 2) with the yellow dot representing the Na\(^+\) and the red dot indicating the Cl\(^-\). Compared with the untreated meat, more dots of Na\(^+\) and Cl\(^-\) in ST treatment suggested that...
ST treatment could promote brine absorption. Regarding the UAT treatments, as the US power increasing, both the dots of Na$^+$ and Cl$^-$ were distributed more uniformly and the number of dots was increased. The results illustrated that UAT treatment could remarkably promote the NaCl penetration and dispersion evenly in meat compared with the ST treatment. Additionally, the results further proved that UAT treatment could lead the meat samples to gain more NaCl during tumbling, thus improving the curing effect of tumbling.

### 3.4. Tumbling yield

The tumbling yield could reflect the brine absorption capacity of pork. As shown in Table 3, the US power, treatment time, and their interaction had a significant impact on the tumbling yield ($P < 0.05$). At the treatment time of 60 min, the tumbling yield had no significant difference between the ST group and UAT-100 W group ($P > 0.05$), which might be due to that the US power of 100 W was lower than the threshold. When US power was at 300 W and 500 W, the tumbling yield was considerably enhanced in comparison with the ST group ($P < 0.05$). On the contrary, when US power was at 700 W, the tumbling yield was...
The tumbling treatment could cause cellular disruption in muscle tissue by applying mechanical energy [33] and facilitating the extraction of the meat proteins to form protein exudates [34]. The total protein extracted from meat to brine is shown in Table 3. Both the US power and the treatment time significantly impacted the protein content (P < 0.05), while the interaction of US power and treatment time had no significant effect on the protein content (P > 0.05). It was apparent that the protein content was considerably increased when UAT treatment was applied (300 and 500 W) for 120 min compared with ST treatment (P < 0.05). However, the protein content of the UAT-700 W group was oppositely reduced to a lower level than that of the UAT-500 W group regardless of the treatment time (P < 0.05). The brine in this study did not contain any protein before usage. Thus, the increase of protein content in brine indicated that moderate UAT treatment could further promote protein extraction and solubilization to brine compared with ST treatment. The results are consistent with McDonnell et al. [22], who employed a series of ultrasonic intensities (4.2, 11, and 19 W cm⁻²) to salting pork meat and found that the protein content in brine was increased with the ultrasonic intensity increase. In addition, as the NaCl content in the UAT group increased, the higher ionic strength could increase myofibrillar proteins extraction, which could bind more external water and form the protein gel to retain water during cooking [35]. Therefore the results of protein content in brine are consistent with the results of tumbling yield and cooking loss. Also, the reduction of protein content under excessive UAT treatment confirmed the occurrence of protein denaturation.

### 3.5. Cooking loss

Table 3 shows the cooking loss of various groups applied by different US power and treatment time. The US power, treatment time, and their interaction notably impacted the cooking loss (P < 0.05). Particularly, compared with the ST group, the cooking loss was considerably reduced when the US power ranged from 300 W to 700 W (P < 0.05), and the cooking loss was significantly decreased along with the increase of US power (P < 0.05). Similarly, when meat samples were treated for 120 min, the cooking loss was significantly decreased at the group of UAT-500 W compared with that of the ST group (P < 0.05). On the contrary, when meat samples were treated by UAT-700 W, the cooking loss was subsequently increased with no significant difference with the ST group (P > 0.05). Therefore, the reduction of cooking loss suggested that the moderated UAT treatment could improve the WHC of pork meat. The previous study by Zou et al. [24] has observed similar results that the US-assisted cooking treatment improved the WHC of beef. Furthermore, our previous study has revealed that the US could exert the cavitation effect to reduce the protein particle size and expose the active region, which increased the protein-water interaction to bind more water thus improving the WHC [17].

### 3.6. Protein content

The tumbling treatment could cause cellular disruption in muscle

### 3.7. Texture profile analysis (TPA)

Table 4 shows the TPA indexes of hardness, springiness, cohesiveness and chewiness of pork meat after various treatments. The US power and treatment time had a significant impact on these indexes (P < 0.05), while the interaction of US power and treatment time only significantly influenced the cohesiveness and chewiness (P < 0.05). As for the hardness, at the short treatment time of 60 min, the hardness of each UAT group was higher than that of ST group (P < 0.05). However, only the UAT-500 W group had higher hardness than the ST group when meat samples were treated for 120 min. Moreover, the excessive UAT treatment (700 W; 120 min) significantly reduced the hardness compared with the UAT-500 W treatment. The increase of hardness under moderate UAT treatment might be related to the fact that the extracted myofibrillar proteins could form a compact thermal-induced gel network which could improve the hardness. Zhang et al. [36] also implied that the moderate US could aid myofibrillar protein gel to form a uniform and dense network, thus strengthening the gel properties. As the result of protein content showed (Table 3), moderate UAT treatment could improve the myofibrillar protein extraction which allowed the formation of protein gel with great structure during cooking, thus improving the hardness of pork meat. In addition, the protein content also proved that excessive US could cause protein denaturation which partly explained the decrease of hardness under long treatment time and excessive UAT treatment.
Table 4

