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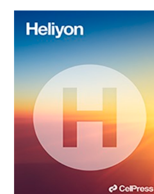

Research article

Based on proteomics probing into the deterioration mechanism of pork batter gel caused by different cooking temperatures

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A R T I C L E I N F O

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A B S T R A C T

The purpose of this experiment was to explore the influence of different cooking temperatures on the deterioration characteristics of pork batter gel by using proteomics, gel electrophoresis, size and chemical bond of aggregates. The results showed that the protein molecules of the pork batter gel was degraded during heating cooking and the protein aggregates were composed of many degraded protein fragments; compared with the control group 75 °C (0 min), the significant degradation of cytoskeleton showed at 110 °C (30 min) and 121 °C (30 min) and the significant degradation of myosin complexonly appeared at 121 °C (30 min). As the heating temperature points increased, compared with the control group 75 °C (0 min), the different temperatures could promote the separation of metal ions with proteins especially at 110 °C (30 min) and 121 °C (30 min), which could ultimately influence quality of pork batter gel by the size of particle. As the increase of heating temperature points, the recombination of aggregates composed of different proteins was not conducive to the retention of capillary water, which reduced the texture of pork batter gel. This research provided theoretical support for improving the process property of the meat products.

1. Introduction

In the time of rapid economic development, the nutritious and portable emulsified meat products had became the first choice for consumers [1,2]. The proteins of the emulsion system were determinant factor for texture and appearance of products [3]. Some researchers had found that the overheat treatment could lead to the aggregation of protein molecules, which resulted in that the structure of protein gel got poor [4,5], so that the high cooking temperature had an adverse effect on the quality of the protein gel. The destruction of gel network affected the retention of the water. The water retention directly affected the quality of meat products (juiciness, texture and color) and the economic benefits of enterprises [6,7] (see Fig. 1).

The micro-structure of myofibrillar protein significantly rearranged during heat treatment, which affected cross-linking and aggregation of proteins [8]. The formed proteins aggregates were basic units to form the network structure of the protein gel. So the properties of the aggregation (charge distribution, conformation and morphology) could directly affect the compactness and sensory properties of the three-dimensional network of gel structure. The particle size had impact on the functional properties of proteins, and

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the changes of aggregate properties could be measured by particle size. The key parameter of granularity was gradually being used as a basic measurement indicator for meat science, because it could help us got a deeper understanding of protein aggregates [9,10]. The particle size reflected the degree of protein aggregation and structural changes and the cross linking between proteins affected the size of particle at different heating temperatures [11,12].

As one of the six hot technologies in the 21st century, the proteomics could be used to analyze composition, modification and complete expression of protein. The proteomics had widely been used in medicine, zootechnics, food science, agricultural research and other fields. The unmarked methods had hugely received attention due to their significant advantages in identifying biomarkers [13,14]. The non-labeled quantitative technology (Label free) had been an important mass spectrometry quantitative method in lately years. At present, the label free had widely been used to compare changes in proteomics. The relevant researchers had used it to measure changes of protein abundance of samples, changes of muscle tissue composition, changes of meat color and dairy products [15,16].

There were few researches about collagen and myofibrillar protein [17,18], however, the meat products were used all components of meat, so this study was based on the phase separation of pork batter emulsion gel and proteomics to analyze the effect of different cooking temperatures on the protein of pork batter gel; at the same time, the impact of different cooking temperatures on the physicochemical and molecular properties of aggregates was analyzed based on the changes of the differential proteins, thus, this research provided theoretical support for improving the processing characteristics of emulsion gel products.

2. Materials and methods

2.1. Materials

The fresh pork longissimus muscle (24–48 h postmortem, water content 70.73 %; fat content 5.03 %; protein content 23.92 %) from Xinlongjia supermarket (Shen Yang, China). The sodium chloride (NaCl) and polyphosphate (including sodium tripolyphosphate (STP), sodium pyrophosphate (SPP), sodium hexametaphosphate (SHMP) in the proportion (1:1:1)) were analytical grade reagents (Aladdin), etc.

2.2. Methods

2.2.1. Meat batter preparation

All the visible connective tissues and fat were removed, and the samples were chopped through a 6 mm plate of a grinder (MM-12, Guangdong, China), cut into 0.5 cm^3, mixed uniformly to ensure homogeneity, vacuum-packed 500 g meat with packing bags, respectively, and all stored at −18 °C.

