PEPTIDOMIC ANALYSIS OF PORCINE LIVER HYDROLYSATES USING SWATH-MS TO SEARCH BIOPEPTIDES

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I. OBJECTIVES

Bioactive peptides are of great interest from a biotechnological point of view, and there is a major concern about their identification and isolation. Peptidomic analysis using SWATH-MS offers a great opportunity to identify and quantify the biopeptides in a complex mixture. On the other hand, protein-rich meat byproducts and enzymatic hydrolysis are being tested to obtain biopeptides with antioxidant, antidiabetic, or antimicrobial activity. Specifically, the porcine liver hydrolysates have proved to have antioxidant and antimicrobial activity, resulting in an effective method to produce added-value products and functional ingredients. Additionally, the use of pork liver and other offal contributes to the sustainability of meat industry.

II. MATERIALS AND METHODS

Livers were cleaned of fat and connective tissues and cut into small pieces. The homogenization was carried out with ice in a cutter machine. Afterwards, enzymatic hydrolysis was performed using 4 different enzymes: papain, bromelain, Alcalase, and Flavourzyme. The conditions of enzymatic reaction were 37°C and pH 6 for papain, 40°C and pH 6 for bromelain, 50°C and pH 8 for Alcalase, and 50°C and pH 5.5 for Flavourzyme. To analyze the peptides by mass spectrometry, digestion of peptide mixtures with trypsin was performed. Twelve replicates were realized for each enzymatic treatment. A shotgun data-dependent acquisition approach using micro-liquid chromatography-mass spectrometry was obtained prior to peptide quantification. Finally, a data-independent acquisition from SWATH-MS analysis was used for peptide quantification for peptide mixtures.

III. RESULTS

A total of 2,022 peptides showed quantitative significant differences between control and hydrolysates from enzymatic digestions ($P < 0.05$). This showed that liver proteins were largely digested by papain, bromelain, Alcalase, and Flavourzyme, indicating that these hydrolysates are an adequate choice to search biopeptides. To develop this approach, we profiled peptide mixtures from enzyme treatments, and the profiles were compared by principal component analysis. This analysis allowed us to reduce a large number of peptide quantifications of the dataset into a smaller number of dimensions without losing significant information. As expected, the principal component analysis reflected that the control samples have a peptide profile quite different from the rest. This suggests that the number of peptides in enzyme samples was far higher due to enzymatic degradation. On the other hand, the porcine liver hydrolysates from Alcalase were particularly different with respect to the other enzymes, thereby generating distinguishing peptides by Alcalase. Among enzymatic treatments, 2 different groups of peptides were found to be differentiated.

IV. CONCLUSION

Therefore, the Alcalase hydrolyzation or the others may be used to search biopeptides with the required activity and subsequent isolation.

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