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# Development of a data-dependent workflow for the selection of peptide targets for robust detection of peanut in spices


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**Keywords**

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## Goal

- Development of a workflow that allows selection of peptide targets that can be measured reproducibility in different food matrices and under differently processed conditions.
- A targeted MS/MS method for the detection of peanut in cumin and garlic.

## Introduction

In the absence of effective cures for food allergies, compulsory labeling of major allergens allows allergic consumers to avoid foods that pose a risk for them. To enable labeling, food manufacturers institute allergen management plans to ensure allergens are contained within intended products. Management of food allergens during food production requires analytical methods that allow for the specific detection of food allergens in complex and processed food ingredients. The diversity of food ingredients, combinations, and processing in a market such as that in the U.S. pose a challenge for analytical method development. A key factor is the ability of analytical methods to unequivocally and reproducibly detect food allergens in a variety of foods with differing processing and matrices. Many current

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methodologies, such as some commercial ELISA (enzyme-linked immunosorbent assay) kits, yield results that are dependent upon food processing and matrix.[1] This matrix-dependence means that reliable quantitation is often difficult and sometimes impossible.

All allergen detection methods use one or more target molecules to serve as analytical proxies for the presence of an allergenic food. For ELISA and MS, these proxies are proteins or peptides, whereas for polymerase chain reaction (PCR) methods, DNA is used. Depending on protein physicochemical properties, extraction efficiency might be different and may be matrix-dependent. We therefore aim to determine the identities of target peptides that are abundant and extract equivalently from different food types. Selection of peptide targets for analysis is left entirely to method developers, with no regulatory guidance. 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, but this approach does not consider the effect of processing and matrix.

Therefore, an alternative workflow was explored for peptide target selection based on utilizing quantitative data-dependent acquisition (DDA) analysis to predict targeted method behavior in food matrices. Although target selection was performed using DDA due to its ability to identify and quantify many peptide targets without prior selection, targeted detection using parallel-reaction monitoring (PRM) was preferred for a final detection method. Targeted detection methods are generally more sensitive and yield less complex data sets. The extent to which DDA analysis correlated with, and therefore could be used to predict, PRM behavior was therefore examined. Both quantitative DDA and PRM work were performed using a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer.

## Experimental

Replicated (three extracts, three analysis) data-dependent experiments were performed on raw and roasted peanut, and these materials were then spiked into cumin and garlic powders at a concentration of 100,000 mg/kg peanut. Extraction was designed to be inexpensive and to accommodate large (0.5 g+) quantities of food sample, and was performed using 50 mM Tris-HCl, 10 mM DTT, 3% (w/v) PVPP.

Following reduction, alkylation, and digestion, data-dependent analysis was performed using one-dimensional (1D) microscale liquid chromatography separation of tryptic peptides (5 µL injection) with a Thermo Scientific™ UltiMate™ 3000 RSLC liquid chromatography (UHPLC) system, equipped with a Thermo Scientific™ Javelin™ direct-connection column filter, 2.1 mm; a Thermo Scientific™ Hypersil GOLD™ aQ C18 1.9 µm, 20 × 2.1 mm pre-column; and a Thermo Scientific™ Hypersil GOLD™ C18 1.9 µm, 100 × 1 mm analytical reversed phase column. Mobile phase A consisted of water containing 0.1% (v/v) formic acid, while mobile phase B was 100% (v/v) acetonitrile containing 0.1% (v/v) formic acid. Five microliters of the sample were injected on-column. Peptides were eluted from the analytical column and separated using a gradient of 2-40% mobile phase B over 60 minutes at a flow rate of 60 µL/min. The analytical column temperature was maintained at 35 °C.

Mass spectrometric analysis utilized a Q Exactive Plus hybrid quadrupole-Orbitrap MS in the data-dependent mode with survey scans acquired at a resolution of 70,000. The target value for the fragment ion spectra was set to a resolution of 17,500. Up to the top 10 most abundant isotope patterns with charge 2 to 5 from the survey scan were selected with an isolation window of 1.5 Da and fragmented by higher-energy collisional dissociation with normalized collision energies of 27. The maximum ion injection times for the survey scan and the MS/MS scans were 100 and 60 ms, respectively, and the ion target values for scan modes were set to 1E6 and 2E5, respectively. Repeat sequencing of peptides was kept to a minimum by dynamic exclusion of the sequenced peptides for 10 s.

Twenty peptides with a range of recovery characteristics for PRM experiments were selected to examine the correlation of label-free quantitation and targeted quantitation methods in food matrices. Peptides were selected using the same column and solvents as described above, but with a 2-40% gradient over 34 min. PRM detection was achieved with a resolution of 70,000, AGC target of 2e5, maximum IT of 50 ms, and normalized CE of 25. An isolation window of 1.6 Da with no offset was used. Three fragment ions per peptide were used to quantify, with these selected based on abundance and chromatographic profile.