Thawed meat at 4 °C for 24 h. All materials were stored in an ice-water bath. Firstly, mixed 800 g lean meat with 30 g salt and 3 g polyphosphate, chopped and mixed in a chopping machine (Hanjiaours) at 3000 r/minute for 1 min, and stopped for 2 min to facilitate the dissolution of salt-soluble proteins; secondly, added 1/3 of the total amount of ice water (200 g), continued to chopped and mixed at 3000 r/minute for 1 min; stopped for 2 min; then, added 2/3 of the remaining total amount of ice water (200 g), chopped and mixed for 1 min at 3000 r/minute to make meat batter samples.

2.2.2. Prepare gel samples

Took about 30 g of meat batter put into a 50 mL screw cap polypropylene centrifuge tube and sealed it, then, centrifuged at low speed 1000 r/minute for 5 min to remove air bubbles in the meat batters. The centrifuge tube containing the meat batters was firstly heated in a constant temperature water bath until the central temperature of the meat batters was 75 °C, then, one group was taken out as a control group75 °C (0 min), and the other groups were heated at constant temperature of 75 °C, 100 °C, 110 °C and 121 °C for 30 min for cooking, respectively, then, took out the centrifuge tubes and inverted those for 60 min until it cooled to room temperature, which was placed in refrigerator at 4 °C overnight before analyzed. The different words represented tested samples. Z0 (75 °C (0 min));

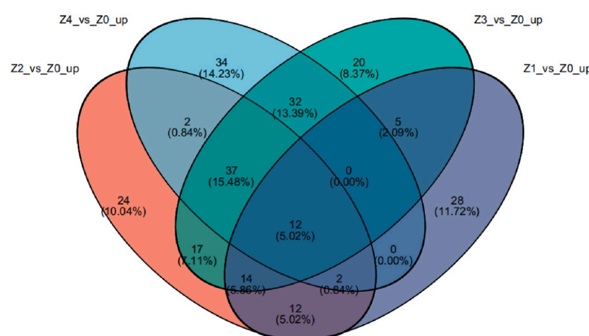

Fig. 1. The analysis of up-regulated differential proteins.
Note: Z0 (75 °C (0 min)); Z1 (75 °C (30 min)); Z2 (100 °C (30 min)); Z3 (110 °C (30 min)); Z4 (121 °C (30 min)).

Z1 (75 °C (30 min)); Z2 (100 °C (30 min)); Z3 (110 °C (30 min)); Z4 (121 °C (30 min))

2.3. The changes of protein types and quantities

2.3.1. Protein extraction

Took out a portion of the sample in a frozen state, transferred it to a grinding tube, and added an appropriate amount of BPP solution; shook 3 times using a Wanbai frozen grinder, each time for 120 s at 60 Hz; centrifuged at 4 °C, 12000 g for 20 min to obtain the supernatant, added equal volume Tris saturated phenol, and vibrated at 4 °C for 10 min; centrifuged 12000 *g* at 4 °C for 20 min to obtain the phenolic phase, added an equal volume of BPP solution, and oscillated for 10 min at 4 °C; centrifuged 12000 *g* at 4 °C for 20 min to obtain phenolic phase, added 5 times the volume of pre-cooled ammonium acetate methanol solution, and precipitated protein at −20 °C overnight; centrifuged at 4 °C, 12000 g for 20 min to discard the supernatant the next day, added 90 % pre-cooled acetone to the sediment, mixed well and centrifuged to discard the supernatant, repeated twice; dissolved the precipitate in protein lysis solution (8 M urea+1%SDS, containing protease inhibitor cocktail); ultrasound on ice for 2 min; centrifuged 12000 *g* at 4 °C for 20 min, and extracted the protein supernatant; BCA quantification, SDS-PAGE electrophoresis.

2.3.2. Peptide segment desalination and quantification

After trypsin digestion, dried the peptide segment with a vacuum pump; dissolved the peptide segments extracted by enzymatic hydrolysis with 0.1 % trifluoroacetic acid (TFA); after desalination of peptide segments using HLB, each sample was divided into two portions and drained using a vacuum concentrator; used Thermo Fisher Scientific (product number 23275) peptide quantification kit for peptide quantification.

