Application of temperature-controlled ultrasound treatment and its potential to reduce phosphate content in frankfurter-type sausages by 50%

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ABSTRACT

The objective of this study was to evaluate the effect of ultrasound treatments with different durations (15, 20, 25, 30, and 35 min) at a low static temperature (12 °C) controlled by an intelligent temperature control and monitoring system on the quality of 50% reduced-phosphate frankfurters. The results show that without ultrasound treatment, phosphate reduction caused some obvious deficits in the textural properties, sensorial parameters, and oxidative stability of frankfurters. Moreover, 25-min ultrasound treatment could significantly lower the cooking loss and enhance emulsion stability, textural properties, and sensorial parameters of reduced phosphate frankfurters, which was also verified by dynamic water distribution analysis and microstructural observation. Additionally, low constant temperature during ultrasound treatment was another crucial factor in retarding lipid oxidation during storage. Therefore, ultrasound treatment with moderate duration and stable low temperature could be considered a successful approach to obtain healthier reduced-phosphate frankfurters under the “clean label” concept.

1. Introduction

Emulsified meat products (such as frankfurter sausage, bologna sausage, vienna sausage, luncheon meats, etc.) are widely consumed in many countries throughout the world [1]. Their popularity is mainly attributed to their unique flavor and taste, convenience, and economic aspects [2]. During the processing of emulsified meat products, diverse additives (either natural or synthetic) are commonly applied to promote textural or sensorial properties and product shelf-life [3]. Phosphates are crucial synthetic food additives and are widely used in the production of emulsified meat products due to their unique beneficial features, such as water holding capacity, emulsifying properties, metal chelating activity, and antibacterial activity [4]. With the aid of phosphates, emulsified meat products show higher cooking yield and improved textural qualities (such as tenderness and juiciness) [5]. In general, the maximum amount of phosphates allowed in meat products is no>5000 mg/kg according to food additive legislation in many countries [6]. However, Pinton et al. [7] reported that the actual daily intake of phosphates per person in many countries significantly exceeded the acceptable daily intake recommended by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). High intake of phosphate is closely associated with enhanced risk of cardiovascular disease mortality, especially for the patients with chronic kidney disease [8]. Block et al. [9] also reported that excessive phosphate intake could potentially result in an increased risk of bone fractures by impacting the balance of calcium, iron, and magnesium in the human body. Thus, it is essential for the meat industry to develop some novel strategies to reduce the phosphate content in emulsified meat products.

More recently, some various functional ingredients, such as rice starch [10], sodium or potassium bicarbonates [11], citrus fiber [12], chia mucilage powder [13], winter mushroom powder [3] and alkaline electrolyzed water [14] have been applied to replace phosphates in processed meat products. However, a crucial issue among these functional ingredients was that they could not completely replace phosphates because of negative effects on appearance, textural properties and sensory characteristics of the final products [4]. Moreover, in the last decade, with increasing public awareness of health and sustainability, a new trend of “clean labelling”, representing shorter ingredient declarations, has been proposed [15]. This trend encompasses the reduction or elimination of phosphates in meat products without introducing other additional ingredients that are unfamiliar to consumers. However, simply reducing or removing phosphates, while...
maintaining the quality of emulsified meat products, is extremely challenging for the meat industry. Furthermore, in order to successfully achieve this goal, some advanced processing technologies with high commercial potential should be taken into consideration.

Ultrasound is considered an emerging and innovative technology with outstanding benefits in promoting the enhancement of meat and meat product quality parameters, such as meat tenderness [16], brining or curing acceleration [17], microorganism or enzyme inactivation [18], and freezing rate acceleration [19]. Moreover, some studies also reported that ultrasound could significantly promote the release of myofibrillar proteins, as well as notably increase the solubility of myofibrillar proteins [20], thereby effectively promoting the water holding capacity and textural properties of emulsified meat products [21]. However, acoustic cavitation generated by ultrasound can gradually elevate the temperature of the liquid medium [22]. Increased temperature during ultrasound treatment could impact the stability of meat batters, and subsequently affect the cooking yield and textural properties of emulsified meat products [23]. As some previous studies indicated, ice was used to maintain the ultrasound water bath temperature when ultrasound processing was applied. Although this method countered heat accumulation, it was not precise enough to maintain a fixed temperature throughout the entirety of the ultrasound application. To rectify this issue, we have customized a temperature-controlled ultrasound experimental apparatus by using a refrigeration unit to precisely maintain the water temperature of the ultrasound bath. Furthermore, no information is available on the systematic application of temperature-controlled ultrasound in order to reduce the phosphate content of emulsified meat products. Therefore, the objective of the present study was to investigate the potential use of temperature-controlled ultrasound on the quality parameters (cooking loss, emulsion stability, color, textural properties, water distribution, etc.) and sensory properties of the frankfurter-type sausages with reduced phosphate content.

2. Material and methods

2.1. Materials and chemicals

Post-rigor lean pork meat and pork back-fat were purchased from Gaojin Meat Corporation (Harbin, Heilongjiang, China). The samples were kept on ice while transported to the laboratory and processed on the same day. Collagen casing (20 mm in diameter) was provided from the total weight of lean meat, back-fat, and ice). Before the production of meat batters, all visible connective tissue was trimmed from the meat, and then the pork lean meat and pork back-fat were ground through 8-mm and 3-mm plates in a mincer, respectively (SZ-12A, Xuzhong Food Machinery Manufacturing Co., Ltd., Guangzhou, Guangdong, China). After that, the chopping process commenced according to the procedure described by Choi et al. [24], and the temperatures of the meat batters were always kept below 12 °C. After chopping, the meat batters were immediately stuffed into collagen casings. The frankfurters were hand linked at 18 cm intervals. The control group (Tc) and the 50% reduced phosphate group (Tr) were formulated with 0.40% and 0.20% phosphate content, respectively.

2.2. Application of ultrasound

A schematic diagram of the temperature-controlled ultrasound experimental apparatus is shown Fig. 1. The size of the ultrasound bath tank was 30 × 22 × 26 cm (W × D × H). The tank was filled with water, and the temperature of the water was monitored by a temperature testing instrument (AT4500, Applent Precision Instrument Co., Ltd., Changzhou, Jiangsu, China), and controlled via an intelligent temperature control system. During the entire ultrasound treatment, a refrigeration unit was used to maintain the temperature of the water at 12 °C under the command of the temperature control system.

