Evaluation of the secondary structure and digestibility of myofibrillar proteins in cooked ham

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ABSTRACT
The present study aimed to evaluate structure and digestibility of dry-cured ham in relation to different cooking temperatures (70, 100 and 120°C), and further assess the relationship between structure and digestibility. The secondary structure analysis showed that the content of α-helix decreased from 55.03% to 20.61%, accompanied by an increase in the content of β-sheet and random coil from the raw to 120°C. The digestibility showed that proteolysis rate of myofibrillar proteins by pepsin and trypsin & α-chymotrypsin (digestive enzymes) significantly increased to 0.62 and 0.51 at 100°C, compared with the raw. Odour and taste were susceptible to cooking temperature, and 100°C enhanced odour and taste attributes of ham. The changes of protein conformation were closely related to the digestibility. Correlation analysis further demonstrated that activities of digestive enzymes for proteins were negatively correlated with α-helical and β-sheet, and positively correlated with β-turn and random coil.

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Evaluación de la estructura secundaria y la digestibilidad de las proteínas miofibrilares en el jamón cocido

RESUMEN
El presente estudio se propuso evaluar la estructura y la digestibilidad del jamón curado en seco a distintas temperaturas de cocción (70, 100 y 120 °C), así como investigar la relación entre estructura y digestibilidad. El análisis de la estructura secundaria mostró que durante el proceso de elevación de la temperatura del jamón crudo a 120 °C, el contenido de hélice-α disminuyó de 55.03% a 20.61%. Esto se acompañó de un aumento del contenido de lámina-β y espiral aleatoria. El análisis de la digestibilidad indicó que, en comparación con el jamón crudo, a 100 °C la tasa de proteólisis de las proteínas miofibrilares aumentó a 0.62 y 0.51 a causa de la pepsina y tripsina & α-quimotripsina (enzimas digestivas), respectivamente. Tanto el olor como el sabor del jamón son susceptibles a la temperatura de cocción: una temperatura de 100 °C mejoró los atributos de olor y de sabor del jamón. Los cambios en la conformación proteica se relacionan estrechamente con la digestibilidad. Además, el análisis de correlación demostró que las actividades de las enzimas digestivas en términos de proteínas se correlacionan negativamente con la hélice-α y la lámina-β, y positivamente con el giro-β y la espiral aleatoria.
1. Introduction

Jinhua ham, an important Chinese dry-cured meat product, is famous for outstanding flavor parameter in the world (Huan, Zhou, Zhao, Xu, & Peng, 2005). It usually is consumed after cooking in China (Zhou et al., 2018). Temperatures of 70°C, 100°C, and 120°C usually represent traditional cooking methods, for example, stewing (70°C), boiling (100°C) and steaming (120°C), respectively. In fact, heat treatments applied to meat and meat products, improve its hygienic quality by inactivation of pathogenic microorganisms and enhance its flavor and tenderness (Bax et al., 2012). However, cooking methods as well as cooking conditions, like cooking time and temperature or end-point temperature, modify the chemical composition of meat with a consequent change of proteins structure (Kosulwat, Greenfield, & Buckle, 2003; Liu, Zhao, Xiong, Xie, & Qin, 2008). Furthermore, the changes of the structure and microenvironment of proteins are responsible for changes in functional properties and digestibility of meat proteins during heating processing (Bax et al., 2012; Xu, Han, Fei, & Zhou, 2011).

Cooking temperature is one of the main determinants of the structure and functional properties of meat proteins, to which attention should be paid after meat products cooking. The effect of temperature on the structure of meat proteins and in turn on product quality has been studied (Xu et al., 2011). Astruc, Gatellier, Labas, Lhoutellier, and Marinova (2010) reported that cooking temperature affected not just the structural properties of muscular proteins but also the bioavailability of their constitutive amino acids. During the cooking of meat products, highly oxidative conditions could accelerate the intermolecular cross-links and the formation of aggregation, which reduces proteins susceptibility to enzymatic proteolysis and decreases the digestibility of proteins (Grune, Jung, Merker, & Davies, 2004). The decrease of digestibility in proteins reduces bio-availability of amino acids, which has a negative effect on the nutritional quality of meat products (Lund, Heinonen, Baron, & Estevez, 2011).

