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In-vitro digestive Peptides Differ by Species

Siying Wen[1], Guanghong Zhou[1], Xinglian Xu[1], Chunbao Li[1]*

1 Key Laboratory of Animal Products Processing, MOA, Key Laboratory of Meat Processing and Quality Control, MOE, Synergetic Innovation Center of Food Safety and Nutrition, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, P.R. China

*Correspondence: Dr Chunbao Li (E-mail: chunbao.li@njau.edu.cn)

Abstract - In this study, we simulated gastrointestinal digestion of cooked pork, beef, chicken and fish, and differentiated their digestion products. Before enzymatic digestion, the four types of cooked meat showed significant differences in SDS-PAGE gel bands. After pepsin digestion, pork and beef had a greater number of fragments in similarity than chicken and fish meat after pepsin digestion, while the species differences were less pronounced after the second digestion with trypsin.

Key words - *in vitro* digestion; meat protein; peptidomics; bioactive peptides

I. INTRODUCTION

Meat is known to be an important source of human nutrition as it contains high-value proteins, lipids, minerals and vitamins.(1) It has been recognized that different species of muscle protein lead to different internal digestion. It remains unknown about the meat-derived peptides difference, especially their effects on human nutrition. The objectives of the present study were to characterize the *in vitro* digestive products of cooked pork, beef, chicken and fish meat with pepsin and trypsin and to explore bioactive peptides.

II. MATERIALS AND METHODS

Pork *longissimus dorsi* muscle, beef *longissimus dorsi* muscle, chicken *pectoralis major* muscle and fish muscle from silver carps were obtained from a commercial meat packing company. All samples were cooked in water bath until the center temperature of meat pieces or pies reached 70 °C. Determination of pH was carried out as described as Jeacocke.(2) The mean values for meat quality traits are shown in Table 1. Cooked meat was *in vitro* digested according to the procedures of Escudero et al. with some modifications.(3)

Table 1 pH and cooking loss (%) of raw pork, beef, chicken and fish meat (mean ± SD, n=8)

species	pH	cooking loss (%)
pork	5.83±0.18^{b}	21.35±3.99^{a}
beef	5.46±0.08^{c}	22.16±2.97^{a}
chicken	5.83±0.05^{b}	8.62±2.28^{b}
fish	6.67±0.17^{a}	22.38±3.18^{a}

Both the pepsin digests and pepsin/trypsin digests were deproteinized by adding three volumes of ethanol and storing for 12 h at 4 °C. The ethanol-soluble and the precipitate fractions were separated after centrifuged at 10,000×g for 20 min.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to characterize the total protein profiles before digestion and ethanol-insoluble fraction profiles after digestion.

The ethanol-soluble fractions of the pepsin digest and the pepsin/trypsin digest were characterized by both MALDI-ToF MS(ultrafleXtreme Bruker, Germany) and a hybrid quadrupole orbitrap mass spectrometer equipped with a

nanoelectrospray ionization source (Q-Exactive, Thermo Fisher Scientific, USA) in duplicate.

The potential biological activity of identified peptides was evaluated with PeptideRanker (http://bioware.ucd.ie/~compass/biowareweb/), and a peptide was labeled as bioactive if it received a score higher than 0.5.(4) In addition, all the potential bioactive peptides were further evaluated using basic local alignment search tool (BLAST) matching against PepBank databases (http://pepbank.mgh.harvard.edu/) which included known bioactive peptides.(5)

III. RESULTS AND DISCUSSION

Before digestion, there are significant differences in band intensities among the four species of cooked meat. After pepsin digestion, all meats showed significant changes. When pepsin-treated samples were further incubated with trypsin, almost all of the bands disappeared, indicative of fragments lower than 5 kDa.

Pork and beef had similar peptide components with an m/z range between 1300 and 2200 after pepsin treatment and from 850 to 1050 after pepsin/trypsin treatment. Chicken showed a higher m/z range than pork and beef, whereas fish had a broader m/z range than the other three meats both after pepsin treatment and after pepsin/trypsin treatment. However, when the samples were treated with both pepsin and trypsin, species differences in the fragments from the ethanol-soluble fractions were weakened.

The nano-LC-MS/MS analysis showed that a total of 630 peptides were identified from the pepsin fragments and 302 peptides were identified from the pepsin/trypsin–treated groups, in which 101, 139, 174 and 135 peptides were specific for pepsin-treated pork, beef, chicken and fish meats, respectively. After pepsin and trypsin digestion, 302 peptides were identified, in which 73, 62, 92 and 35 peptides were specific for pork, beef, chicken and fish meat, respectively. Again, pork and beef showed the highest similarity in peptide sequences.

According to PeptideRanker and PepBank matching, there were 25, 9, 25 and 13 species-dependant bioactive peptides found in pork, beef, chicken and fish meat after pepsin digestion as the venn diagram showed (fig1a). Four peptides from pork and two peptides from chicken were predicted to have ACEI activity. Thirty-one peptides from one or more species could have antioxidative activity. In addition, the bioactive functions of forty-seven peptides were not characterized. However, after further digestion by trypsin all of these bioactive peptides disappeared, but 36 new ones were found, of which 4, 13, 9 and 5 were specific for pork, beef, chicken and fish, respectively(fig1b). Of these bioactive peptides, 14 peptides were predicted to have antioxidative activity.

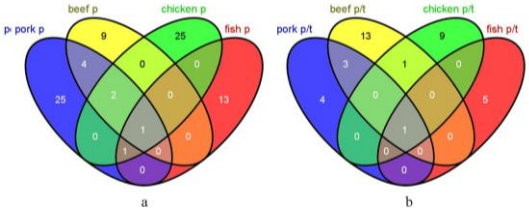

Figure 1. Venn diagrams of bioactive peptides obtained from pork/beef/chicken/fish meat hydrolysates with a PeptideRanker score of >0.5.

Pork p, beef p, chicken p and fish p represented the peptides digested by pepsin from pork, beef, chicken and fish, and pork p/t, beef p/t, chicken p/t and fish p/t represented the peptides digested by pepsin and trypsin from pork, beef, chicken and fish. (a) showed the interaction of the pepsin digests of the four kinds of meat, and (b) showed the interaction of the pepsin and trypsin digests.

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REFERENCES

(1) Pereira, P. M. D. C. C., & Vicente, A. F. D. R. B. (2013). Meat nutritional composition and nutritive role in the human diet. Meat science, 93(3): 586-592.

(2) Jeacocke, R. E. (1977). Continuous measurements of the pH of beef muscle in intact beef carcases. International Journal of Food Science & Technology, 12(4): 375-386.

(3) Escudero, E., Sentandreu, M. A., & Toldra, F. (2010). Characterization of peptides released by in vitro digestion of pork meat. Journal of agricultural and food chemistry, 58(8): 5160-5165.

(4) Mooney, C., & Pollastri, G. (2011). SCLpred: protein subcellular localization prediction by N-to-1 neural networks. Bioinformatics, 27(20): 2812-2819.

(5) Panchaud, A., Affolter, M., & Kussmann, M. (2012). Mass spectrometry for nutritional peptidomics: how to analyze food bioactives and their health effects. Journal of proteomics, 75(12): 3546-3559.