Frankfurters of the Tr group were sealed in plastic zipper bags (27 cm × 28 cm, Miaojie Daily Necessities Co., Ltd., Jiaxing, Zhejiang, China) and placed into the US bath, and sonicated for 15, 20, 25, 30, and 35 min, respectively. The ultrasound parameters were fixed at constant frequency of 25 kHz and a power of 240 W with 10 sec on/10 sec off cycles. The five groups under different ultrasound times were named as Tr-15, Tr-20, Tr-25, Tr-30, and Tr-35, respectively.

2.2.2. Cooking treatment

After ultrasound treatment, the frankfurters were transferred to an automatic smoking chamber (BYXX-50, Expo Meat Processing Machinery Manufacturing Co., Ltd., Jiaxing, Zhejiang, China) with the following treatment: drying at 45 °C for 20 min, smoking at 60 °C for 30 min, and cooking until the frankfurker core temperatures reached 72 °C (monitored by thermocouples). After that, the frankfurters were quickly immersed in ice water until the temperature was below 20 °C. The frankfurters were vacuum packed and stored at 4 °C in the refrigerator until sensory and instrumental evaluations were carried out.

2.3. Raman spectroscopic determination

Raman analysis of each frankfurter was measured using the same procedure and testing parameters of Kang et al. [25]. The determination was conducted via a Raman spectrometer (HR8000, Horiba/Jobin, Yvon, Longjumeau, France). Before measurement, the sliced frankfurters were carefully spread on glass slides. Raman spectra were obtained in the range of 400 to 3600 cm⁻¹. The essential parameters of Raman spectrometer were setting as follows: 100 mW of laser power, 2 cm⁻¹ resolution, 10 s exposure time, and 10 scans. After that, the spectral data obtained from the scans of each sample was smoothed and baseline using Labspec software, and its intensity was normalized against the phenylalanine peak near 1001 cm⁻¹. The relative amounts of different secondary structures of each sample were determined from amide I spectra by computing the areas under the bands. The levels of secondary structures of each sample were expressed as percentages of α-helix, β-sheet, β-turn, and random coil and were calculated using the method of Alix et al. [26].

2.4. Water distribution

The water distributions within frankfurters were determined according to the method of Han et al. [27] by using a low field nuclear magnetic resonance (LF-NMR) analyzer (PQ001, Shanghai Niumag Electronic Technology Co., Ltd, Shanghai, China) with a magnetic field strength of 0.47 T and a proton resonance frequency of 22 MHz. The spin–spin relaxation time (T2) was measured via the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with an interpulse spacing τ-value (time between 90° pulse and 180° pulse) of 80 μs and 16 scans obtained at a 3500 ms interval with 5000 echoes in each sample. After that, T2 was analyzed using MultiExp InV Analysis software. The relaxation components were expressed as T2a, T2b, and T2c, and their related
area fractions were expressed as \( P_{20}, P_{21} \) and \( P_{22} \), respectively.

### 2.5. Cooking loss

The cooking loss was measured and calculated according to the method of Tahmasebi et al. [28]. Briefly, after refrigerated storage at 4 °C overnight, the cooked frankfurters were weighed, and the cooking loss (%) was calculated as a percentage from the known weights of frankfurters before cooking.

### 2.6. Emulsion stability

The emulsion stability, expressed as total released liquid, released fat and released water, was determined and calculated according to the procedure of Bolger et al. [29] with some modifications. Briefly, approximately 35 g of each meat batter sample was placed and weighed in plastic centrifuge tubes and then centrifuged at 3,500 \( \times \) g for 5 min under 4 °C. After that, each tube was placed in a water bath at 80 °C for 30 min and then cooled to room temperature (20–22 °C). Subsequently, each tube was left to stand upside-down for 1–2 h to release the exudates to the pre-weighed aluminum dish. Total released liquid (%) was calculated as the difference value between the weight of the aluminum dish after draining of the sample and the weight of the empty aluminum dish expressed as a percentage of the initial uncooked meat batter weight. Released water (%) was calculated as the difference value between the weight of the aluminum dish before and after drying in an oven at 105 °C for 16 h expressed as a percentage of the initial uncooked meat batter weight. Released fat (%) was calculated as the difference value between the weight of the aluminum dish after drying in an oven at 105 °C for 16 h and the weight of the empty aluminum dish expressed as a percentage of the initial uncooked meat batter weight.

### 2.7. Determination of pH

Briefly, 10.0 g of each frankfurter was homogenized with 100.0 mL distilled water. A pH meter (FE20, Mettler Toledo Instruments Co., Ltd., Shanghai, China) was used to determine the homogenate at room temperature (20–22 °C).

### 2.8. Color measurement

The color of frankfurters was determined by using our previous reported method [2] via a ZE-6000 colorimeter (Nippon Denshoku, Kogyo Co., Ltd., Tokyo, Japan). Where the \( L^* \)-value, \( a^* \)-value and \( b^* \)-value represent lightness, redness/greenness and yellowness/blueness, respectively. This instrument was equipped with a D65 light source, a 10° observer with an 8 mm diameter measuring area, and a 50 mm diameter illumination area. A white standard plate \( (L^* = 95.26, a^* = -0.89, b^* = 1.18) \) was used for calibration.

### 2.9. Texture profile analysis (TPA)

Before determination, all frankfurters were equalized to room temperature (20–22 °C) for at least one hour. TPA was measured using a TA-XT plusC texture analyzer (Stable Micro Systems Co., Ltd., Godalming, UK) with a P/2 cylindrical probe (2.0 mm diameter). The testing parameters were as follows: test mode was Hold & Penetration, pre-test speed was 1.50 mm/s, test speed was 1.5 mm/s, post-test speed was 10.0 mm/s, and trigger force was 10.0 g. Additionally, according to the device instructions, the testing procedure was divided into two consecutive cycles as follows: (1) the 1st cycle should not break into the surface of the frankfurters at 15.0% strain, which mainly reflects the hardness (g), springiness (%) and resilience (g); (2) the 2nd cycle should puncture into the interior of the frankfurters at 75.0% strain, which mainly reflected the chewiness (g.sec), fracturability (g) and tightness (g.sec). The holding time between two cycles was 5 s.