The proteomics was completed in Meiji Biology company (Shang hai).

2.4. Distribution of the mass of protein aggregates

The gel electrophoresis followed the below method with slightly modifications [22]. Accurately weight 3 g gel sample, chopped it up and placed it in a centrifuge tube containing 27 mL 5 % SDS solution at 85 °C, and homogenized with a high-speed homogenizer for 1 min, then, incubated in a water bath at 85 °C for 1 h, after the homogenate was cooled naturally, centrifuged at 25 °C and 10,000 g for 8 min to take the supernatant, afterwards, the concentration of the supernatant was adjusted to 4 mg/mL by the biuret method, then, mixed with the loading buffer at a ratio of 1:1 (v/v), and boiled for 5 min. The volume of the samples was 10 μL. The concentration of the stacking gel was 5 % and the separating gel was 12 %. After separating gel stained with a staining solution (0.25 % Coomassie brilliant blue G250, 10 % glacial acetic acid and 40 % ethanol) for 40 min, immediately, used a de-staining solution (10 % glacial acetic acid and 40 % ethanol), then, using a gel imager scan the stain gel to get distribution of protein molecular weight.

2.5. Distribution of the size of protein aggregates

According to the experimental method [19]and with certain adjustments, a certain amount sample was taken and placed into a laser particle size analyzer. The concentration of the sample was adjusted to 2 mg/mL, and the protein aggregation trend was measured using a laser particle size analyzer. The temperatures were set at 25 °C, and the equilibrium time and scanning time were both 10 s.

2.6. The changes of chemical bond of protein aggregates

The chemical forces of ionic bonds, hydrogen bonds and hydrophobic bonds were determinutesed by the methods [20]with slightly modifications. 4 g of the prepared meat batters samples were mixed with 20 mL of 0.05 mol/L NaCl (SA), 0.6 mol/L NaCl (SB), 0.6 mol/L NaCl+1.5 mol/L urea (SC) (atomic ratio 2:5) and 0.6 mol/L NaCl+8 mol/L urea (SD) (atomic ratio 3:40), respectively, after homogenizing with a T25 homogenizer at 5000 r/minute for 2 min, then, rested at 4 °C for 1 h, then, centrifuged at 10,000 g for 15 min. The protein content of the supernatant was determined, and the contribution of each chemical force to the meat batters system was investigated at each temperature point. The contribution of ionic bonds was expressed as the difference between the protein content dissolved in SB solution and SA solution; the contribution of hydrogen bonding was expressed as the difference between the protein content dissolved in SC solution and SB solution; the contribution of hydrophobic interaction was expressed as the difference between the protein content dissolved in SD solution and SC solution.

Referring to the below method [21], the specific method with 5,5′-dithiodinitrobenzoate (DNTB) as followed: 15 mg of the gel sample was suspended and dissolved in 5 mL Tris-Gly buffer (pH = 8.0, 8 M urea), then, 50 μL of Ellman's reagent (DTNB, Tris-Gly buffer, 4 mg/mL) was added, and the reaction was incubated at 25 °C for 60 min, afterwards, the samples were centrifuged (5000 g, 15 min), the absorbance of the supernatant was measured at 412 nm. The tris-Gly buffer containing Ellman's reagent was used as a blank control. The SH represented sulfydryl thiol group. The formula for calculating the thiol content was as followed:

$$\mathrm{SH}\ (\mu\ \mathrm{mol}\ /\ \mathrm{g}) = 73.53 \times \mathrm{A} \times \mathrm{D}/\mathrm{C}$$

in the formula: A - the absorbance value after the sample removing the reagent blank; D - the dilution ratio; C - the sample concentration (mg/mL).

2.7. Effect of different cooking temperatures on aggregated capillary water

In each group of samples, randomly used a sampler to select samples with a diameter of 1.0 cm and a height of 1.0 cm, and then placed the taken gel samples (C1) into a sealed vial of a qualitative filter paper with a diameter of 2.0 cm laid at the bottom. After balancing, sealed the vial with a wooden stopper, and then balanced it at 20 °C for 3 days, and the mass was recorded as C2. The capillary water retention (CS) was calculated according to the following formula:

$$CS / \% = C2/C1 * 100$$

3. Statistical analysis

The values were marked in the form of mean ± standard deviation. The Statistical Package for the Social Sciences was used to execute data for Windows v19.0. In order to evaluate the statistical significance ($P < 0.05$), one-way analysis of variance was carried out, moreover, Duncan's test and Pearson's correlation (R) were applied to all the targets. The graphs were plotted using Origin software.

