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METHOD DEVELOPMENT FOR PEPTIDOME CHARACTERIZATION

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The concept of dietary bioactive peptides brings a new dimension that should be considered in the description of dietary protein quality. Bioactive peptides have been defined as protein fragments that have, in addition to their nutritional value, a positive impact on body functions (vascular system, immunity, metabolism …) and may ultimately influence health. Most dietary proteins contain bioactive sequences, but the corresponding peptides need to be released by proteolysis during digestion, and absorbed from the gut.

The purpose of this study was to develop the analytical tools required for peptide isolation, characterization and identification by mass spectrometry, in plasma and serum samples.

A rapid and robust sample preparation method, based on a protein precipitation using acetonitrile, was optimized. Peptides profiles were then determined using Ultra Performance Liquid Chromatography coupled on-line to a LTQ-Orbitrap Velos mass spectrometer. The separation was performed on an Aeris peptideXB-C18 column and using a 17 min gradient. Data were collected in positive ion mode in scan and data dependent MS/MS modes. Two approaches depending on peptide masses were developed to perform the data processing and the identification. Peptides of masses between 400-800 m/z were identified with proteomic tools: ions were extracted using Progenesis LC-MS software, and Mascot (http://www.matrixscience.com/) was used to identify peptides in the merged peaklist generated by Progenesis. For peptides of lower masses between 100-500 m/z, not listed in proteomic databases, metabolomic tools were used: the raw data were transformed to centroid mode and mass corrected before being analyzed using XCMS The data were peak detected and noise-reduced. Identification was performed using an in-house database built from peptide data extraction from the Human Metabolome DataBase ((http://redpoll.pharmacy.ualberta.ca/hmdb/HMDB/) and from the BIOPEP website (http://www.uwm.edu.pl/biochemia). The validation of the putative identification was performed by analysis of their fragmentation with the combination of CID and HCD.

The developed method allowed us characterizing the peptide fraction in plasma or serum samples, to study the bioavailability of peptides and their kinetics of appearance in blood. following the intake of a complete meal. ((http://redpoll.pharmacy.ualberta.ca/hmdb/HMDB/) and from the BIOPEP website (http://www.uwm.edu.pl/biochemia).

The validation of the putative identification was performed by analysis of their fragmentation with the combination of CID (collision induced dissociation) and HCD (Higher-energy C-trap dissociation) a good compromise will be possible to obtain better fragmentation informations.

The developed method allowed us characterizing peptide fraction from plasma or serum samples, to study the bioavailability of peptides and their kinetics of appearance in blood following the intake of a complete meal.