Although the changes of proteins structure have been widely reported during the cooking process of meat such as Longissimus dorsi muscle of pig and beef, dry-cured ham differ from other meat products and it is processed according to the special protocols mainly including salting, ripening, and post-ripening. The effect of cooking temperature on the structure and digestibility of myofibrillar proteins of dry-cured ham is not evaluated, and the relationship between the structure and digestibility of myofibrillar proteins is not further established.

The main purpose of this study, therefore, was to investigate the changes of structure and digestibility of myofibrillar proteins in cooked ham. The digestibility of myofibrillar proteins was further discussed in relationship to protein structure (α-helix, β-sheet, β-turn, and random coil). This work provides an available information on the changes in digestibility and nutritional quality of cooked ham in traditional cooking methods.

2. Materials and methods

2.1. Sampling and cooking of Jinhua ham

Jinhua ham was processed with 10 months processing according to the traditional technology in Jinzi Ham Company which is located in Jinhua City (119° 84’ E and 29° 28’ N), Zhejiang Province. Sixteen Jinhua hams, which were from two batches (each batch included 8 hams) were collected. Biceps femoris muscle was removed from each ham and cut into small strips (6 * 4 * 2 cm³, respectively). A total of 96 strips (6 * 16 hams) were sampled from 16 hams. The 80 strips (no visible connective tissue) were selected. These selected strips were packaged using high-temperature cooking bag (each bag included 4 strips) and divided into four equal groups (the raw, 70°C, 100°C, and 120°C groups) (each group included 20 strips from 4 hams). The samples of 70°C and 100°C groups were cooked in a water bath for 30 min at 70°C and 100°C, respectively. The samples of 120°C group were cooked in a digital temperature-controlled steam cooker (MSC-600, Cuisinart, USA) for 30 min at 120°C. At the end of heating, the core temperature was immediately recorded by digital thermometer. The core temperatures of strips were 68.2°C, 98.7°C, and 116.8°C, for 70°C, 100°C, and 120°C groups, respectively. The heat treatment stopped by placing samples in ice. The 20 raw strips did not undergo any heat treatment as the control. The samples of control group were placed in ice all the time. All of the samples were frozen in liquid nitrogen and stored at –80°C until samples analysis.

2.2. The preparation of myofibrillar proteins

Myofibrillar proteins were prepared according to the previous procedure of Zhou et al. (2016). The myofibrillar proteins were suspended in 50 mM sodium phosphate buffers (PBS) at pH 8.0 containing 0.5 M NaCl. The concentration of myofibrillar proteins was determined by bicinchoninic acid (BCA, 4,4′-dicarboxi-2,2′-biquinolinia) proteins assay kit (Thermo Scientific, MA) and bovine serum albumin as the standard. The myofibrillar proteins were used immediately for the experiment or stored at –80°C for further use.

2.3. Raman determination

Raman measurements were performed according to Berhe, Engelsen, Hvid, and Lametsch (2014) with slight modifications. The samples of each group were cut into slices (5 mm-thickness) across the fiber direction. The measurements of Raman spectroscopy were performed on the surface of the slices with Renishaw in Via-reflex instrument (Renishaw Inc., Gloucestershire, England). The slices were placed on a quartz slide under a microscope with a 10 × objective (Renishaw Inc., Gloucestershire, England) emitted at a wavelength of 785 nm was used as excited source. The laser was focused on each sample, and typical spectra were recorded at 1 cm⁻¹ resolution with 400–2000 cm⁻¹ scans with 120 mW of laser power. Each Raman spectrum represented the average of five different replicates on one single surface of samples. The average of the 9 spectra of each sampling point was used for data analysis (4 groups × 9 presented spectra per sampling point = 36 spectra). Occasional Raman spectra (high fat content) were removed in order to focus on the structural changes of proteins. Labspec 5.0 software was used for signal processing. Phenylalanine with a background spectrum at 1004 cm⁻¹ was defined as internal standard for the normalization of spectra. The relative contents of secondary
structures (α-helix, random coil, β-sheet, β-turn) were calculated according to Susi and Byler (1988) using the secondary derivative in PeakFit 4.12 software (SeaSolve Software Inc., USA) to identify protein amide I component peak frequencies and using a multipeak fitting program with Lorentz function to quantify the multicomponent peak areas in proteins amide I band.

