Enhanced identification of bioactive peptides in meat hydrolysates by 4D peptidomics

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Introduction

- Food proteins encrypt biologically active peptide sequences that can positively modulate health, and which may be released by processing or consumption.
- Meat hydrolysates are known to contain peptides with desirable biological functions.
- Current peptidomics approaches via LC-MS/MS analysis (3D methods) provide qualitative and quantitative analysis of free peptides but have encountered peptide identification limitations.

Aim

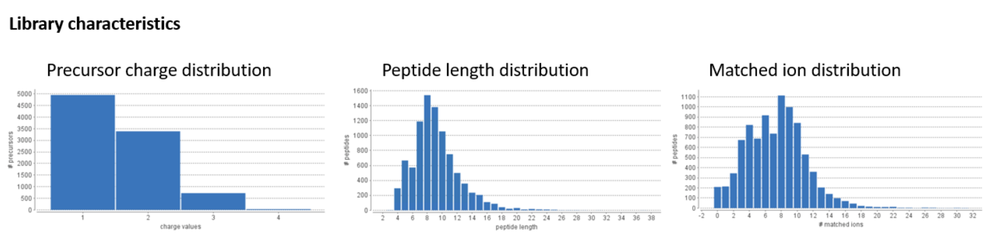
This preliminary study aimed to see if recently developed 4D acquisition methods have the potential to enhance bioactive peptide identification in nutraceutical research.

Methodology

- Peptides were produced by enzymatic hydrolysis of fresh bovine meat using pepsin.
- A nanoElute UHPLC was coupled online to a Bruker timsTOF Pro and peptides were separated on a reversed-phase column (15 cm x 75 µm i.d.) (IonOpticks) using a linear gradient from 2 to 28% B (0.1% formic acid/acetonitrile/water), where solvent A was 0.1% formic acid.
- A discovery PASEF method was used to build the spectral library, including fragmentation of singly charged ions.
- The instrument firmware was adapted to perform diaPASEF, with data independent isolation of multiple precursor windows within a single TIMS separation (85 ms) and opening up the DIA range with singly charged ions.
- Analysis of the 4D data space was performed using PEAKS X+ (Bioinformatics Solutions Inc.).
- In-house software was used for prediction of bioactivity.

Results

- A large-scale 4D peptidomics analysis of a meat hydrolysate was performed followed by *in silico* identification of bioactive peptides
- To identify and quantify peptides from diaPASEF data, a project specific library was built using 12 high-pH reversed-phase peptide fractions acquired via DDA PASEF.
- A PEAKS X+ workflow was used to analyse the DDA runs and generate ion mobility-enabled spectral libraries directly from the PASEF runs, which were used for targeted data extraction.
- The library build with the DDA data comprises more than 8300 peptides, with most of them being singly or doubly charged ions.



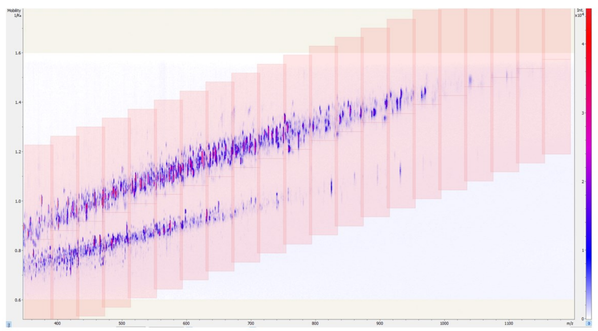


Figure 1: DiaPASEF acquisition isolation scheme using of 50 Th windows and selection of both 1+ ions (top part) and 2+ and 3+ ions (lower part).

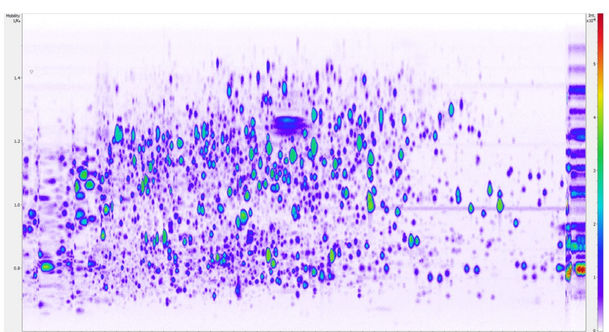
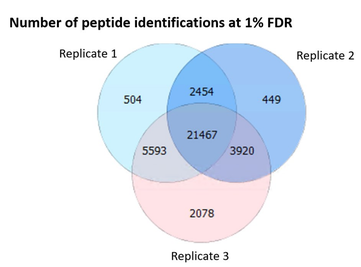


Figure 2: Heatmap visualising the time vs. mobility of all precursor ions in the diaPASEF run of a single meat hydrolysate peptidomics sample.

- With the library, we investigated the performance of diaPASEF in the peptidome analysis of *Bos taurus* meat hydrolysate.
- Overall, we identified more than 2618 unique peptide sequences at 1% FDR from triplicate injections.



Discussion

- In this work, a new acquisition method was developed to allow the analysis of small peptides, including singly charged, by the application of 4D peptidomics with diaPASEF technology.
- Previous results acquired on a Impact II Q-TOF system and characterised via Peaks X+ with similar parameters, identified 1810 peptides in this meat hydrolysate, of which 24 sequences can be linked to potential bioactive properties as exact or partial matches.
- With our custom library, containing 8378 peptides, we identified 102 peptides with predicted bioactivity as an exact or partial match.
- Compared to our 3D systems, the increase in peptidome depth by a 4D approach not only increases numbers of identified unique peptides, but also numbers of bioactives, which now can be studied in greater depth.
- Overall, these results demonstrate the potential for 4D data acquisition to deliver crucial insights of the functionality of meat.

Conclusion

Our preliminary study demonstrates the value of the enhanced 4D data acquisition method for characterising bioactive peptides present in a potential functional foods in much more depth than was previously possible.

Acknowledgements

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