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EFFECTS OF *IN VITRO* SIMULATED GASTROINTESTINAL DIGESTION ON THE PEPTIDE PROFILE OF JINHUA DRY-CURED HAM BROTH

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I. INTRODUCTION

The fermentation of dry-cured ham induces protein degradation through the combined action of endogenous enzymes and microbial activity, which leads to higher concentrations of bioactive peptides [1]. With salt concentrations ranging from 6% to 15%, Chinese dry-cured hams are typically incorporated into stewed broths rather than consumed directly [2]. After proteins enter the human body, they are processed by digestive enzymes such as pepsin in the stomach, and trypsin and chymotrypsin in the intestine, hydrolyzing into smaller fragments [3] and releasing more peptide fragments with high bioactivity [4]. Therefore, this study aimed to investigate the effects of *in vitro* simulated gastrointestinal digestion on the peptide profile of dry-cured ham broth.

II. MATERIALS AND METHODS

2.1 Extraction of Jinhua ham broth peptides

The 200 g of ham samples were added to 800 mL of water and boiled at 2,100 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 °C for 12 h, they were centrifuged (12,000 × g, 10 min), followed by rotary evaporation. The resulting product was then freeze-dried at -80 °C in a freeze dryer and stored at -20 °C.

2.2 *In vitro* simulated gastrointestinal digestion

Following the method of Minekus et al. [5], simulated gastric fluid (pH=2) and intestinal fluid (pH=7) were prepared. Then 5 mL ham broth was mixed with 4 mL gastric fluid and homogenized four times (12,000 rpm/min, 10 s each) to simulate gastric peristalsis. After adjusting the pH to 2, pepsin was added (EA=2,000 U/mL) and incubated at 37 °C with shaking (75 rpm/min) for 2 h. Part of the sample was centrifuged (8,000 g/min) to collect the supernatant, while the remainder continued for further digestion. The 5 g of gastric digesta was mixed with 4 mL intestinal fluid and similarly homogenized four times. After adjusting pH to 7, trypsin (EA=100 U/mL) and chymotrypsin (EA = 25 U/mL) were added and incubated at 37 °C for 2 h. The mixture was heated at 100 °C for 3 min to inactivate enzymes, cooled, adjusted to pH 7, and centrifuged to collect the supernatant.

2.3 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.

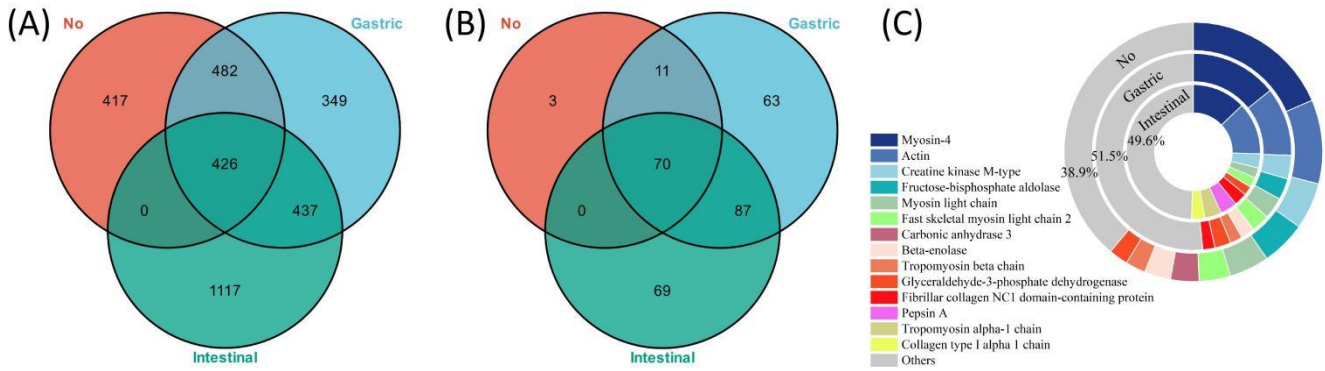
2.4 Bioinformatics analysis

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 a FDR≦1%.

III. RESULTS AND DISCUSSION

There were 1,306, 1,352, and 1,980 peptide sequences for undigested, simulated gastric digestion, and simulated intestinal digestion, respectively (Figure. 1A). The 426 of these peptides were repeated in the five groups, while the unique peptide sequences were 417, 349, and 1,117 for each group, respectively. Hence, there were 70 identical source proteins in the three groups of which 3, 63, and 69 source proteins were unique to the respective three groups (Figure. 1B). The number of distinctive source proteins was dependent on the digestion phase. In identical source proteins, myosin and actin were the most prevalent sources of peptides (Figure. 1C). Peptides derived from fibrillar collagen NC1 domain-containing protein, tropomyosin alpha-1 chain, and collagen type I alpha 1 chain significantly increased after simulated gastrointestinal digestion.



IV. CONCLUSION

This study demonstrates that in vitro simulated gastrointestinal digestion significantly alters the peptide profile of dry-cured ham broth. The digestive process increases peptide diversity, with intestinal digestion yielding the highest number of unique peptides (1,117) and source proteins. While myosin and actin remain predominant peptide sources throughout digestion, significant increases were observed in peptides derived from fibrillar collagen NC1 domain-containing protein, tropomyosin alpha-1 chain, and collagen type I alpha 1 chain after digestion. These findings suggest that gastrointestinal processing enhances the bioactive potential of dry-cured ham broth through increased peptide release and diversification.

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