### 2.10. Scanning electron microscopy (SEM)

Before SEM observation, the samples were prepared according to the method of Wu et al. [30] with some modifications. Briefly, frankfurters were cut into cubic samples (about 2 \( \times \) 2 \( \times \) 2 mm) and fixed in 0.1 M phosphate buffer solution (pH 6.8) which including 2.5% glutaraldehyde (v:v) for at least 24 h at 4 °C. Then, the fixed samples were washed three times by using 0.1 M phosphate buffer solution (pH 6.8) for 10 min, and post-fixed with 1% osmium tetroxide for 1 h and washed. After that, all the samples were dehydrated with a gradually increasing concentration of alcohol (50%, 70%, and 90%) for 10 min for each step, and then transferred to the mixed solution of ethyl alcohol and tertiary butanol (v:v = 1:1) for 15 min, and following transferred to pure tertiary butanol for 15 min. Subsequently, all the samples were freeze-dried, and sputter-coated with 10 nm of gold/palladium. Finally, the microstructures of frankfurters were observed by SEM (S-3400 N, Hitachi, Co., Ltd., Tokyo, Japan) with scanned images magnified to 2000 times.

### 2.11. Sensory evaluation

The sensory evaluation of frankfurters was carried out through a
sixteen-member sensory panel (consisting of 8 females and 8 males) in a sensory laboratory. All the panelists were trained through three preliminary sessions for sample familiarization by an expert in the meat science laboratory in the Northeast Agricultural University. Each frankfurter was evaluated by sensory descriptive analysis according to the following parameters with a 7-point descriptive scale: for interior color of frankfurters (1 = yellowness; 7 = pinkness), for degree of uniformity of frankfurters (1 = low; 7 = high), for flavor intensity of frankfurters (1 = less intense, 7 = most intense), for juiciness of frankfurters (1 = extremely dry; 7 = extremely juicy). Furthermore, the overall acceptability (1 = low; 7 = high) of each frankfurter was also provided by the panelists. Before sensory evaluation, some preparatory work was conducted according to the same procedure of Chen et al. [2].

2.12. Lipid oxidation

The lipid oxidation of frankfurters during 21 days of storage at 4 °C, expressed as the value of thiobarbituric acid reactive substances (TBARS), was determined according to the method described by Jia et al. [31] with some modifications. Briefly, approximate 5.0 g frankfurters samples were transferred to 15 mL of 7.5% trichloroacetic acid which containing 0.10% propyl gallate and 0.10% ethylene diamine tetraacetic acid, and followed by homogenization using a homogenizer (IKA, T18 digital Ultra-turrax, Germany) for 1 min at 11,000 rpm. Then, each of the homogenate was filtered via filter paper, and then 2.5 mL of each filtrate was mixed with 2.5 mL of 20 mM thiobarbituric acid in a glass test tube with stopper and vortexed via a vortex mixer (3030A, Scientific Industries, INC., Bohemia). After that, all the glass test tubes are frozen at −30°C and stored at −70°C until analysis. The TBARS level was determined using a spectrophotometer (Shimadzu, UV-1800, Japan) within 1 day of the analysis. The absorbance of each sample was measured at 532 nm, and the TBARS values were calculated using a standard curve of 1,1,3,3-tetraethoxypropane (TEP).
were placed in a rack which located in a covered water bath at 100 °C for 50 min, and then cooled to room temperature (20–22 °C). Subsequently, the absorbance of the mixture was read at 532 nm. The TBARS value was calculated as mg of malonaldehyde (MDA) per kg for each sample as follows:

\[ TBARS (\text{mg/kg}) = \frac{A \times V \times M}{a 	imes L \times m} \]

where \( A \) was the absorbance at 532 nm, \( V \) was the volume of sample (mL), \( M \) was the molecular weight of malonaldehyde (72.063 g/mol), \( \varepsilon \) was the molar extinction coefficient (156,000 M\(^{-1}\)cm\(^{-1}\)), \( L \) was the length of the optical path (1 cm in our present test), \( m \) was the weight of the sample (kg).

2.13. Statistical analysis

Three independent batches of frankfurters (replicas) were prepared. For each batch of frankfurters, measurements of related traits were carried out in triplicate. All data were expressed as the mean ± standard deviations (SD) and were analyzed using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St Paul, MN, USA). One-way analysis of variance (ANOVA) with Tukey’s multiple comparison procedure was performed to evaluate the significance of the main effects (\( P < 0.05 \)). Hierarchical cluster analysis (HCA) was performed among all characteristics (cooking loss, emulsion stability, pH, color, textural properties, sensory evaluation) of frankfurters treated via R software (version 3.6.3, Tsinghua University, Beijing, China) to identify similarities and differences among different groups.

3. Results and discussion

3.1. Protein secondary structures

The Raman spectra of frankfurters from each group were measured in the frequency range from 800 to 1800 cm\(^{-1}\). As depicted in Fig. 2A, the bond around 1650 cm\(^{-1}\) is considered the amide I vibrational mode, which mainly includes C = O stretching and to a lesser extent N–H in-plane bending of peptide groups [32]. Alix et al. [26] indicated that the changes in relative intensity of this band mainly reflected the \( \alpha \)-helix content of meat proteins. Moreover, two additional major bands near 1530 cm\(^{-1}\) and 1150 cm\(^{-1}\) respectively included C=N stretching of the trans peptide group of the amide II vibration and N–H in-plane bending, and the C=N stretching vibration of protein. The changes in relative intensities of these two features mainly reflects \( \beta \)-sheet formations of meat proteins during the heating treatment [33]. Meanwhile, both the C–H and C–H\(_2\) bending vibrations at about 1450 cm\(^{-1}\), and the CH stretching region from 2800 to 3100 cm\(^{-1}\) as seen in Fig. 2B, mainly represents changes to aliphatic and aromatic residues, and increased relative intensity commonly implies the burial of those residues [34].

