AOAC Food Allergen Community

NEWSLETTER

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NEWSLETTER ARCHIVE

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Editorial Comment

Image composite: Carmen Diaz-Amigo (bottle of milk picture: Jultud / envato)

Where Food Allergens and Food Fraud Converge

One year after the publication of the Guest Editor section of the Journal of AOAC International on Food Allergens, a new Guest Editor section is already available online. On this ocassion, the special section focuses on the Detection of Food Allergens and Food Fraud by Mass Spectrometry. All 12 articles are open access and we have made the abstracts and links to each article available in this issue of the Newsletter. Enjoy the read!

Carmen Diaz-Amigo Editor in Chief

Mass Spectrometry: Status Quo in Food Allergen and Food Authenticity Applications

Bert Popping & Carmen Diaz-Amigo

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From Signal to Analytical Reporting for Allergen Detection by Mass Spectrometry

Philip Johnson & Melanie Downs

MS offers a flexible and precise alternative to traditional methods for allergen detection and quantitation. However, this flexibility also engenders many ways of acquiring information and translating it to simple, clear data useful to end-users. Currently, methods for performing data analysis for allergen detection by MS are unstandardized, and it is therefore difficult to compare different analytical methods. We identify three key components of data analysis: detection of positive signals, calibration, and signal integration. For each of these components, there are multiple pathways available for method developers. We discuss these alternative methods, giving examples from literature. Assuming that the end result of an allergen analysis should be clear, unambiguous, and understandable to all relevant stakeholders, we pay particular attention to the consequences of each choice to the analysis in question and, where appropriate, suggest best practices. We also identify data analysis criteria that should be clearly delineated in the reporting of a method. Establishment of community-wide standards for unambiguous reporting of data analysis workflows will improve the evaluation, comparability, and transferability of methods.

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Selection of Tree Nut Allergen Peptide Markers: A Need for Improved Protein Sequence Databases

Weili Xiong, Melinda McFarland, Cary Pirone and Christine Parker

Background: To effectively safeguard the food-allergic population and support compliance with food-labeling regulations, the food industry and regulatory agencies require reliable methods for food allergen detection and quantification. MS-based detection of food allergens relies on the systematic identification of robust and selective target peptide markers. The selection of proteotypic peptide markers, however, relies on the availability of high-quality protein sequence information, a bottleneck for the analysis of many plant-based proteomes. Method: In this work, data were compiled for reference tree nut ingredients and evaluated using a parsimony-driven global proteomics workflow. Results: The utility of supplementing existing incomplete protein sequence databases with translated genomic sequencing data was evaluated for English walnut and provided enhanced selection of candidate peptide markers and differentiation between closely related species. Highlights: Future improvements of protein databases and release of genomics-derived sequences are expected to facilitate the development of robust and harmonized LC–tandem MS-based methods for food allergen detection.

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Investigation of Reduced ELISA Recovery of Almond and Hazelnut Traces from Roasted Nut Samples by SDS-PAGE and Mass Spectrometry

Sebastian Perner, Linda Heupel, Lisa Zimmermann, Yasmin Peters, Kai Vongehr, Hesham El-Bedewy, Susanne Siebeneicher, Thomas Weiß, Thomas Hektor, Bernd Lindemann, Simone Loos-Theisen and Klaus Schneider

Western society is facing an increase in the number of food-allergic individuals, with rising incidence in the past years. Therefore, allergen-free food and accurate and reliable analysis of allergen contamination are essential for the protection of consumers. Yet, there is limited understanding on the effect of food processing on allergenicity and on the ability of available methods to detect trace contamination in processed food. Available studies addressing this have relied on sample processing on a laboratory scale. In this study, industry-like processing under precisely defined conditions (ranging from 110 to 150°C roasting temperatures) was employed to better understand the limitations of state-of-the-art methods for detecting traces of hazelnut and almond in processed food. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis indicated an overall reduction in extracted proteins from roasted nut samples, and with matrix-assisted laser desorption ionization time-of-flight Cor a 9 and Prunin, were identified as majorly expressed proteins for hazelnut and almond, respectively. A commercial ELISA kit detected nut traces only up to a 130°C roasting temperature. Untargeted MS (Orbitrap) analysis was able to detect traces of nuts roasted up to 150°C while also confirming Cor a 9 and Prunin as the major expressed proteins for hazelnut and almond, respectively. Preparing cookie dough spiked with roasted nut samples, a complex food matrix was simulated. Analysis by ELISA showed the same limitations encountered for pure nuts samples, hardly detecting traces of nuts roasted above 130°C. Targeted MS (linear ion trap) using multiple reaction monitoring methods for one proteotypic peptide for Cor a 9 and Prunin, respectively, enabled a detection of nut traces up to 150°C. The results indicated that a reduced extractability because of temperature-related effects (e.g., protein denaturation, cross-linking, poor solubility) caused the significant differences between the ELISA and MS analysis. Overall, the results of this study may form the basis to improve allergen detection after roasting through improved extraction methods and refined ELISA formats.

