P-07-10

Enzymatic effects on peptide aggregation following plastein reaction of porcine hemoglobin and meat by LC-MS/MS (#208)

Qian Li, René Lametsch

Department of Food Science, University of Copenhagen, Frederiksberg C, Denmark

Introduction

Protein hydrolysates can aggregate into less soluble and higher molecular substances which is called plastein when additional protease are added at high substrate concentration. Plastein reaction was reported to enhance the nutritional value of proteins, reduce the bitterness and improve the bioactivities of protein hydrolysates. Hydrolysates from different protein sources such as milk, wheat, soybean, etc. have been used as materials for plastein reaction. However, plastein reaction of meat proteins or meat by-products is less investigated, and the related mechanism of plastein reaction has not been thoroughly elucidated. Three potential mechanisms have been proposed (peptide condensation, transpeptidation and physical forces). In-depth knowledge of the mechanisms behind plastein reaction is necessary in the development of plastein production and its further application in food processing industry.

Methods

The present work aimed to compare the effects of microbial and plant-derived proteases (Alcalase and papain) on plastein reaction (0, 3, 6 and 24 h) of protein hydrolysates of porcine meat and hemoglobin under the optimal conditions. Four groups of plastein samples with alacalase-treated hemoglobin, papain-treated hemoglobin, alcalase-treated meat and papain-treated meat were abbreviated as AH, PH, AM and PM. Correspondingly, their controls with inactivated proteases were named AHC, PHC, AMC and PMC, respectively. The yield of hydrolysates, free amino groups, particle size distribution, turbidity, chemical interactions of non-covalent bonds and disulfide bonds, and peptide characterization of plastein and the control group were compared to illustrate the mechanisms of plastein reaction in porcine meat and hemoglobin.

Results

The yields of protein hydrolysates of porcine hemoglobin catalyzed by alcalase and papain were significantly higher ($P < 0.05$) than the yields of protein hydrolysates derived from meat, and alcalase produced higher yields of protein hydrolysates of both hemoglobin and meat. As reaction time prolonged, a single peak in alcalase-treated samples towards an increasing particle size proved the formation of plastein, and meanwhile, no obvious difference in distribution patterns were observed in control group. Similarly, a rise in turbidity in all groups indicated the increase of insoluble substances that formed from plastein reaction. Decrease in free amino groups usually represents peptide condensation but was not obvious in our study, suggesting that the condensation was not the key factor in the present work. The percentage of hydrophobic interaction of all plastein samples of hemoglobin and meat was above 60%, reflecting its significant role in enzyme-induced peptide aggregation. In LC-MS/MS analysis, precursor proteins including porcine hemoglobin subunit alpha (P01965) and subunit beta (P02067), as well as skeletal muscle alpha-actin (P68137) and myosin-1 (Q9TV61) were chosen for study. A few peptides prepared from hemoglobin at some regions of amino acid sequences were more likely to aggregate, and highly participated in plastein reaction treated by both enzymes.

Conclusion

In conclusion, porcine hemoglobin was a better substrate than meat for plastein reaction when considering higher rate of aggregation formation. Covalent bonds including peptide bonds and disulfide bonds were not key factors in aggregation of hydrolysates, whereas non-covalent interactions especially hydrophobic interaction were considered as the major mechanism in plastein reaction. The visualization of peptide profiles illustrated the amino acid position of aggregative peptides located in precursor proteins, and the outlined peptides will be synthetized and validated for aggregation in further study.

Notes