4. The results and discussions

4.1. The analysis of differential protein types

4.1.1. The analysis of up-regulated differential proteins

The up-regulated protein referred to the amount of protein in the experimental group was more than that in the control group. Compared to the control group Z0, the common up-regulated proteins of each experimental group mainly came from the cytoplasm, nucleus and cytoskeleton.

Compared to the control group Z0, the up-regulated proteins contained in Z1 mainly came from the cytoplasm, nucleoplasm, and cytoplasm, which indicated that the heating temperature at 75 °C (30 min) promoted the degradation of proteins of the cytoplasm, nucleoplasm, and cytoplasm. Compared to the control group Z0, the up-regulated proteins of the Z2 group mainly came from the membrane, mitochondria and cytoplasm, which indicated that the heating conditions at 100 °C (30 min) could promote the degradation of proteins of the membrane, mitochondria, and cytoplasm. Compared to the control group Z0, the up-regulated proteins of the Z3 group mainly came from the cytoplasm, nucleus, cytoplasm and cytoskeleton. This indicated that the heating temperature at 110 °C (30 min) could promote the decomposition of the cytoskeleton and the degradation of proteins of the cytoplasm, nucleus, and cytoplasm. Compared to the control group Z0, the up-regulated proteins of the Z4 group mainly came from the cytoskeleton, myosin complex, cytoplasm and troponin complex. This indicated that the condition at 121 °C (30 min) promoted the degradation of the cytoskeleton, myosin complex, and troponin complex, as well as the degradation of proteins of the cytoplasm.

4.1.2. The analysis of down-regulated differential proteins

The down-regulated protein indicated that the number of proteins in the control group was higher than that in the experimental group. Compared to the control group Z0 group, the down-regulated differential proteins of each experimental group mainly came from the membrane and cytoplasm, indicating that different heating conditions promoted the degradation or aggregation of proteins of the membrane and cytoplasm.

Compared with the Z1 group, the down-regulated proteins of the Z0 group mainly came from endoplasmic reticulum membrane and collagen trimer, which indicated the ribosomal receptors lysine and collagen type XII from the endoplasmic reticulum membrane and collagen trimer α1 chain were involved in excessive aggregation or degradation of protein molecules with the heating condition at 75 °C (30 min); compared with the Z2 group, the down-regulated proteins of the Z0 group mainly came from mitochondrial respiratory chain complex I and cytoplasm, which indicated that the protein molecules from mitochondrial respiratory chain complex I and cytoplasm were mainly involved in the excessive aggregation or degradation of proteins with the temperature at 100 °C (30 min);

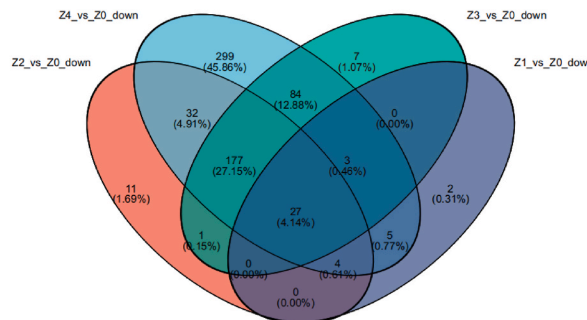

Fig. 2. The analysis of down-regulated differential proteins.
Note: Z0 (75 °C (0 min)); Z1 (75 °C (30 min)); Z2 (100 °C (30 min)); Z3 (110 °C (30 min)); Z4 (121 °C (30 min)).

compared with the Z3 group, the down-regulated proteins of the Z0 group mainly came from the cytoplasm and actin cytoskeleton, which indicated that the protein molecules from the cytoplasm and actin cytoskeleton were mainly involved in the excessive aggregation or degradation of proteins at a heating temperature of 110 °C (30 min); compared with the Z4 group, the down-regulated proteins of the Z0 group mainly came from protein molecules of the cytoplasm, mitochondria, and cytoskeleton, which meant that the proteins molecules from cytoplasm, mitochondria, and cytoskeleton mainly participated in the phenomenon of excessive protein aggregation or degradation.

