PEPTIDE PROFILING ANALYSIS OF JINHUA HAM BROTH PEPTIDES AT DIFFERENT COOKING TIMES

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I. INTRODUCTION

Dry-cured ham is a premium product with a long history. Its production process can last for 6-24 months [1]. Some of the peptides produced during proteolysis are biologically active and thus potentially beneficial for human health [2]. Numerous reports have demonstrated that Chinese dry-cured ham, including Jinhua and Xuanwei, as well as Spanish dry-cured ham, contain peptides with antioxidative properties [3]. It is also worth noting that the traditional Chinese dry-cured ham has a salt concentration of 6-15%, which is substantially greater than dry-cured ham from European countries [4]. Because of its high salt content, Chinese consumers more commonly use Jinhua ham to prepare broth rather than consume it directly. However, the effects of Jinhua ham broth cooking on the release of peptides have not been reported yet. Therefore, this study aimed to examine the effects of various cooking times on peptide profiles by simulating traditional soup-making conditions.

II. MATERIALS AND METHODS

2.1 Extraction of Jinhua ham broth peptides

The peptides extracted from uncooked ham are referred to as 'uncooked' JHBP-0. Four 'cooked' groups (JHBP-1, JHBP-1.5, JHBP-2, JHBP-2.5) were obtained by cooking with slight modifications based on the method of Zhang et al. [5]. To do so, 200 g of meat were added to 800 mL of water in a ratio of 1:4 (w/v) and boiled at 2100 W using induction cookers, after which the power was adjusted to 300 W to maintain a simmer for 2 h. Water was replenished periodically during cooking to maintain a ratio of 1:4 of meat-to-water, ensuring constant total volume. After filtering, the ham broth was passed through a double-layered gauze to remove insoluble impurities. The ham broth was cooled to room temperature, after which 100 mL of ham broth was mixed with three times its volume of 40% ethanol. After resting the samples at 4℃ for 12 h, centrifugation (12000 g, 10min) followed by rotary evaporation was performed, then freeze-drying at -80℃ in a freeze dryer, and storing of the dried samples at -20℃.

2.2 Characterization of peptide sequences

The desalted peptides were diffused in 0.1% formic acid (solvent A) before being analyzed by Nano LC system coupled with Orbitrap Exploris 480 mass spectrometer with FAIMS (High-Field Asymmetric Waveform Ion Mobility Spectrometry). The analytical columns were applied to perform the chromatographic separation using a 30-minute linear gradient of 3-35% buffer B (80% acetonitrile with 0.1% FA) at a flow rate of 0.3 μL/min. FAIMS had a compensation voltage (CV) of -45 V and -65 V. The mass spectrometer was set to data-dependent analysis (DDA) mode with a dynamic exclusion of 30 s and full-scan MS spectra (m/z 350-1,500) with a resolution of 60,000 (m/z 200) and a resolution of 15,000 (m/z 200) in MS/MS scans.

2.3 Bioinformatics analysis

The peptides extracted from the various broths were identified using PEAKS Studio X Pro (Version 10.6). The UniProt database was used to identify peptides and proteins of origin, with a parent mass error tolerance of 10 ppm and a fragment mass error tolerance of 0.2 Da. Proteomes from *Sus scrofa* (pig) and 'no enzymes' were set as options. For peptide sequences, the processed data used database searching with an FDR≦1%.

III. RESULTS AND DISCUSSION

There were 1,306, 1,352, 1,431, 1,500, and 1,556 peptide sequences found for JHBP-0, JHBP-1, JHBP-1.5, JHBP-2, and JHBP-2.5, respectively (Figure. 1A). Fifty of these peptides were repeated in the five groups, while the unique peptide sequences were 873, 411, 523, 567, and 745 for each group, respectively. Hence, there were 13 identical source proteins in the five groups of which 6, 10, 13, 16, and 20 source proteins being unique to the respective five groups (Figure. 1B). The number of distinctive source proteins was dependent on the cooking time process. In identical source proteins, myosin and actin were the most prevalent sources of peptides (Figure. 1C). The number of peptides from the four source proteins, including myosin, actin, mLC1f and aldolase-fructose-diphosphate A, accounted for more than 50% in each group.

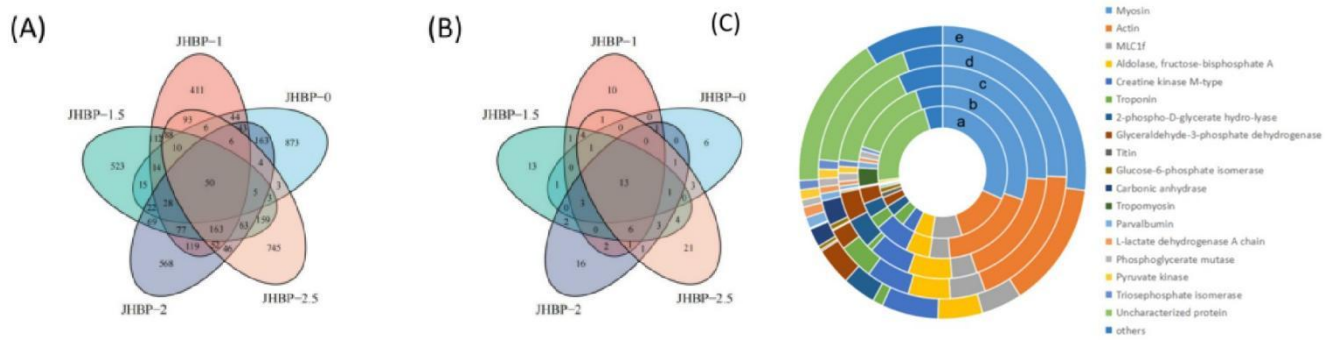

Figure 1. Venn diagram of characteristic peptides (A). Source proteins for peptides (B). Distribution in percentages of peptides according to the origin proteins in muscle of *Sus scrofa*: a. JHBP-0; b. JHBP-1; c. JHBP-1.5; d. JHBP-2; e. JHBP-2.5 (C).

IV. CONCLUSION

The current research revealed that different cooking times altered the peptide profile of Jinhua ham broth peptides. Cooking increased the proportion of <1 kDa peptides and significantly reduced the proportion of peptides in all molecular weight ranges. Meanwhile, cooking increased the abundance of peptides released from myosin and actin, thereby further demonstrating that cooking promotes proteolysis.

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