The relative percentages of protein secondary structures of each frankfurter group, expressed as \( \alpha \)-helix, \( \beta \)-sheet, \( \beta \)-turn and random coil contents, are shown in Table 1. Compared with the Tc group, the Tr group showed a slightly lower \( \alpha \)-helix content and higher \( \beta \)-sheet, \( \beta \)-turn and random coil contents. This indicates that a different phosphate level produced no obvious differences in protein secondary structures. Moreover, with the increasing ultrasound treatment time, \( \alpha \)-helix content decreased from 54.63% (0 min) to 41.83% (25 min), and then increased to 50.97% (35 min), respectively. Meanwhile, \( \beta \)-sheet, \( \beta \)-turn and random coil contents increased from 19.90%, 15.28%, and 10.30% (0 min) to 29.71%, 17.29%, and 11.08% (25 min), and then declined to 22.70%, 15.86%, and 10.53% (35 min), respectively. According to Zhang et al. [35], the decline of \( \alpha \)-helix content was mainly induced by the unfolding of myofibrillar protein molecular structures, such as sulfhydryl and hydrophobic groups, whereas, the increase of \( \beta \)-sheet content was due to the aggregation of myofibrillar protein closely associated with the formation of the 3-D gel network. Wang et al. [36] suggested that moderate ultrasound treatment (3–15 min) could significantly decrease the \( \alpha \)-helix content and increase the \( \beta \)-turn content of chicken myofibrillar protein gels after the heating process. Alarcon-Rojo et al. [22] also reviewed that ultrasound treatment could effectively lead to fewer \( \alpha \)-helices and more folded \( \beta \)-sheets in muscle protein gels, subsequently enhancing gel strength, water or fat holding capacities and elasticity-viscosity. In addition, as suggested by Zhao et al. [37], the \( \beta \)-sheet mainly depended on the internal hydrogen bonds between peptide chains in myofibrillar protein gels. However, with longer ultrasound treatment time, hydrogen bonds might be partially ruptured and possibly result in the disruption of \( \beta \)-sheets [36].

3.2. LF-NMR relaxation analysis

LF-NMR, a non-destructive testing technology, is commonly used to determine the water distribution in meat products. It also provides some useful information about the interactions between meat protein and water within a gel matrix [38]. Zhang et al. [39] also stated that LF-NMR analysis could more clearly illustrate the changes of water mobility in meat products than the conventional centrifugal method to determine the water holding capacity. After data analysis by using a multi-exponential fitting, the spin–spin \( \left(T_2\right) \) relaxation time was separated into three components \( T_{2b}, T_{21}, \) and \( T_{22} \). \( T_{21} \) (0–10 ms) represents the water tightly bound to macromolecules. \( T_{22} \) (10–100 ms) reflects immobilized water considered to be the dominating water component among muscle fibers. \( T_{2b} \) (100–1000 ms) corresponds to free water considered to be extra-myofibrillar water. As exhibited in Table 2, although there were no obvious differences or regularities to the changes in \( T_{2b}, T_{21}, \) and \( T_{22} \) among all the groups, the corresponding relaxation peak area proportions \( (P_{2b}, P_{21}, \) and \( P_{22} \)) of each relaxation time obviously changed. For example, compared with the Tc group, the Tr group showed a slightly higher \( P_{22} \), but had basically the same in \( P_{2b} \) and \( P_{21} \), indicating that more free water was released due to the reduction of phosphates in the frankfurters. Moreover, with increasing ultrasound treatment time, \( P_{2b} \) and \( P_{21} \) increased from 0.57 and 89.44 (15 min) to 1.06 and 93.54 (25 min), and then declined to 0.67 and 91.27 (35 min), respectively. Meanwhile, the \( P_{22} \) decreased from 9.12 (15 min) to 4.73 (25 min), and then increased to 7.29 (35 min), respectively. Our results imply that ultrasound treatment for 25 min could effectively increase the proportion of immobilized water and decrease the proportion of free water in frankfurters. Alarcon-Rojo et al. [22] also summarized that ultrasound treatment duration was a crucial factor in influencing the water mobility or water holding capacity of meat products, and that too long of a sonication time would generate a negative effect. Additionally, Stadnik et al. [40] indicated that the extent of structural changes to myofibrillar proteins induced by ultrasound treatment could lead to

### Table 1

| Treatments | \( \alpha \)-helix (%) | \( \beta \)-sheet (%) | \( \beta \)-turn (%) | Random coil (%) |
|------------|----------------------|----------------------|-------------------|----------------|
| Tc         | 56.45 ± 3.15\(^a\)   | 18.50 ± 2.41\(^c\)   | 15.01 ± 0.49\(^e\) | 10.19 ± 0.19\(^f\) |
| Tr         | 54.63 ± 3.15\(^b\)   | 19.90 ± 2.41\(^c\)   | 15.28 ± 0.49\(^b\) | 10.30 ± 0.19\(^b\) |
| Tr-15      | 49.14 ± 3.17\(^a\)   | 24.11 ± 2.43\(^b\)   | 16.14 ± 0.50\(^b\) | 10.64 ± 0.20\(^b\) |
| Tr-20      | 47.31 ± 3.17\(^b\)   | 25.51 ± 2.43\(^b\)   | 16.43 ± 0.50\(^b\) | 10.75 ± 0.33\(^b\) |
| Tr-25      | 41.83 ± 3.46\(^b\)   | 29.71 ± 4.19\(^b\)   | 17.29 ± 0.86\(^b\) | 11.08 ± 0.34\(^b\) |
| Tr-30      | 45.48 ± 3.46\(^b\)   | 26.91 ± 4.19\(^b\)   | 16.72 ± 0.86\(^b\) | 10.86 ± 0.19\(^b\) |
| Tr-35      | 50.97 ± 3.46\(^b\)   | 22.70 ± 4.19\(^b\)   | 15.86 ± 0.50\(^b\) | 10.53 ± 0.20\(^b\) |

Values are given as means ± SD from triplicate determinations. Different letters (a-c) in the same column indicate significant differences \( (P < 0.05) \).
positive or negative effects on the dynamics of water in meat products. Zhang et al. [38] found that more immobilized water (indicated as increased P22) was trapped in the myofibrillar protein gels when a moderate intensity of ultrasound was applied, meanwhile more free water (indicated as decreased P22) was attracted by the negative charges. Amiri et al. [41] also pointed out that smaller cavities or pores of homogenous meat protein gels induced by moderate duration ultrasound treatment could efficiently and firmly trap water molecules, and then lower the mobility of immobilized water (expressed as higher P22).

