A data-dependent workflow for selection of peptide targets for robust detection of allergens in difficult food matrices

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OVERVIEW

• An experimental and bioinformatic pipeline for the selection of surrogate peptides for allergen detection based on generating quantitative untargeted data from allergens in food matrices is described.

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- This method differs from traditional surrogate peptide discovery in that quantitative recovery of potential surrogate peptides from food matrices is the primary (first) selection criteria.
- The ability of our novel pipeline to generate surrogate peptides was trialed using peanut in cumin and garlic as a testbed. The analysis of peanut in spices has been highlighted due to product recalls, particularly in the US.
- 15 peanut peptides were identified as potential surrogates for peanut in both cumin and garlic matrices.

Samples for DDA

- Raw peanut
- Raw peanut in cumin
- Raw peanut in garlic
- Roasted peanut in cumin
- Roasted peanut in garlic
- Cumin

Garlic

- Designed for large scale (0.5 g +)
- samples and inexpensive components

Sample preparation

METHODS

- for use in food analysis :
- 50 mM Tris-HCl, pH 8.8
- 20 mM DTT
 - 4% (w/v) PVPP

DDA MS analysis

Thermo Scientific[™] Q Exactive[™] Plus Hybrid Quadrupole-Orbitrap MS coupled to a Thermo Scientific[™] UltiMate[™] 3000 RSLC system, equipped with a Thermo Scientific[™] Hypersil GOLD[™] C18 1.9 µm, 100 x 1 mm analytical reversed phase column

Comparison of DDA to PRM performance

• Select peptides with varying robustness in food

Data analysis Identification and label-free quantitation using Thermo Scientific™

- Subsequent PRM analysis demonstrated the comparability of peptide performance in targeted and untargeted experiments, and therefore validated our workflow for the selection of peptides for allergen quantitation.
- matrices from varying allergenic proteins.
- Targeted (PRM method) using Q Exactive Plus Hybrid Quadrupole-Orbitrap MS to assess relative abundance across all peanut-containing samples and matrix controls.
- Proteome Discoverer[™] software ver. 2.6.
- Comparative analysis to identify peanut peptides which occur with
- -Similar abundance in all peanut-containing samples
- -High overall abundance
- -Do not occur in garlic and cumin control analyses

INTRODUCTION

Management of food allergens during food production requires analytical methods allowing for the specific detection of food allergens in complex and processed food ingredients. A key factor is the ability of analytical methods to equivalently detect food allergens in a variety of foods with differing processing and matrices. Many current methodologies, such as some commercial ELISA kits yield results that are dependent upon food processing and matrix (see right).

For MS methods, the current paradigm for selecting peptides to act as surrogates for food allergens is to use primary bioinformatic analysis with supporting untargeted MS experiments, and does not consider the effect of processing and matrix. This results in methods which can give different results for the same amount of allergen in different foods. We therefore explored an alternative workflow for peptide target selection for food allergen detection based on utilizing quantitative DDA analysis to predict targeted method behavior in food matrices.

prolamin family).

cupin family and

upon thermal

others)



- A commercial peanut ELISA kit was used to analyze peanut (raw or roasted) spiked into cumin powder.
- The presence of raw peanut in cumin samples was overrepresented by 4-6 fold, indicating that correct quantitative analysis would be impossible.



1. Effect of peanut roasting

2. Effect of the food matrix

• When cumin and garlic are





used as a matrix, more peptides fall in abundance compared to an unprocessed peanut sample.

- This loss of abundance is considerably more severe with cumin than with garlic.
- The effect of thermal processing (Fig. 1) and matrix is synergistic – heating + matrix have a greater effect than would be anticipated from either effect alone.

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4. Can DDA analysis predict matrix robustness in PRM ?



- A comparison of peptide robustness using DDA vs that obtained with PRM (A) demonstrates the utility of DDA as a predictor of targeted method behavior.
- The recovery of selected robust \bullet

- More potential peptide targets could be identified for garlic than for cumin.
- Interestingly, the peptides robustly quantified in cumin primarily belong to the prolamin family of proteins.
- The peanut peptides which are robustly detected in cumin are also robustly detected in garlic. These peptides are suitable for development of a method for detection in both matrices.



peptide and a non-robust peptide are shown in (B) and (C) respectively. The peptide shown in (C) is frequently used for MS detection of peanut.

CONCLUSIONS

FUNDING

- Processing and food matrix are essential considerations when developing an MS-based method for allergen detection.
- We can predict the robustness of peptide recovery from food matrices using DDA data.
- Peptide target selection based on label-free DDA data can be used to generate robust targets to analyze processed allergens in problematic food matrices using inexpensive large-scale extraction methodology.

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