2.4. Determining the residues of aromatic amino acids by fluorescence spectra

The residues of aromatic amino acids of myofibrillar proteins were measured using fluorescence spectroscopy according to Zhou, Zhao, Su, and Sun (2014) with slight modifications. Myofibrillar proteins were suspended in 20 mM PBS (pH 6.0) and the concentration of proteins was measured using BCA proteins assay kit. The intrinsic fluorescence of proteins was assayed at a constant proteins concentration (0.8 mg/mL) with 0.4 mg/kg methional (Sigma-Aldrich, Saint Louis, USA) in 20 mM PBS (pH 6.0) using a 96-Well Plate Reader M200 (Tecan, Austria). The spectra were collected from 300 to 400 nm at 283 nm (excitation wavelengths). The slit widths of excitation and emission were set at 5 nm. The fluorescence spectra of the PBS were subtracted from the respective spectra of the samples. The exposure of aromatic amino acids was expressed by the change of fluorescence intensity (a.u.).

2.5. Measurement of digestibility of myofibrillar proteins in vitro

Proteins digestibility was performed according to the procedure of Bax et al. (2012) and Zhou et al. (2018). Myofibrillar proteins (0.8 mg/mL) were prepared in 33 mM glycine buffer at pH 1.8. Proteins were first digested by gastric pepsin (5 U/mg of myofibrillar proteins) for 1 h at 37°C. The digestive time was chosen based on the changes of proteolysis rate in our previous study (Zhou et al., 2018). Digestion was stopped by the addition of 15% (final concentration) trichloroacetic acid (TCA). After centrifugation at 4,000 g for 10 min, the content of hydrolyzed peptides in the soluble fraction was measured by absorbance at 280 nm. The proteolysis rate was defined as the changes of optical density units per hour (ΔOD/h). The nonsoluble fractions were washed twice in 33 mM glycine buffer at pH 8.0. The final concentration of nonsoluble fractions was adjusted at 0.8 mg/mL in same glycine buffer (pH 8.0). The nonsoluble fractions were hydrolyzed for 1 h at 37°C by trypsin & α-chymotrypsin (6.6 and 0.33 U/mg of proteins), which were purchased from Sigma-Aldrich company (Saint Louis, USA). Digestion was terminated by the addition of 15% TCA (final concentration) at 1 h. The proteolysis rate was determined as previous description. Five time repeats were performed in the assays, respectively.

2.6. Sensory analysis of cooked ham

The sensory analysis was performed according to the method of Tomović et al. (2013) with some modifications. Samples were tasted by a panel of 10 expert sensory assessors, balanced in terms of gender, varying in tasting experience, and previously trained in the assessment of ham. During different sessions, the descriptive analysis panel generated a list of color, juiciness, texture, taste and flavor attributes using the consensus training. Rating of the attribute’s intensities was done using a linear unstructured 1 mm scale anchored at their extremes (0: absence of sensation; 10: maximum of sensation intensity) and results were expressed as the mean of five replicates. Each of the five sensory characteristics were assessed on a 1 mm unstructured line marked optimum (right end) and unacceptable (left end). The samples were presented in the laboratory for sensory analyses, where the assessors were asked to evaluate the following characteristics: color, juiciness, texture, taste, and flavor.