3.3. Cooking loss and emulsion stability

Cooking loss is an important indicator closely associated with the water or fat holding capacities of frankfurters during heating treatment. As shown in Table 3, the Tr group shows much higher cooking loss than the Tc group (P < 0.05), which indicates that more water or fat was released from the meat protein matrix when the phosphate addition amount was reduced. Resconi et al. [42] suggested that the addition of phosphates in emulsified meat products not only effectively increased the pH value and ionic strength of muscle, but also promoted better extraction and solubilization of myofibrillar proteins during chopping or blending, ultimately facilitating the formation of stronger meat protein gel networks with lower cooking loss during heat treatment. Moreover, except for the Tr-15 group, the cooking loss of samples subjected to ultrasound application exhibited lower cooking loss when compared with the Tr group (P < 0.05). However, the Tr-25 group not only showed the lowest cooking loss among all ultrasound treated groups (P < 0.05), but also showed no obvious difference compared to the Tc group (P > 0.05). Cooking loss declined 2.17%, 16.54%, 38.06%, 13.34%, and 12.31% compared to the Tr group without ultrasound treatment when the ultrasound treatment time increased from 15 min to 35 min, respectively. Thangavelu et al. [4] indicated that the ultrasound treatment could considerably promote the water and oil holding capacities of meat proteins, which was beneficial to the quality of processed meat products. Pinton et al. [7] also pointed out that the ultrasound treatment could lead to some structural modifications of myofibrillar proteins, which may have promoted the interaction between the polar/nonpolar groups and water/fat, respectively. And our above results as regards to the changes of protein secondary structures can strongly confirm their statement. Meanwhile, as reported by Inguglia et al. [43], wave sonication or wave propagation generated via ultrasound treatment might efficiently improve the diffusion of phosphates in meat batter, which may significantly promote higher solubility of myofibrillar proteins. Additionally, Zhang et al. [39] suggested that moderate ultrasound treatment could contribute to the formation of finer, denser, and more homogeneous myofibrillar protein gels that could retain water or fat more firmly. However, longer durations of ultrasound treatments probably caused denaturation of myofibrillar proteins. McDonnell et al. [44] declared that a stronger microjet effect caused by ultrasonic cavitation possibly induced irreversible denaturation of myosin, which mainly led to less myosin participating in the formation of a gel network and subsequently decreased the water or fat holding capacity.

Emulsion stability, which is expressed as total released liquid, released fat and released water, is an important indicator for evaluating the quality of frankfurters. As shown in Table 3, compared with the Tc group, the Tr group shows significantly higher total released liquid, released fat and released water (P < 0.05), which implies that reduced phosphate addition led to lower emulsion stability of frankfurters. The synergistic effect of salt and phosphates could notably promote the solubility of salt-soluble myofibrillar protein, and subsequently enhance the water holding ability of meat products [45]. Glioreux et al. [46] noted that phosphates could effectively dissociate the complex of actomyosin, as well as efficiently chelate protein-bound Mg$^{2+}$ and Ca$^{2+}$ between meat proteins, which subsequently resulted in a certain degree of the release of myosin. Thus, more proteins could be extracted and exert their emulsifying capacities to the greatest extent, which was beneficial to promote the stability of meat emulsion systems. Moreover, the changes of emulsion stability among all the ultrasound treated groups might be due to the speed of unfolding of myofibrillar proteins participated in the formation of interfacial layer and increased the emulsifying efficiency and stability. Cichoski et al. [23] also indicated that ultrasound treatment could significantly decrease the size of fat globules, which subsequently promote the emulsion stability of meat batters. Additionally, declining emulsion stability in the Tr-30 and Tr-35 groups might be due to the speed of unfolding of myofibrillar proteins was faster than aggregation after longer durations of ultrasound treatment [39], and subsequently leading to a coarser and more nonhomogeneous microstructures of frankfurters with lower emulsion stability.

### Table 2

| Treatments | T2b (ms) | T12 (ms) | P2b (%) | P21 (%) | P22 (%) |
|------------|----------|----------|---------|---------|---------|
| Tc         | 4.72     | 52.07    | 242.54  | 0.52    | 90.78   |
|            | ±0.10ab  | ±4.68ab  | ±19.99ab| ±0.73ab | ±4.21ab |
| Tr         | 4.67     | 57.60    | 266.60  | 0.68    | 90.55   |
|            | ±0.30ab  | ±0.66ab  | ±1.73ab | ±1.05ab | ±0.58ab |
| Tr-15      | 5.60     | 56.88    | 264.94  | 0.57    | 89.44   |
|            | ±0.65ab  | ±0.58ab  | ±1.15ab | ±0.96ab | ±0.73ab |
| Tr-20      | 6.47     | 57.53    | 254.07  | 0.72    | 90.10   |
|            | ±0.56ab  | ±0.58ab  | ±1.99ab | ±0.55ab | ±0.44ab |
| Tr-25      | 6.02     | 49.58    | 242.57  | 1.06    | 93.54   |
|            | ±0.31b   | ±0.31b   | ±1.94b  | ±1.54b  | ±1.52b  |
| Tr-30      | 5.86     | 54.55    | 278.86  | 0.59    | 92.66   |
|            | ±0.46ab  | ±0.46ab  | ±2.26ab | ±0.41ab | ±0.59ab |
| Tr-35      | 5.47     | 57.40    | 292.12  | 0.67    | 91.27   |
|            | ±0.31b   | ±0.31b   | ±2.26ab | ±0.72ab | ±0.59ab |
|            | 1.51b    | 0.99b    |         |         |         |

Values are given as means ± SD from triplicate determinations. Different letters (a-e) in the same column indicate significant differences (P < 0.05).

### Table 3

| Treatments | Cooking loss (%) | Emulsion stability |
|------------|------------------|--------------------|
|            | Total released liquid (%) | Released water (%) | Released fat (%) |
| Tc         | 6.21 ± 0.28d     | 5.24 ± 0.38d      | 4.86 ± 0.37d     | 0.38 ± 0.01d     |
| Tr         | 9.67 ± 0.03b     | 8.01 ± 0.55b      | 7.41 ± 0.51b     | 0.58 ± 0.04b     |
| Tr-15      | 9.46 ± 0.49b     | 7.90 ± 0.51b      | 7.33 ± 0.48b     | 0.56 ± 0.03b     |
| Tr-20      | 8.07 ± 0.22b     | 6.42 ± 0.72b      | 5.95 ± 0.67b     | 0.46 ± 0.05b     |
| Tr-25      | 5.99 ± 0.77b     | 5.56 ± 0.56ed     | 5.16 ± 0.52ed    | 0.39 ± 0.04ed    |
| Tr-30      | 8.38 ± 0.63b     | 7.23 ± 0.64ab     | 6.72 ± 0.57ab    | 0.45 ± 0.01ab    |
| Tr-35      | 8.48 ± 0.69b     | 7.29 ± 0.17ab     | 6.84 ± 0.17ab    | 0.50 ± 0.06ab    |