## Results and discussion

Peptides extracted from unroasted and roasted peanut broadly fell into two categories (Figure 1): those which were largely unaffected by thermal processing and those which were not. The peanut seed proteome is dominated by cupin- and prolamin-family storage proteins, both of which are allergenic. Peptides derived from prolamins were largely more extractable from roasted peanut.

When either unroasted or roasted peanut is spiked into cumin or garlic, loss of peanut-derived peptides abundance is observed (Figure 2). This loss of abundance is considerably more severe with cumin (A) than with garlic (B). The effect of thermal processing and matrix is synergistic, with only few peanut peptides successfully extracted and analyzed when roasted peanut is spiked into cumin. Examination of peptides that are both abundant and recover well from roasted peanut in cumin or garlic (Figure 3) showed that prolamin peptides, particularly those of the major allergens Ara h 2 and Ara h 6, make good targets for robust detection. Most peptides that perform well in a cumin matrix also perform well in garlic.

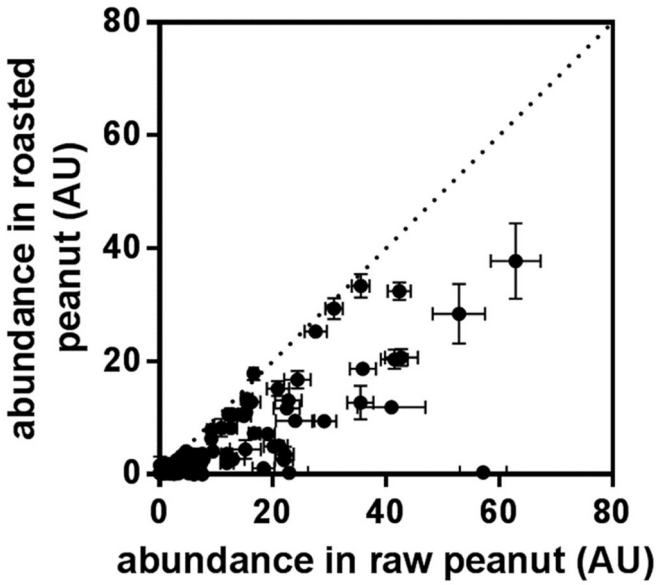


**Figure 1. Abundance of peanut peptides from extracts of raw peanut compared to those from roasted peanut.** Peptide quantitation was performed using label-free analysis of DDA data (n=9).

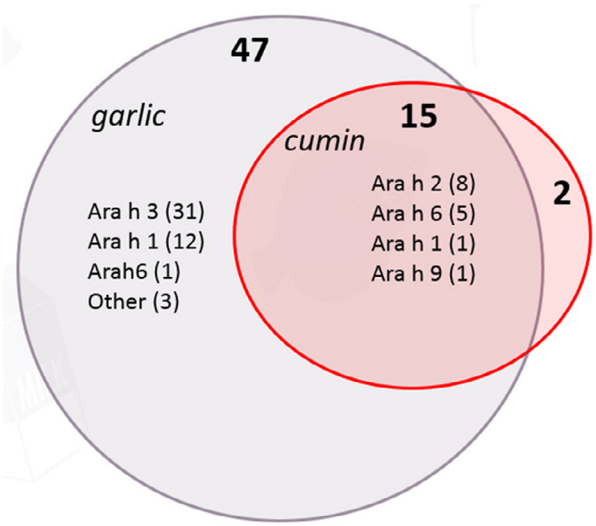


**Figure 3. Selection of suitable peptides (high abundance, high recovery from roasted peanut in a food matrix) for detection of peanut in cumin and garlic.** Protein origin of peptide targets is shown, with the number of peptides from each protein given in parentheses.