2.7. Statistical analysis

The content of secondary structure, the intensity of S-S, the intensity ratio of tryptophan and tyrosine Doublet, proteolysis rate and sensory parameters were analyzed by one-way analysis of variance using SAS 8.0 (SAS Institute Inc., Cary, NC). Significant differences (p < 0.05) between mean were identified using Duncan’s multiple range tests. The relationship between the structure and digestibility of myofibrillar proteins was assessed by calculation of Pearson correlation coefficients.

3. Results and discussion

3.1. Analyzing the raw Raman spectra

The raw Raman spectra of samples (the raw, 70°C, 100°C, and 120°C) in the 400–2000 cm⁻¹ region are presented in Figure 1. The spectra were variously colored so as to uncover any systematic difference in the original spectra on account of the cooking temperature. The characteristic peaks including phenylalanine peak (1004 cm⁻¹), amide I band (1600–1700 cm⁻¹), amide III band (1225–1350 cm⁻¹) and C-H bending peak (1452 cm⁻¹) were clearly observed in cooked ham. Moreover, the spectra from 120°C samples showed higher background fluorescence than others groups;
the frequency at 1672 cm$^{-1}$ showed a higher intensity in 120°C samples among the four treatments.

Raman spectroscopy has been widely used for monitoring structural changes of proteins (Berhe et al., 2014; Xu et al., 2011). Beattie, Bell, Farmer, Moss, and Patterson (2004) reported that the secondary structure of proteins and quality parameters of roasted beef showed a good relationship by Raman spectra analysis. The principle of Raman spectroscopy is that monochromatic laser light interacts with a molecule of the irradiated samples. The changes of frequency and intensity in the Raman band were the main indicators of changes in the secondary structure, tertiary structure and the local environments of myofibrillar proteins (Sun et al., 2011). In our studies, the assignments of the Raman band have been carried out according to these references (Herrero, 2008; Wei, Zhang, Halas, & Hartgerink, 2008). The high intensities of peaks were clearly observed near 1004, 1246, 1340, 1452 and 1672 cm$^{-1}$ in Jinhua ham from the raw Raman spectra. These peaks ascribe to phenylalanine peak, amide II band amide, C-H bending and amide I band, respectively. The higher background intensity in samples cooked at 120°C could be explained by the denaturation of myofibrillar proteins. The denaturation of proteins results in exposure of the residues of aromatic amino acids primarily including tryptophan, phenylalanine, and tyrosine, which are known to cause fluorescence (Boyer, Joandel, Ouali, & Culioli, 1996). The higher intensity near 1672 cm$^{-1}$ in 120°C groups indicated that cooking temperature could dramatically change the secondary structure of proteins in amide I band.

### 3.2. Analysis of amide I spectra profile

Previous studies have suggested that the frequencies of the amide I (1600–1700 cm$^{-1}$) band were closely related with the types of protein backbone conformation (Careche, Herrero, Rodríguez-Casado, Del Mazo, & Carmona, 1999; Zeng, Xu, & Wang, 1997). The assignments of the amide I (1600–1700 cm$^{-1}$) band were carried out according to Herrero (2008) and Berhe et al. (2014). Curve-fitted amide I band of proteins is presented in Figure 2. For the raw, the major peak near 1657 cm$^{-1}$ band was observed in amide I; it implied the predominance of the α-helical structure. Cooking induced the unfolding of α-helical, which was affirmed by the shift of the major band to 1670 cm$^{-1}$ (Berhe et al., 2014). The obvious peaks near 1670–1680 cm$^{-1}$ (Herrero, 2008) assigned to the β-sheet structure evidently increased at 70°C, compared with the raw. As the cooking temperature increased, the peaks near 1670–1680 cm$^{-1}$ gradually became dominant at 100°C and 120°C.

In order to further analyze the content of secondary structures, the corresponding results of percentage areas of secondary structures of protein are shown in Figure 3. There was a decrease in the α-helix (p < 0.001) content accompanied by an increase in the β-sheet (p < 0.001) among all of the cooked...
The changes of secondary structure of myofibrillar proteins in raw and cooked hams.