Values are given as means ± SD from triplicate determinations. Different letters (a-d) in the same column indicate significant differences (P < 0.05).
excepted, addition of 50% reduced amount of phosphates (Tr group) caused an obviously decrease in pH value when compared with Tc group ($P < 0.05$) (Fig. 3). However, all the ultrasound treated samples had higher pH values than Tr group ($P < 0.05$), which probably associated with protein denaturation. Alarcon-Rojo et al. [22] noted that the changes of protein structures or release of ions from the cellular structure induced by ultrasound treatment could lead to significant changes in the position of ionic functionalities which could effectively alter the pH values of meat. Moreover, with the increasing ultrasound treatment time, the pH values of the frankfurters gradually increased and then decreased, and the Tr-25 group had the highest pH value among all of the ultrasound treated samples ($P < 0.05$). Amiri et al. [41] indicated that moderate ultrasound treatment could efficiently promote the formation of free radicals, which probably reacted with the side chains of myofibrillar proteins, thus decreasing the number of acidic groups and increasing the pH values. Furthermore, the decreased pH values of the Tr-30 and Tr-35 groups compared to the Tr-25 group might be due to the stronger acoustic cavitation effect induced by longer ultrasound treatment possibly decreasing the net charges among the meat proteins and subsequently driving the pH towards the isoelectric points of meat proteins. Additionally, Pinton et al. [7] found that changes of pH values were positively associated with the cooking loss and emulsion stability of cooked meat batters, which strongly supported our results above.

3.5. Color determination

Color is a significant factor in determining the quality of frankfurters and subsequently influences the organoleptic evaluation of the consumer. The color parameters ($L^*$-value, $a^*$-value and $b^*$-value) of each frankfurter group are shown in Table 4. Compared with the Tc group, the Tr group showed lower $L^*$-value and higher $b^*$-value ($P < 0.05$), as well as slightly lower $a^*$-value (no obvious difference was detected) ($P > 0.05$). In the study of Choe et al. [3], they indicated that higher cooking loss in reduced-phosphate emulsion-type sausages commonly led to lower $L^*$-values and higher $b^*$-values, which was mainly attributed to weaker water or fat holding capacities. Moreover, when compared with the Tr group, the $L^*$-values of ultrasound treated samples were slightly enhanced and then followed by an obvious decline when the ultrasonic time increased from 15 to 35 min. Meanwhile, the Tr-25 group had the highest $L^*$-value among all the ultrasound treated samples ($P < 0.05$), which was positively related to enhanced emulsion stability. Furthermore, except for the Tr-35 group, the $a^*$-values of all the other ultrasound treated samples showed no obvious differences between the Tr group ($P > 0.05$). And the higher $a^*$-value of the Tr-35 group was mainly attributed to the longer ultrasonic time. de Lima Alves et al. [47] suggested that ultrasound had no obvious effect on heme pigments or metmyoglobin in some pork-meat products, but longer ultrasound time might exhibit a positive effect on redness ($a^*$-value). In addition, the $b^*$-values of all the ultrasound treated samples were lower than that of the Tr group ($P < 0.05$). However, not only no obvious differences of $b^*$-values were detected among all the ultrasound treated samples ($P > 0.05$), but also almost the similar with the $b^*$-value of the Tc group ($P > 0.05$). Peña-Gonzalez et al. [48] also suggested that no obvious differences of yellowness ($b^*$-value) were found between cooked meat or meat products with or without ultrasonicated treatment. Overall, the above results imply that the 25 min ultrasound treatment had a positive effect on the color parameters of reduced-phosphate frankfurters.

![Fig. 3. pH values of frankfurters with different phosphate levels and ultrasound treatment under different durations. Different letters (a-c) indicate significant differences ($P < 0.05$).](image-url)
3.6. Tpa

As shown in Table 5, frankfurters with 50% phosphate reduction (Tr group) have greater hardness and chewiness, as well as lower tightness, springiness, fracturability and resilience than the Tc group (P < 0.05). However, in contrast with our results, some previous studies reported that the hardness of sausage significantly decreased with a reduction of phosphates [3,7,12]. In one aspect, our contrary results might be associated with different frankfurter formulations. In another aspect, G流氓 et al. [46] suggested that higher cooking loss would also induce greater hardness and chewiness of emulsified meat products. Moreover, with the increasing ultrasound treatment time, the hardness and chewiness of the frankfurters initially decreased and then increased, and the Tr-25 group had the lowest hardness and chewiness among all of the ultrasound treated samples (P < 0.05). These results are mainly attributed to the lower cooking loss and higher water or fat binding capacities of the samples when subjected to 25 min ultrasound treatment. In contrast, with increasing ultrasound treatment time, the tightness, springiness, fracturability and resilience of frankfurters initially increased and then declined, and the Tr-25 group had the highest of the above textural parameters among all the ultrasound treated samples (P < 0.05). In addition, the Tr-25 group had higher tightness than the control group (P < 0.05), while no obvious differences in springiness and resilience were detected between the Tr-25 group and the control group (P > 0.05). As reported by Cichoski et al. [23], the springiness, resilience and tightness are crucial factors affecting the quality of emulsified meat products, since they are also closely associated with slice-ability. Zhao et al. [37] indicated that ultrasound treatments could generate higher structural densities of pre-emulsified meat batters, as well as reduce the size of fat droplets, facilitating extracted myofibrillar proteins to coat the fat droplets and enhance the gelling properties of the meat protein gel networks. Saleem and Ahmad [20] also declared that the cavitation effect of ultrasound could lead to the structural changes of myofibrillar proteins (mainly for myosin), thereby efficiently increasing the gelation of myofibrillar proteins and promoting and modifying the gelling properties of the 3-dimensional gel network. Thus, ultrasound treatment with appropriate treatment time is a potentially promising technology that can modify the textural properties of reduced-phosphates frankfurters.