4.2. The effect of different cooking temperatures on the molecular mass of aggregates

The influence of different heating temperatures on the molecular weight of protein of pork batter gel was shown in Fig. 3 (see Fig. 2). The main proteins of pork batter gel system included myosin heavy chain (220 kDa), connexin (100 kDa), actin (43 kDa), tropomyosin (35 kDa), myosin light chain and a variety of micro regulatory proteins. From the picture 3, it could be seen that there had protein aggregates with a molecular weight greater than 220 kDa in the separation gel, compared to the control group Z0, the number of aggregates gradually increased with the heating temperature points increased. This phenomenon might be due to the degraded and dissociated protein fragments formed large molecular weight aggregates as the increase of heating temperature point. The relevant scholars had also reached similar conclusions [23,24]. Compare to Z3, the number of aggregates with weight greater than 220 kDa decreased at Z4, the reason for this phenomenon might be that, under this heating condition 121 °C (30 min), the formed aggregates undergo further degradation, thus, resulting in a decreasing trend of aggregates content.

Compared with the control group Z0, the content of actin (43 kDa) showed an increasing trend at Z2 and Z3, which might be due to the aggregation of small molecular weight protein molecules of pork batter gel or the degradation of large molecular weight protein molecules to increase the content of aggregates at 43 kDa; however, the band at 43 kDa showed a weakening trend at Z4, which might be due to excessive aggregation of actin molecules to form larger aggregates or further degradation of the formed aggregates, thus, resulting in a decrease of the content of 43 kDa. Compared with the control group Z0, the band at 35 kDa gradually showed a weakening trend as the heating temperature point increased. The reason for this phenomenon might be that, as the heating temperature point increased, myosin molecules gradually excessively aggregated to form macro-molecular aggregates or underwent degradation, thus, resulting in a gradually weakening trend of the band at 35 kDa. The protein bands with a molecular weight less than 35 kDa correspond to low or no protein content, possibly due to the excessive aggregation of low molecular weight protein molecules to form higher molecular weight aggregates or degradation into low mass peptides as the heating temperature points increased.

4.3. The effect of different cooking temperatures on the particle size of aggregates

The particle size was a fundamental measurement indicator of the meat science [9,12]. The particle size could reflect the changes and aggregation degree of protein molecules. From Fig. 4, it could be seen that, compared with the control group Z0, that the particle size of pork batter protein gel showed a gradually significant increased trend with the increase of cooking temperature points ($P < 0.05$), which might due to the pH change of pork batter gel was more conducive to the aggregation of protein molecules with the increase of cooking temperature point, so that the particle size of pork batter protein gel showed an increasing trend. The researchers had found that protein cross-linking and aggregation between myofibril protein molecules were prone to occur under acid treatment conditions, thus, leading to an increase in particle size [25]. Compared to control group Z0, the significant differences began to appear at Z2. The reason for this phenomenon might be that the rate of denaturation and aggregation of up-regulated proteins from the membrane, mitochondria and cytoplasm and down-regulated proteins from the mitochondrial respiratory chain complex I and cytoplasm of the pork gel was unbalanced, so that the rate of protein aggregation gradually increased to form disordered aggregates with large particle sizes, ultimately, the particle size increased with significant differences (see Fig. 5).

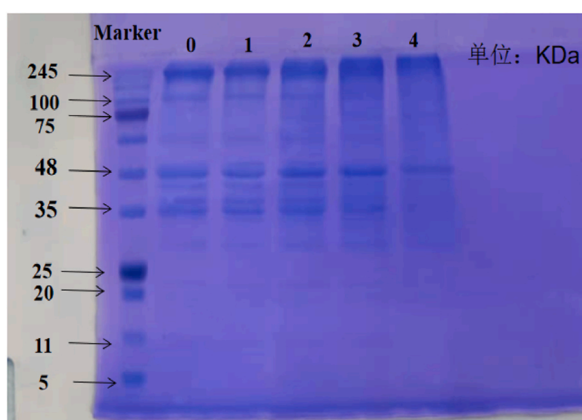

Fig. 3. The effect of different cooking temperature on molecular weight of aggregates.
Note: 0, 1, 2, 3, and 4 represented 75 °C (0 min), 75 °C (30 min), 100 °C (30 min), 110 °C (30 min), and 121 °C (30 min), respectively.