The assignments of the amide I (1600–1700 cm\(^{-1}\)) bands are carried out according to Herrero (2008) and Berhe et al. (2014). Different lowercase letters mean significant difference among four groups on α-helical (p < 0.001), β-sheet (p < 0.001), β-turn (p < 0.001), random coil contents (p < 0.001) or others (p > 0.05), respectively.

Random coils content showed a significant increase at 70°C and 100°C groups (p < 0.001), and then no obvious change was observed at 120°C group. Compared with the raw, α-turn content showed an increasing tendency (p < 0.001) at 70°C and 120°C. These results were in accord with the changes of amide I spectra in Figure 3, and further implied that cooking over 100°C accelerated the transformation of α-helix to β-sheet and random coils.

The amide I of Raman band is the most useful band to analyze secondary structural information of proteins. Bertram, Kohler, Böcker, Ofstad, and Andersen (2006) demonstrated that an increased β-sheet structure was observed in pork myofibrillar proteins by heat treatments using Fourier Transformed Infrared Spectroscopy. Herrero, Carmona, López-López, and Jiménez-Colmenero (2008) reported that the stability of gel network was closely linked to β-sheet structure of proteins during the heating processing. In the present study, the changes in frequency or intensity of the Raman band were in line with these reports that β-sheet gradually increased and α-helix decreased during heat processing in intact muscle (Beattie, Bell, Borggaard, & Moss, 2008) and extracted myofibrillar proteins (Xu et al., 2011). Interestingly, an increased level of random coils was observed from 70°C to 100°C, which implied that the dramatic denaturation of myofibrillar proteins contributed to the formation of random coils after cooking of Jinhua ham (Yoshidome & Kinoshita, 2012). The decrease of α-helical content indicated the uncoiling of myofibrillar proteins and exposure of nonpolar amino acids to the surface of proteins in the cooked ham (Chelh, Gatellier, & Santeclhouettelier, 2006). The exposure of nonpolar amino acids contributed to the enhancement of surface hydrophobicity, and surface hydrophobicity of proteins was sensitive to the changes in cooking temperature (Zhou et al., 2018). When cooking over 100°C, the interaction of nonpolar amino acids significantly enhanced and contributed to the aggregation of proteins (Xie, Qin, Cao, & Wang, 2011). The aggregation of proteins dramatically changes the conformation of proteins. Previous studies suggested that β-sheet was closely related to the aggregation of proteins (Nault, Vendrely, Brechet, Bruckert, & Weidenhaupt, 2013). In our study, the content of β-sheet significantly increased from the raw to 120°C groups, and gradually became dominant at 120°C group, which could be attributed to the rebuilding of unfolded myofibrillar proteins and the aggregation of myofibrillar proteins by hydrophobic interaction between nonpolar amino acids, which was caused by the effects of heating and pressure (Bouraoui, Nakai, & Li-Chan, 1997; Okuno, Kato, & Taniguchi, 2007).

3.3 Evaluating protein tertiary structure by the normalized intensities of S-S, tryptophan, and tyrosine doublet

In order to further investigate the effect of cooking temperature on local microenvironment of proteins in cooked ham, tertiary structure of proteins was evaluated by the normalized intensities of S-S, tryptophan and tyrosine doublet. Table 1 shows these normalized intensities including S-S, tryptophan and tyrosine doublet using only the frequently detected band as the variables. The intensity near 530 cm\(^{-1}\) (I\(_{\text{530}}\)) showed a significant decrease (p < 0.001) with the increase of cooking temperatures. The intensity ratio for tyrosine doublet (I\(_{1004}/I_{\text{530}}\)) only presented a significant decrease at 70°C groups, while no obvious difference was observed at the intensity ratio of tryptophan (I\(_{1240}/I_{\text{1004}}\)) among all of the groups. These results indicated that cooking temperature significantly affected the local microenvironment of proteins.