3.7. Microstructure observation

In order to better assess the changes induced within the meat protein 3-D gel network by the reduction of phosphates and ultrasound treatment, the microstructures of frankfurters were observed using SEM. As shown in Fig. 4, compared with the Tc group, the Tr group shows a less compact and less uniform meat protein gel network, as well as some larger water channels or porous cavities throughout the gel matrix (which are clearly labeled by the white arrows in Figs. A and B). Huang et al. [49] suggested that the addition of higher levels of phosphates could obviously decrease the pore diameter within the myofibrillar protein gel network, as well as promote the formation of a finer and more ordered microstructure. Cámara et al. [13] also indicated that higher amounts of phosphates could efficiently extract more myofibrillar proteins, increase the solubility of myofibrillar proteins during emulsified meat product processing, and subsequently create a denser meat protein gel network that holds more water after heating treatment. The most positive effect on the quality of reduced-phosphate frankfurters is considered to occur with moderate ultrasound treatment duration (25 min), thus among ultrasound treated groups, only the Tr-25 group was observed using SEM. As shown in Fig. 4C, the Tr-25 group has a more compact, uniform, and denser meat protein gel network than the Tc and Tr group gel networks, which is mainly due to the decrease in protein particle size during the cavitation that occurs with ultrasound treatment [41]. Zhang et al. [39] also declared that ultrasound treatment could reduce the total thylakoid group content and increase the surface hydrophobicity of myofibrillar proteins, which significantly promoted the interactions of protein molecules through disulfide bonds or hydrophobic force, thus a more uniform and compact meat protein network could be formed.

3.8. Sensory evaluation

Frankfurters from each group were evaluated in terms of interior color, uniformity, flavor, juiciness, and overall acceptability through sensory evaluation. As presented in Table 6, the 50% phosphates reduction (Tr group) led to a significant impact on the sensorial parameters of the frankfurters and encompassed the lowest scores when compared with other groups (P < 0.05). This result is closely associated with the changes in physical characteristics and textural properties of frankfurters. Tam et al. [50] also suggested that lower levels of phosphates in comminuted meat products could not effectively extract myofibrillar proteins, which impacted the formation of the meat protein gel network, which led to a direct decrease in scores of juiciness and tenderness. Moreover, with increasing ultrasound treatment time, the uniformity, juiciness and overall acceptability of frankfurters were at first gradually enhanced and then negatively impacted with the Tr-25 group having the highest scores of the noted sensorial parameters among all of the ultrasound treated samples (P < 0.05). Pinto et al. [7] indicated that ultrasound treatment could efficiently increase the scores of sensorial parameters (such as color, texture and overall acceptance) of reduced-phosphate emulsified meat products. In general, the results of sensory analysis indicate that the 25 min ultrasound treated sample group with 50% phosphate reduction did not show obvious differences from the control samples (Tc group).

3.9. Tbars

Lipid oxidation is the most important factor that can impair the quality of meat products during storage. Melton [51] pointed out that a TBARS value higher than 0.30 mg/kg could significantly affect consumer perception in the form of unpleasant flavor and taste. As shown in Table 7, the TBARS values for all the samples did not exceed the value mentioned above during the 21-day storage. However, with the increasing storage time from 1 to 21 days, the TBARS values gradually increased (P < 0.05). Compared with the Tc group, the Tr group showed significantly higher TBARS values after 7, 14, and 21 days of storage (P < 0.05). Although alkaline phosphates are not classified as antioxidants, they promote the interactions of protein molecules through disulfide bonds or hydrophobic force, thus a more uniform and compact meat protein network could be formed.

Table 5

| Treatments | Hardness (g) | Resilience (g) | Springiness (%) | Chewiness (g.sec) | Tightness (g.sec) | Fracturability (g) |
|------------|-------------|---------------|----------------|------------------|-----------------|-------------------|
| Tc         | 74.64 ± 0.40a | 52.58 ± 0.68b | 69.03 ± 0.49a | 971.05 ± 3.42b | 45.32 ± 0.14d | 302.73 ± 6.19b |
| Tr         | 84.03 ± 0.95a | 37.97 ± 2.28d | 60.77 ± 1.32d | 1117.37 ± 16.70d | 37.29 ± 0.82d | 182.34 ± 4.00d |
| Tr-15      | 82.98 ± 0.66ab | 46.27 ± 2.30c | 62.23 ± 1.74ed | 1000.31 ± 8.11d | 41.19 ± 0.48b | 241.36 ± 0.84d |
| Tr-20      | 81.46 ± 1.05bc | 54.43 ± 1.32ab | 65.70 ± 0.20b | 966.16 ± 29.12d | 45.09 ± 0.99d | 253.99 ± 6.55d |
| Tr-25      | 71.68 ± 0.40c | 55.39 ± 1.17c | 67.37 ± 0.48b | 729.50 ± 11.36c | 52.32 ± 0.39c | 313.82 ± 7.74c |
| Tr-30      | 78.97 ± 1.74d | 53.71 ± 0.74ab | 67.58 ± 0.63a | 840.19 ± 17.48c | 49.50 ± 1.16b | 242.99 ± 8.94d |
| Tr-35      | 80.33 ± 1.68ed | 52.49 ± 0.19b | 63.52 ± 0.12c | 951.46 ± 11.40c | 47.83 ± 0.35c | 226.87 ± 2.50c |

Values are given as means ± SD from triplicate determinations. Different letters (a-f) in the same column indicate significant differences (P < 0.05).
they may potentially retard lipid oxidation or oxidative rancidity as a metal chelator [3]. Moreover, except at day 1, with increasing ultrasound treatment time, TBARS values gradually increased after 7, 14, and 21 days of storage (P < 0.05) and remained significantly lower than the Tr group (P < 0.05) at these storage times. Kang et al. [52] indicated that ultrasound treatment could promote protein and lipid oxidation, which subsequently increases the carbonyl formation and TBARS values of meat products. However, this phenomenon was mainly attributed to various factors, such as ultrasonic power, ultrasonic duration, temperature, ultrasound setting, and operating modes [22]. Cichoski et al. [23] also reported that the cavitation effect of ultrasound could significantly result in an increase of temperature, subsequently impacting emulsion stability, texture, and oxidative stability of emulsified meat products. In our present study, when compared with the Tc group, it seems that our temperature control of the ultrasound treatment with the appropriate parameters did not lead to an increase in the lipid oxidation of frankfurters.