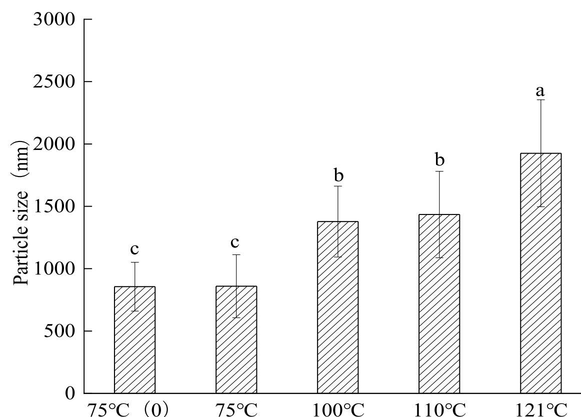

Fig. 4. The effect of different cooking temperatures on the size of aggregates.
Note: The result was the mean ± standard deviation, the different letters represented the significance of differences between groups($P < 0.05$).

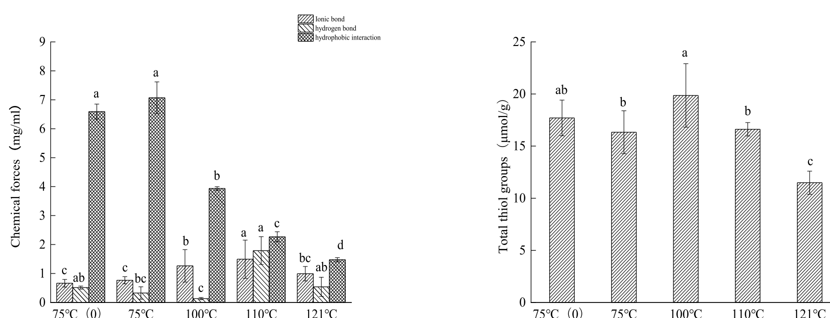

Fig. 5. The effect of different cooking temperatures on the chemical effort of aggregates.
Note: The result was the mean ± standard deviation, the different letters represented the significance of differences between groups($P < 0.05$).

4.4. The effect of different cooking temperatures on the chemical bonding of aggregates

The balance of forces between protein-protein and between protein-solvent played an important role in the process of protein forminutesg gel network. The interactions between protein molecules of the gel network structure were mainly hydrogen bond, ionic bond, disulfide bond and hydrophobic interaction [16,26].

From the graph 5, it could be seen that, compared to the control group Z0, the hydrophobic interaction between aggregates showed a significant downward trend overall ($P < 0.05$) as the cooking temperature point increased, and there was a significant negative correlation between surface hydrophobicity and hydrophobic interaction ($P < 0.05$, $r = -0.641$), which meant that the reason for the decrease of the hydrophobic interaction might be that the enhancement of surface hydrophobicity of monomer aggregates could facilitate the strengthening of hydrophobic interaction forces within monomer aggregates with the increase of heating temperature point, thereby, weakening the hydrophobic interaction between aggregates. The correlation indicated that the hydrophobic interactions and β-sheet showed a significant negative correlation ($P < 0.05$, $r = -0.802$), and had a significant negative correlation with irregular curling ($P < 0.05$, $r = -0.917$), which indicated that the increase β-sheet content and irregular curling might promote the strengthening of internal hydrophobic interactions between monomer aggregates, thereby, reducing the hydrophobic interactions between aggregates.

Compared to the control group Z0, the number of hydrogen bonds showed a trend of decrease firstly, then, increased as the increase of cooking temperature point. Compared to the control group Z0, the content of hydrogen bonds of the aggregates showed a decreasing trend at Z1 and Z2 ($P < 0.05$), the reason for this phenomenon might be that the formation of hydrogen bonds was an exothermic reaction, however, the formation of hydrogen bonds was dominated by reverse reactions under these heating condition, thus, resulting in a decrease of their quantity. During the heating working process, the hydrogen bonds were the mainly force to form β-sheet. Compared to the control group Z0, the content of the hydrogen bonds showed an increasing trend at 110 °C (30 min). It might because the up-regulated differential proteins from cytoplasm, nucleus and cytoskeleton, and down-regulated proteins from cytoplasm and actin cytoskeleton at Z3 promoted formation of the β-sheet, thereby, leading to the increase of hydrogen bonds content of the aggregates; as heating temperature point at Z3, the formation of gelatin increased formation sites of hydrogen bonds, thereby, increasing the content of hydrogen bonds. Compared to the control group Z3, the hydrogen bond content of the aggregates showed a decreased trend at a heating temperature at Z4. The researchers had found that, as the heating temperature increased, the content of alpha helices