Proteins have a precise tertiary structure that directs their function. Processing treatments such as salting or cooking all affect in the changes of secondary and tertiary structure of proteins. Determining the structures of various proteins would aid in our understanding of the mechanisms of protein functions and of the changes of proteins digestibility (Wang, Huang, Lin, & Chen, 2016). Disulfide bonds stabilize the tertiary structure of proteins; 500–550 cm\(^{-1}\) are usually assigned to the S-S stretching vibrations of disulfide bonds, which are formed between the cystine of proteins (Brandt et al., 2008). Band located at 530 cm\(^{-1}\) has been assigned to disulfide bonds in the gauche-gauche-trans (Wang et al., 2016). Interestingly, the intensity of S-S at 530 cm\(^{-1}\) significantly decreased from the raw to 120°C, which could be explained that cooking temperatures caused the transformation from gauche-gauche-trans conformation to all-gauche and trans-

| Treatments | I\(_{1240}/I_{\text{1004}}\) | I\(_{1004}/I_{\text{530}}\) | I\(_{530}\) |
|------------|-----------------|-----------------|-------|
| Raw        | 0.48 ± 0.09a    | 0.80 ± 0.12c    | 0.81 ± 0.11a |
| 70°C       | 0.45 ± 0.04a    | 1.06 ± 0.15b    | 0.58 ± 0.15b |
| 100°C      | 0.43 ± 0.08a    | 1.32 ± 0.11a    | 0.49 ± 0.08b |
| 120°C      | 0.44 ± 0.06a    | 1.34 ± 0.16a    | 0.50 ± 0.11b |

The assignments of these bands are carried out according to Herrero (2008) and Berhe et al. (2014). a-d Different lowercase letters mean significant difference in every column among four groups (p < 0.05), respectively.

Las asignaciones de estas bandas se llevaron a cabo siguiendo a Herrero (2008) y Berhe et al. (2014). a-d Las distintas letras minúsculas indican diferencias significativas entre los cuatro grupos en cada columna, (p < 0.05), respectivamente.
The change of residues aromatic amino acids in the raw and cooked α1973 shows the fluorescence emission spectra of β2014 significantly are assigned to tryptophan residues, which possess intrinsic fluorescence, the conformation change of myofibrillar proteins could be further investigated by the fluorescence quenching (Zhou et al., 2014). Methional revealed no fluorescence emission in the range of 300–400 nm under the experimental conditions (Zhou et al., 2014), which did not affect proteins intrinsic fluorescence, and the interaction between aromatic amino acids and methional could result in fluorescence quenching of proteins. Compared with the raw, the decreased fluorescence intensity in cooked samples could be due to the facts that cooking enhanced the binding between aromatic amino acids and methional, and then quenched the intrinsic fluorescence of myofibrillar proteins. The high fluorescence intensity in 100°C samples implied that there was a weaker interaction between aromatic amino acid residues of proteins and methional in comparison to other cooked samples. However, the weaker interaction needs to be further investigated in the future study. The results of fluorescence quenching further confirmed the change of the local microenvironment of proteins in cooked samples.