| Treat time (min) | Ultrasound power (W) | SE | P-Values |
|-----------------|----------------------|----|----------|
|                 | ST  | UAT-100 | UAT-300 | UAT-500 | UAT-700 |
| Hardness (N)    | 60  | 105.06** | 112.79** | 113.55** | 119.29** | 116.11** | 3.16 | <0.001 | 0.11 | <0.001 |
|                | 120 | 114.69** | 110.30** | 112.21** | 125.22** | 90.52** |      |        |      |
| Springiness     | 60  | 0.54**   | 0.58**   | 0.61**   | 0.56**   | 0.53**   | 0.014 | <0.001 | 0.25 | <0.001 |
|                | 120 | 0.59**   | 0.59**   | 0.53**   | 0.54**   | 0.53**   |      |        |      |
| Cohesiveness    | 60  | 0.52**   | 0.56**   | 0.58**   | 0.56**   | 0.54**   | 0.011 | <0.001 | 0.027 | <0.001 |
|                | 120 | 0.56**   | 0.54**   | 0.55**   | 0.53**   | 0.52**   |      |        |      |
| Chewiness (N)   | 60  | 30.04**  | 30.01**  | 30.84**  | 32.88**  | 30.89**  | 1.35  | <0.001 | 0.0046 | <0.001 |
|                | 120 | 32.45**  | 32.06**  | 36.84**  | 33.77**  | 25.79**  |      |        |      |

1. P-Values indicate the level of significance including high-intensity ultrasound power, treatment time and their interaction.
2. SE: standard error.
3. a-d in the same row indicate a significant difference between ultrasound power (P < 0.05).
4. x-z in the same column indicate significant difference between treatment time (P < 0.05).

Compared with the ST group, the change in springiness was on an upward trend when treated by UAT treatment, especially in UAT-100 W and UAT-300 W for 60 min. However, the long treatment time (120 min) combined with strength UAT treatment (300–700 W) declined the springiness. In addition, the trends of cohesiveness and chewiness coincided well with the springiness. In short, compared with the ST group, the mild UAT treatment (100–150 W) could significantly increase the cohesiveness and chewiness (P < 0.05). In comparison, the excessive UAT treatment (700 W) treated meat samples for a long time (120 min) could obviously decrease those indexes (P < 0.05). This phenomenon might be due to the increased content of myofibrillar proteins under moderate UAT treatment ensured enough formation of protein-protein interaction which could improve the texture of protein gel [37] and the protein denaturation under strength UAT treatment might deteriorate the protein gel [38], thus decreasing the springiness, cohesiveness and chewiness.

4. Conclusion

In this study, the gradual enhancement of NaCl content and the NaCl diffusion coefficient after UAT treatment implied that UAT treatment could accelerate the NaCl diffusion into pork. Furthermore, the images of SEM-EDX identified that compared with ST treatment, UAT treatment was able to facilitate the NaCl to enter into the meat and distribute uniformly. The results of SEM explained the advantage of UAT treatment that moderate ultrasonic cavitation (300 W) could destroy the meat fibers and enlarge the interfilament spaces which could uptake more brine, thus significantly increasing the tumbling yield. However, the SEM images showed that excessive UAT treatment could cause the formation of granular aggrsse. In addition, the results of protein content in the brine indicated that moderate UAT treatment could improve the extraction of protein which further resulted in the improvement of WHC and texture profile. Therefore, UAT treatment could enhance the curing efficiency and improve the WHC and texture profile under moderate US conditions.

CRediT authorship contribution statement

Ruyu Zhang: Conceptualization, Data curation, Methodology, Software, Formal analysis, Writing – original draft, Writing – review & editing, Investigation. Jian Zhang: Data curation; Methodology. Lei Zhou: Writing – review & editing, Data curation; Methodology. Lin Wang: Data curation; Methodology. Wangang Zhang: Supervision, Resources.

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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