decreased [7,27,28]. This phenomenon might because that the up-regulated differential proteins from namely cytoskeleton, myosin complex, cytoplasmic and troponin complex, and the down-regulated proteins from mitochondria, sarcoplasmic reticulum, cytoplasm caused the content of α-helix decreased, and the tail of the α-helix had a large number of hydrogen bonds, thus, the decreased content of α-helix led to the decrease content of hydrogen bonds.

Compared to the control group Z0, the number of ion bonds of the aggregates showed an increase trend overall as the cooking temperature point increased ($P < 0.05$), the reason for this phenomenon might be that the free ions between the aggregates increased as the cooking temperature point increased, which strengthened the electrostatic force between the aggregates and ultimately led to an increase of ion bonds content. The correlation relationship indicated the content of ion bonds had a negative correlation with the content of the α-helix ($P < 0.05$, r = −0.607), and had a significant negative correlation with the content of β-turns ($P < 0.05$, r = −0.541), which meant that the decrease content of α-helix and β-turns was beneficial for the formation of free ions, ultimately, facilitating the formation of ion bonds. Compared with the control group Z2, the number of ion bonds showed a decreasing trend at Z4, this phenomenon might because that the up-regulated differential proteins from namely cytoskeleton, myosin complex, cytoplasmic and troponin complex, and the down-regulated proteins from mitochondria, sarcoplasmic reticulum, cytoplasm caused changes of the quantity and distribution of charges on peptide chains and side chains, thereby, altering the electrostatic interactions between protein molecules and reducing the content of ion bonds.

The total sulfhydryl content referred to the sum of sulfhydryl groups exposed on the surface of a protein and buried within the protein molecule [38]. From the graph 5, it could be seen that, compared to the control group 75 °C (0 min), the content of total thiol groups of the aggregate showed a significant decrease overall with the increase of cooking temperature point ($P < 0.05$), the main reason for this phenomenon was that the thiol groups were oxidized into disulfide bonds during the heating process to maintain the stability of the aggregate, thus, resulting in a decrease of the total number of thiol groups. The researchers had also found that myofibrillar proteins undergo corresponding structural changes under oxidation, leading to the oxidation of free thiol groups into disulfide bonds, ultimately, leading to a decrease of total thiol content [26]. Compared to the control group Z0, the total thiol content showed a significant increased at a cooking temperature Z2 ($P < 0.05$), this phenomenon might because that the up-regulated proteins from membranes, mitochondria, and cytoplasm, and down-regulated proteins from mitochondrial respiratory chain complex I and cytoplasm at Z2 resulted in the disconnection of a large number of disulfide bonds, ultimately, increasing the total number of thiol groups. The correlation indicated the content of total thiol groups had a significant positive correlation with the content of the ionic bonds ($P < 0.05$, r = 0.545), which meant that the formation of ionic bonds and thiol groups was mutually reinforcing, finally, the formation of ionic bonds was beneficial for the formation of thiol groups.

4.5. The effect of different cooking temperatures on capillary water of aggregates

The water embedded in the gel network structure was considered as capillary water. The protein of the muscle would rearrange and aggregate into a three-dimensional protein gel matrix, and inside the gel matrix would produce a diameter of 0.1–1.0 μM hole, which would generate capillary suction during the processing and heating of meat products; the binding force of water of meat products was almost generated by capillary, so capillary water and gel water retention were closely related [29–32].