3.4. The analysis of aromatic amino acids residues using fluorescence quenching

Residues of aromatic amino acids are a useful indicator to evaluate the change of the local microenvironment of proteins. To investigate the contribution of aromatic amino acids residues of proteins, methional was employed as a model to further assay the residues of aromatic amino acids. Figure 4 shows the fluorescence emission spectra of raw and cooked hams with 0.4 mg/kg methional. Evidently, all of these samples exhibited strong fluorescence emission at 330 nm following an excitation at 283 nm. Compared with the raw, the fluorescence intensity of cooked samples at 70°C, 100°C, and 120°C significantly decreased. Moreover, the higher fluorescence intensity was observed at 100°C group among three groups of cooked hams. Fluorescence spectroscopy is a useful tool to study the conformation change of proteins by fluorescence quenching (Wan, Wang, Wang, Yang, & Yuan, 2013). Fluorescence quenching is widely caused, such as excited state reaction, ground state complex formation, molecular rearrangement and collision quenching (Wang et al., 2007). Since myofibrillar proteins have aromatic amino acids, especially tryptophan residues, which possess intrinsic fluorescence, the conformation change of myofibrillar proteins could be further investigated by the fluorescence quenching (Zhou et al., 2014). Methional revealed no fluorescence emission in the range of 300–400 nm under the experimental conditions (Zhou et al., 2014), which did not affect proteins intrinsic fluorescence, and the interaction between aromatic amino acids and methional could result in fluorescence quenching of proteins. Compared with the raw, the decreased fluorescence intensity in cooked samples could be due to the facts that cooking enhanced the binding between aromatic amino acids and methional, and then quenched the intrinsic fluorescence of myofibrillar proteins. The high fluorescence intensity in 100°C samples implied that there was a weaker interaction between aromatic amino acid residues of proteins and methional in comparison to other cooked samples. However, the weaker interaction needs to be further investigated in the future study. The results of fluorescence quenching further confirmed the change of the local microenvironment of proteins in cooked samples.

3.5. Evaluating protein tertiary structure by the normalized intensities of S-S, tryptophan and tyrosine doublet

In order to establish the relationship between the structural changes and digestibility of proteins, myofibrillar proteins were incubated with pepsin and trypsin & α-chymotrypsin in vitro. As shown in Figure 5, the hydrolysis rate of myofibrillar proteins significantly increased at 70°C and 100°C, and decreased at 120°C (p < 0.001) during the pepsin incubation. The similar hydrolysis rate of myofibrillar proteins was also observed among the four groups during the trypsin & α-chymotrypsin incubation. Interestingly, the incubation by pepsin and trypsin & α-chymotrypsin both showed higher proteolysis rate at 100°C than other treatments; it indicated that cooking at 100°C could be a critical point to gain the digestibility of Jinhua ham.
The digestibility of proteins which determines amino acid bioavailability and shapes whole-body metabolism of the absorbed amino acids is a key parameter to evaluate the nutritional quality of high proteins food. Previous studies have indicated that heat treatments significantly influenced proteins digestibility. Santélhoutellier, Astruc, Marinova, Greve, and Gatellier (2008) implied that 100°C caused a sharp decrease in the proteolysis rate of myofibrillar protein by gastric pepsin incubation. Montoya et al. (2008) did not find significant changes in the degree of pepsin hydrolysis compared with raw phaseolins in vitro. Evenepoel et al. (1998) reported a 77% increase in true ileal digestibility for cooked eggs together with improved protein assimilation on egg proteins in an in vivo study. Bax et al. (2012) observed an increased proteolysis rate by trypsin & α-chymotrypsin activities with the increase of cooking temperature in vitro. In the present study, the digestion profile with trypsin and α-chymotrypsin appears to mirror the pepsin digestion profile. Compared with raw group, the incubation of pepsin and trypsin & α-chymotrypsin on myofibrillar proteins both showed that proteolysis rate significantly increased at 70°C and 100°C than decreased at 120°C. The results implied that cooking at 100°C could be a critical point to maximize digestion profile and amino acid bioavailability after pepsin and trypsin & α-chymotrypsin incubating.