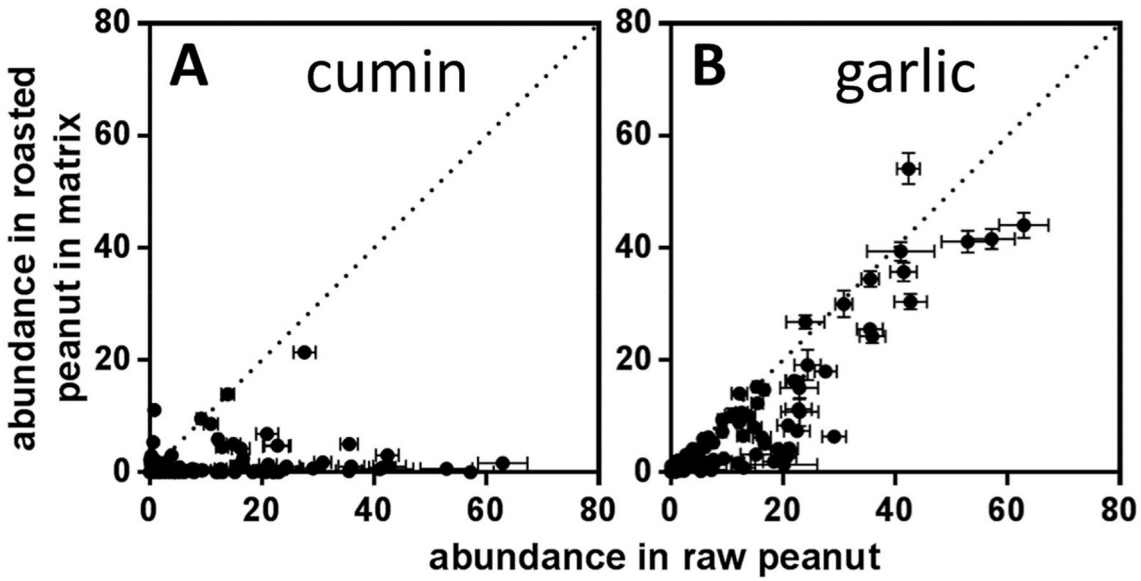


**Figure 2. Abundance of peanut peptides from extracts of raw peanut compared to those from roasted peanut in either a cumin (A) or garlic (B) matrix.** Peptide quantitation was performed using label-free analysis of DDA data (n=9).

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The untargeted MS method uses peptide quantitation based on the peak area of extracted ion chromatograms (EIC) of parent ions with ±5 ppm mass accuracy. The final allergen detection will use a targeted PRM workflow using secondary/fragment ion quantitation. Because quantitation workflows differ from target selection to final method, the ability of parent ion EIC to predict PRM performance was evaluated. Twenty peptides were selected (with utilization of three transitions/peptide) to give a range of recoveries (from 0% to the maximum observed) from roasted peanut in cumin (our most difficult analytical material) as a percentage of that from raw peanut. Recoveries of these peptides using parent ion EIC was compared to those using PRM (Figure 4).

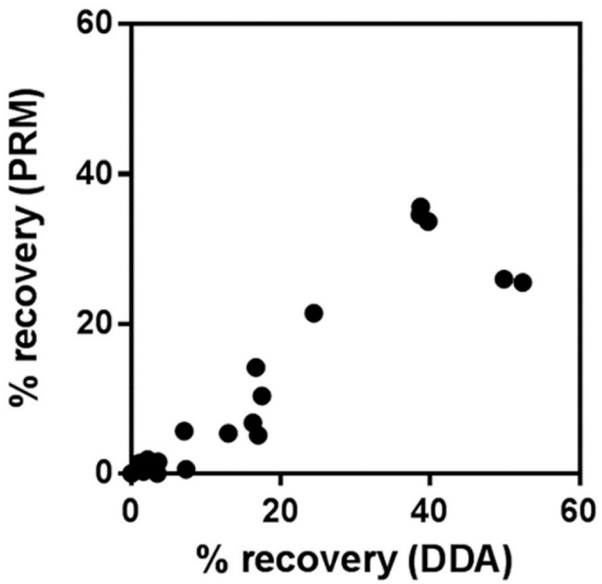


**Figure 4. Comparison of % recovery of peanut peptides from roasted peanut in cumin using DDA with parent EIC quantitation and PRM.** Data from 15 peptides is shown (mean of three replicates).

The untargeted quantitation provided good predictive power for the final PRM method, suggesting that parent ion EIC may be used to effectively select peptide targets for subsequent quantitation with a PRM method.

## Conclusions

Untargeted MS (here DDA) with label-free quantitation is an effective tool for the prediction of abundant, robust peptide targets for allergen detection in difficult matrices. Workflows based on these observations may be used to generate allergen detection methods which exhibit less variability in the range of processing conditions and food matrices, which may be expected to be encountered in routine food allergen analysis. MS-based allergen detection methods are almost exclusively targeted, largely due to increased sensitivity and selectivity, as well as decreased data analysis complexity compared to untargeted methods. Here, it was demonstrated that target selection using DDA can predict the performance of a PRM method. Use of a single instrument for target selection and for the final targeted detection method, such as that described here, maximizes the likelihood that careful target selection will result in a robust method that functions equivalently in varying food matrices. Furthermore, particular protein families (e.g., prolamins in peanut), or peptides derived therefrom, may possess inherent physicochemical characteristics making them the most suitable targets.

### Reference

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