From Fig. 6, it could be seen that, compared with the control group Z0, the capillary water content generally showed a significant downward trend with the increase of cooking temperature point ($P < 0.05$). This phenomenon might because that the increase of heating temperature points degraded the cytoskeletal proteins such as sarconectin and myosin of the pork gel network, thus, causing the collapse and degradation of the gel network structure, furthermore, the pore size of the network formed by aggregates became larger, ultimately, leading to a decrease in the capillary system's hydraulic capacity. The researchers had found that the content of capillary water of meat tended to decrease due to excessive aggregation of protein molecules during the cooking process [6,33]; the

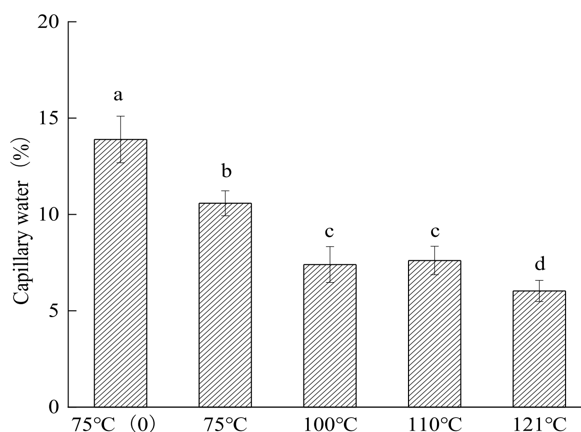

Fig. 6. The effect of different cooking temperatures on capillary force of aggregates.
Note: The result was the mean ± standard deviation, the different letters represented the significance of differences between groups($P < 0.05$).

content of capillary water at Z3 was higher than that at Z2. The reason for this phenomenon might be that the changes of the internal composition and the overall composition of the membrane at Z3 were conducive to the formation of gelatin. The formation of gelatin could improve the water holding capacity of pork gel, ultimately, which increased the content of capillary water.

The correlation indicated the content of capillary water had significant negative correlation with the content of irregular curls ($P < 0.05$, $r = -0.944$), and had significant negative correlation with the content of β-sheet ($P < 0.05$, $r = -0.838$); there was a significant negative correlation between the content of capillary water and the content of ionic bonds ($P < 0.05$, $r = -0.515$); the content of capillary water had significantly positively correlated with hydrophobic interactions ($P < 0.05$, $r = 0.812$), which meant that the aggregates would be reorganized as the increase of content of irregular curls and β-sheet, then, the weakening of hydrophobic interaction between the reorganized aggregates and the increase of ionic bonds would not be conducive to the retention of capillary water, finally, increasing the proportion of juice loss and leading to deterioration of pork gel.

5. Conclusions

The protein molecules of pork gel was degraded during heating cooking (supplement figure 1) and the protein aggregates were composed of many degraded protein fragments. As the heating temperature at Z3, the degradation of the up-regulated proteins mainly came from the cytoplasm, nucleus, cytoplasm, and cytoskeleton, and down-regulated proteins mainly from the cytoplasm and actin cytoskeleton; as the heating temperature at Z4, the up-regulated proteins mainly came from the cytoskeleton, myosin complex, cytoplasm, and troponin complex, and down-regulated proteins mainly from mitochondria, sarcoplasmic reticulum and cytoplasm, which could promote the increase of the particle size of aggregates by the increase content of disulfide bonds, the increase content of neutralization between metal ions and disorder of gel networks (Supplement Figure 3,4,5), thus, the continuity of gel networks worse with the increase of aggregate particle size, finally, which was ultimately detrimental to the retention of capillary water.

As the cooking temperature increased, the degradation of cytoskeletal proteins at Z3 and cytoskeletal proteins, myosin complexes, supramolecular fibers, and supramolecular polymers at Z4 could significantly lead to the content of ion bonds, irregular curls and β-sheet increased; as the cooking temperature increased, the role of hydrophobic interactions of the excessive aggregation of protein molecules decreased; based on the comprehensive analysis of the entire text, it could conclude that, as the heating temperature increased, the content of ionic bonds and disulfide bonds of the aggregates composed of differential proteins increased as well as β-sheet, which led to the recombination of aggregates to form different shapes and excessive aggregation that it was not conducive to the retention of capillary water. Finally, the reduction of capillary water content reduced the texture of pork emulsified gel.

Data availability statement

The data in this article could be provided upon request.

CRediT authorship contribution statement

Jinyue Zheng: Writing – original draft. **Yvxin Ding:** Software. **Lingling Zhao:** Validation. **Zhichao Xiao:** Investigation. **Jun-Hua Shao:** Resources, Conceptualization.

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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Appendix A. Supplementary data

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