EVALUATION OF THE RELATIONSHIP BETWEEN STRUCTURE AND DIGESTIBILITY OF MYOFIBRILLAR PROTEINS IN COOKED JINHUA HAM

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I. INTRODUCTION

Jinhua ham isn't usually consumed in raw ham way in China. Cooking not only is used to inactivate pathogenic microorganisms, but also develop sensorial attributes. In China, temperatures of 70, 100 and 120 °C are usually employed for cooking; these temperatures represent traditional cooking methods, for example stewing (70 °C), boiling (100 °C) and steaming (120 °C), respectively. Cooking of meat and meat products results in structural changes of meat proteins; the changes of proteins structure, especially myofibrillar proteins, will have an effect on tenderness, texture and flavor parameters, and are responsible for changes in functional properties and digestibility of meat proteins during heating processing [1]. Although the changes of proteins structure have been widely reported during the cooking processing, the relationship between structure and digestibility of myofibrillar proteins is not further investigated. The main purpose of this study, therefore, was to investigate the changes of structure and the digestibility of myofibrillar proteins and to expound the relationship between structure and digestibility of myofibrillar proteins.

II. MATERIALS AND METHODS

Sampling and cooking of Jinhua ham

Biceps femoris was excised from sixteen Jinhua ham and cut into small strips along the direction of muscle fibers. Eighty strips with standard sizes and without visible connective tissue were collected from the above strips. The strips were packaged using high temperature cooking bag at room temperature and divided into four equal groups (the raw, 70, 100 and 120 °C). The samples of two groups (70 and 100 °C groups) were placed in a digital temperature-controlled water bath at 70 and 100 °C for 30 min, respectively. Another group was placed in a digital temperature-controlled steam cooker at 120 °C for 30 min. The 20 raw strips didn't undergo any heat treatment as the control (Raw group).

Raman measurements

Raman measurements were performed according to Berhe *et al.* [2] 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 to collect the Raman scattering from the samples. An Argon Ion Laser (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 400-3200 cm^{-1} scans with 120 mW of laser power. The contents of secondary structures were calculated using PeakFit 4.12 software (SeaSolve Software Inc., USA).

Evaluation of digestibility of myofibrillar proteins in vitro

Proteins digestibility was assessed as described by Bax *et al.* [3] with slight modifications. 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. Digestion was stopped by the addition of 15% (final concentration) trichloroacetic acid. 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). The proteolysis rate was determined as above description.

III. RESULTS AND DISCUSSION

Analysis of amide I spectra profile

The assignments of the amide I (1600-1700 cm^{-1}) bands were carried out according to Berhe *et al.* [2]. The

corresponding results of secondary structures of proteins were shown in Figure 1A. 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 groups. Random coils content showed a significantly increase at 70 and 100 °C ($p<0.001$), and then no obvious change was observed at 120 °C. Compared with the raw, β-turn content showed an increase tendency ($p<0.001$) at 70 and 120 °C. These results further implied that cooking over 100 °C accelerated the rebuilding of proteins structure, affirmed by the transformation of α-helix to β-sheet and β-turn.

Evaluating the relationship between the structural changes an digestibility of proteins

The pepsin activity on myofibrillar proteins (Figure 1B) significantly increased at 70 and 100 °C, and then decreased at 120 °C during the whole hydrolysis. The similar activities of trypsin & α-chymotrypsin on myofibrillar proteins were also observed among four groups. Interestingly, 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 nutrition of Jinhua ham. Amide I is most useful band to analyze secondary structural information of proteins. In order to clarify the relationship between the structural changes and the digestibility of proteins, Pearson correlation was performed. Correlation analysis showed that pepsin activities were significantly and negatively correlated with α-helical and β-sheet in amide I bands ($r = -0.522$, $P = 0.0379$; and $r = -0.887$, $P < 0.001$, respectively), and were positively correlated with β-turn and random coil in amide I bands ($r = 0.579$, $P = 0.019$; and $r = 0.920$, $P < 0.001$, respectively), and that trypsin & α-chymotrypsin activities were also significantly and negatively correlated with α-helical and β-sheet in amide I bands ($r = -0.833$, $P < 0.001$; and $r = -0.579$, $P = 0.019$, respectively), and were positively correlated with β-turn and random coil in amide I bands ($r = 0.513$, $P = 0.042$; and $r = 0.913$, $P < 0.001$ respectively).

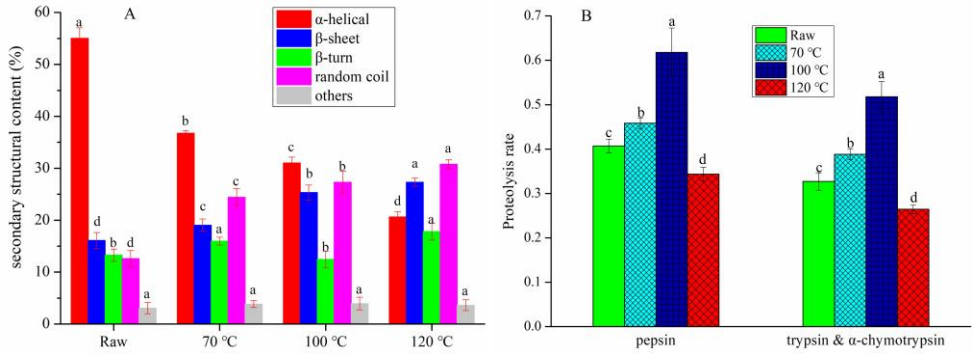

Figure 1 The effect of cooking temperature on secondary structural contents and proteolysis rate of myofibrillar proteins

IV. CONCLUSION

Cooking at 70 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. Proteolysis rate significantly increased at 70 and 100 °C, and then decreased at 120 °C after the pepsin and trypsin & α-chymotrypsin incubation, indicating that cooking at 100 °C could be a critical point to gain the nutrition of Jinhua ham. Correlation analysis further showed that the digestive enzymes activities were negatively correlated with α-helical and β-sheet, and were positively correlated with β-turn and random coil. High proteolysis rate at 100 °C could be explained by the fact that the unfolding of proteins maximized the recognition of digestive enzymes.

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REFERENCES

1. Santé-Lhoutellier V., Astruc T., Marinova P., Greve E., and Gatellier P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. Journal of Agricultural and Food Chemistry 56:1488-1494.
2. Berhe, D. T., Engelsen, S. B., Hviid, M. S., & Lametsch, R. (2014). Raman spectroscopic study of effect of the cooking temperature and time on meat proteins. Food research international, 66, 123-131.
3. Bax M. L., Aubry L., Ferreira C., Daudin J. D., Gatellier P., Rémond D., and Santé-Lhoutellier Vr. (2012). Cooking temperature is a key determinant of in vitro meat protein digestion rate: investigation of underlying mechanisms. Journal of agricultural and food chemistry 60:2569-2576.