3.10. HCA analysis

HCA is an effective method to establish a matrix that can clearly describe the level of multivariate correlation among different samples in hierarchical form. The results of an HCA analysis are generally expressed as heat maps, which can explicitly exhibit the proximity and relationship of different samples. As shown in Fig. 5, the clustering results indicate that in cluster 1 the Tr group shows an impact toward down-regulating pH, textural properties (springiness, resilience, tightness, and fracturability), L*-value, a*-value, and sensorial parameters (juiciness, uniformity, interior color, and flavor), whereas the Tc group and the Tr-25 group exhibits an up-regulation trend. Moreover, in cluster 2, obvious up-regulation of b*-value, emulsion stability (total released liquid, released fat and released water), cooking loss, and textural properties (hardness and chewiness) appears in the Tr group. However, the Tc group and the Tr-25 group have significant down-regulation of these aspects. In addition, the clustering results show that the Tr-25 group is grouped with the Tc group, which indicates that the 25 min ultrasound treatment has the greatest potential to promote the quality of frankfurters with 50% phosphate reduction over any other ultrasound durations. It also implies that precise temperature-controlled ultrasound processing with moderate duration seems to be a successful strategy to apply to reduced-phosphate frankfurters under the “clean label” concept.

4. Conclusion

Phosphate reduction (reduced by 50% in our present study) can significantly increase the cooking loss and lower the emulsion stability of frankfurters, as well as lead to shortcomings in textural properties, sensorial parameters, and oxidative stability. With trending healthiness and the increasing demand for “clean label” meat products, a temperature-controlled ultrasound bath with an intelligent temperature control and monitoring system was applied to overcome the above detrimental aspects of reduced-phosphate frankfurters. The results indicate that meat batter subjected to 25-min ultrasound treatment post filling, had lower cooking loss and higher emulsion stability following the heating and cooling process. The 25-min ultrasound treatment results also show enhanced textural properties (such as tightness, springiness, and

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**Fig. 4.** Scanning electron microscopy (SEM) images of frankfurters with different phosphate levels and ultrasound treatment. (A) Frankfurters formulated with normal phosphate levels (0.40%, w/w), (B) Frankfurters formulated with 50% reduced phosphate levels (0.20%, w/w), (C) Frankfurters formulated with 50% reduced phosphate levels (0.20%, w/w) combined with 25 min ultrasound treatment. The magnification of A-C was 2000 × .

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**Table 6**

| Treatments | Interior color | Uniformity | Flavor | Juiciness | Overall acceptability |
|------------|---------------|------------|--------|-----------|----------------------|
| Tc         | 5.13 ± 0.35a | 5.50 ± 0.15a | 5.06 ± 0.25a | 4.76 ± 0.25a | 5.26 ± 0.05ab    |
| Tr         | 3.10 ± 0.10b | 2.22 ± 0.25a | 3.26 ± 0.25a | 3.28 ± 0.25a | 3.60 ± 0.10c    |
| Tr-15     | 4.86 ± 0.47a | 3.46 ± 0.05a | 4.86 ± 0.25a | 4.23 ± 0.25a | 4.76 ± 0.25b    |
| Tr-20     | 5.26 ± 0.32a | 5.06 ± 0.25a | 5.20 ± 0.25a | 4.73 ± 0.25a | 5.10 ± 0.36ab   |
| Tr-25     | 4.96 ± 0.36a | 5.96 ± 0.25a | 4.96 ± 0.25a | 5.66 ± 0.25a | 5.46 ± 0.40a    |
| Tr-30     | 4.90 ± 0.30a | 5.26 ± 0.25a | 5.10 ± 0.25a | 4.60 ± 0.25a | 5.30 ± 0.17a    |
| Tr-35     | 4.86 ± 0.31a | 4.46 ± 0.25a | 5.06 ± 0.25a | 4.10 ± 0.25a | 4.76 ± 0.30b    |

Values are given as means ± SD from triplicate determinations. Different letters (a-e) in the same column indicate significant differences (P < 0.05).

**Table 7**

| Treatments | 1d | 7d | 14d | 21d |
|------------|----|----|-----|-----|
| Tc         | 0.017 ± 0.0014a | 0.047 ± 0.0011a | 0.053 ± 0.002a | 0.064 ± 0.003a |
| Tr         | 0.020 ± 0.0015a | 0.082 ± 0.0011a | 0.091 ± 0.002a | 0.166 ± 0.004a |
| Tr-15      | 0.025 ± 0.0015a | 0.060 ± 0.0011a | 0.066 ± 0.002a | 0.091 ± 0.003a |
| Tr-20      | 0.028 ± 0.0015a | 0.065 ± 0.0011a | 0.077 ± 0.002a | 0.099 ± 0.003a |
| Tr-25      | 0.031 ± 0.0015a | 0.070 ± 0.0011a | 0.082 ± 0.002a | 0.103 ± 0.004a |
| Tr-30      | 0.033 ± 0.0015a | 0.073 ± 0.0011a | 0.085 ± 0.002a | 0.115 ± 0.004a |
| Tr-35      | 0.038 ± 0.0015a | 0.075 ± 0.0011a | 0.088 ± 0.002a | 0.131 ± 0.005a |

Values are given as means ± SD from triplicate determinations. Different letters (a-f) in the same column indicate significant differences (P < 0.05). Different letters (A-D) in the same row indicate significant differences (P < 0.05).
resilience), which were verified by dynamic water distribution analysis and SEM observation. Moreover, sensory evaluation scores were essentially the same in both the control group (Tc) and the 25-min ultrasound treated group (Tr-25) with 50% reduced phosphate, which implies that our optimal ultrasound parameters could efficiently reduce the textural or sensorial defects caused by a 50% phosphate reduction. In addition, ultrasound treatment did not increase the lipid oxidation of reduced-phosphate frankfurters during 21 days of storage, which is probably attributed to the low static temperature controlled by our intelligent temperature control and monitoring system. Thus, ultrasound treatment with moderate duration and a precisely controlled low temperature could be considered a successful strategy to obtain healthier reduced-phosphate frankfurters under the “clean label” concept. In our future research, we will focus on the effect of different ultrasound temperatures on the textural properties and sensorial parameters of reduced-phosphate frankfurters.

CRediT authorship contribution statement

**Fengxue Zhang**: Methodology, Investigation, Writing - original draft. **Honglei Zhao**: Software, Investigation, Validation. **Chuanai Cao**: Investigation, Visualization. **Baohua Kong**: Data curation, Formal analysis. **Xiufang Xia**: Visualization, Resources. **Qian Liu**: Conceptualization, Funding acquisition, Supervision.

**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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![Fig. 5. HAC analysis of frankfurters with different phosphate levels and ultrasound treatment under different durations.](image-url)
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