3.6. Establishing the relationship between the structure and digestibility of proteins

Amide I of Raman band is the most useful band to analyze secondary structural information of proteins. In order to further clarify the relationship between the structure and the digestibility of proteins, the Pearson correlation was performed. Correlation matrix between parameters of proteins structures and protease activities is shown in Figure 6. Correlation analysis showed that pepsin activity was significantly correlated with α-helical and β-sheet in amide I band ($r = -0.833, P < 0.001$; and $r = -0.579, P = 0.019$, respectively), and was positively correlated with β-turn and random coil in amide I band ($r = 0.513, P = 0.042$; and $r = 0.913, P < 0.001$, respectively). For the tertiary structure, pepsin and trypsin & α-chymotrypsin activity was significantly and negatively correlated with S-S (I$_{1330}$) and was positively correlated with tryptophan. Heat treatments easily cause denaturation and the changes of conformation and local microenvironment of proteins; the changes of structure and local microenvironment of proteins are closely related with the digestibility (Santélhoutellier et al., 2008). In order to further support the hypothesis, a correlation study was performed to establish links between the structure (α-helical, β-sheet, β-turn, random coil, S-S (I$_{1330}$) and tryptophan) and proteolysis rates. In our results, a negative and highly significant correlation was observed between digestible enzymes activities and α-helical and β-sheet; a positive and significant correlation was also found between digestive enzymes activities and β-turn and random coil. The correlation analysis was a good expectation to explain the increased proteolysis rate at 70°C and 100°C because heating treatments enhanced the uncoiling of proteins and then maximized protein surface exposure to hydrophobic zones and thus promoted the accessibility of digestive enzymes to cleavage sites (Meersman, Smeller, & Heremans, 2000). The uncoiling of proteins was confirmed by the decrease of α-helical and the increase of random coil at 70°C and 100°C in amide I band. However, the further increase in cooking temperature (120°C) resulted in the decrease of proteolysis rate of myofibrillar proteins. The decrease of proteolysis rate could be due to the rebuilding of proteins structure, especially the aggregation of proteins (Zhou et al., 2018) and then decreasing the recognition site for proteases (Mession, Sok, Assifaoui, & Saurel, 2013); the increase of β-sheet content affirmed the rebuilding of proteins structure at 120°C. The correlation
between S-S (I_{530}) and digestive enzymes further implied that cooking temperature changed the transformation of the disulfide bridges and then affected the formation of protein aggregates, which have a significant effect on accessibility of digestive enzymes to cleavage sites.

### 3.7. Sensory characteristics of cooked ham

Sensory characteristics of hams sampled from pieces of biceps femoris muscle in different cooking temperatures are shown in Figure 7. The sensory scores of color, juiciness, and texture did not show a significant difference from the raw to 100°C, while there was a significant decrease from 100°C to 120°C (p < 0.001). The scores of odor and taste showed a significant increase from the raw to 100°C, and then decrease from 100°C to 120°C (p < 0.001). The results of sensory analysis implied that cooking at 100°C would have a higher sensory attributes among the four treatments.

Colour, texture, juiciness, odor, and taste are very important quality characteristic of dry-cured ham (Tomovic et al., 2013). Cooking has a key effect on sensory attributes. In the present study, cooking with different temperatures showed that odor and taste were more significant in describing dry-cured ham than color, texture, and juiciness. These results were not in line with previous studies presented in literature which found that the largest difference between cooked samples with respect to color and texture was caused by the cooking temperature (Barbieri et al., 2016). The difference could be explained by the fact that Jinhua ham owned an outstanding flavor parameter (Huan et al., 2005).

### 4. Conclusions

Cooking at 70°C and 100°C caused that α-helix gradually transformed into β-sheet and random coil, while cooking at 120°C accelerated the rebuilding of the unfolded myofibrillar proteins and the aggregation of myofibrillar proteins became dominant morphology. The aggregation of proteins contributed to α-helix transforming into β-sheet, which resulted in a dramatic decrease in proteolysis rate from 100°C to 120°C. Sensory analysis demonstrated that cooking at 100°C significantly enhanced the taste and odor attributes of Jinhua ham. Proteolysis rate and sensory analysis implied that cooking at 100°C could be a critical point to gain the digestibility and sensory attributes of Jinhua ham. Correlation analysis further suggested that the digestive enzymes activities were negatively correlated with α-helical and β-sheet, and positively correlated with β-turn and random coil. This study in vitro approach makes it possible to understand the relationship between structure and digestibility of proteins in cooked ham.

### Disclosure statement

All authors declare that there are no conflict of interest.

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### Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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