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German Government Official Methods Board Points the Way Forward: Launch of a New Working Group for Mass Spectrometry for Protein Analysis to Detect Food Fraud and Food Allergens

Manfred Stoyke, René Becker, Jens Brockmeyer, Wolfganag Jira, Bert Popping, Steffen Uhlig, Stefan Wittke

The detection of food fraud and undeclared food allergens is one of the major challenges for competent authorities. Because adulterations are continuously adapted to the methods used to uncover them, the accomplishment of this task has become increasingly difficult over time. In recent years, various new promising methods for the detection of multiple food adulterants and multiple food allergens have been developed. Some of them utilize LC–MS to identify specific marker peptides. However, these methods have yet to be validated and standardized. For this reason, the German officials have established a working group with the objective of validating methods through multilaboratory validation studies. The experts of the working group also aim for the first time to standardize validated methods and to develop general validation criteria. This manuscript will highlight the current work of the group. For this purpose, an overview is given on the principles and applications of the new mass spectrometric methods. Moreover, requirements and the present work of other institutions regarding method validation are described.

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Gluten Analysis in Processed Foodstuffs by a Multi-Allergens and Grain-Specific UHPLC-MS/MS Method: One Method to Detect Them All

Jean Henrottin, Mélanie Planque, Anne-Catherine Huet, Riccardo Marega, Amandine Lamote, Nathalie Gillard

Background: Celiac disease, a complex, long-term autoimmune disorder and gluten intolerance, is estimated to affect from 1 to 5% of the world's population. Objective: As a consequence, to protect gluten-sensitive consumers, the development of reliable analytical methods allowing the detection of gluten in various food products is needed. Methods: Currently, ELISA is probably the most widespread used methodology. The method based on the R5 antibody has received type I status in Codex Alimentarius. However, the ELISA method suffers from some limitations, especially concerning quantification of nonwheat gluten. As a consequence, the development of another complementary methodology such as LC–tandem MS (MS/MS) is considered to be essential. Furthermore, this method could also be used for the simultaneous detection of gluten with other allergens, which will constitute a great additional benefit for producers of "free-from" food products and/or having a management policy integrated for several allergies and/or intolerances. Results: A multi-allergen and grain-specific ultra-HPLC coupled to MS/MS method allowing the identification and the discrimination of gluten from seven cereals, simultaneously with the detection and identification of 10 allergens in only one analysis, is thus described here. Conclusions: This method can be used for the analysis of a broad range of foodstuff matrices containing wheat and/or its derivatives, including cereals, flours, heat-treated and foodstuffs, but also more complex samples having undergone fermentation processes (such as beers).

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Food Fraud: A Simple and Efficient LC–MS/MS Approach for Peptide-Based Food Authentication

Monika Ruhland, Richard Klinger

Food fraud includes the addition of inferior components or the substitution of substances. Besides being of economic concern, this might furthermore pose a health risk for consumers. Therefore, the detection of replacements is of high importance. For the identification of species, in recent years proteomic methods gained more and more importance. In this work, an easy and efficient approach of targeted peptide analytics for revealing food fraud is presented. One of the most common workflows for protein analytics was improved by the application of urease to hydrolyze the urea in the extraction buffer; therefore, no further cleanup is required. By considering only selected compounds and the use of open-source databases for the selection of the target peptides for the adoption of the analytical methods, no time-consuming basic research is required. For the detection of the substitutes, it is not necessary to know the absolute concentration of the component. In these cases, the calculation of the proportion of a species in relation to the total content of all analyzed compounds is sufficient.

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Advances in LC–MS/MS Methods for Allergen Testing, Meat Speciation, and Gelatin Speciation

Jianru Stahl-Zeng, Ashley Sage, Philip Taylor, Jeremy Dietrich Netto, Tuo Zhang

Background: Food authenticity is demanded by the consumer at all times. The consumer places trust in the manufacturer that the food product is genuine in terms of what is recorded on the packaging label. Objective: Recent advancements in LC–tandem MS methodology in the detection of allergens, meat, and gelatin speciation in raw food products and processed foods are detailed in this paper. Method: For each of the three methods, initial proteome analysis and the screening leading to the determination of unique tryptic peptides were conducted using a high-resolution, accurate tandem mass spectrometer. Having identified the unique markers, the method was transferred to a tandem quadrupole mass spectrometer for a higher-sensitivity quantitative study, multiple reaction monitoring transition analysis. Results: For

the allergens method a detection limit of at least 10 ppm was attained across the 12 allergen peptides in this workflow. In the gluten workflow the resulting chromatograms show good detection down to 5 ppm, with no interference from the food matrices. The meat speciation method details that signature peptides could be readily identified at 1% w/w with no matrix interference. Conclusions: These single-injection workflows with cycle-time optimization enable wide coverage of analytes to identify multiple species within challenging matrix samples.

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Simultaneous Detection of 13 Allergens in Thermally Processed Food Using Targeted LC–MS/MS Approach

Tairo Ogura, Robert Clifford, Uwe Oppermann

Food allergy is a major concern for public health and food industries. Because of the large numbers of food ingredients to be tested, MS is considered an alternative to existing techniques in terms of high selectivity, sensitivity, and capability to analyze multiple allergens simultaneously. In this study, we developed the method for monitoring significant peptides derived from 13 food allergens (milk, eggs, cod, shrimp, lobster, almonds, brazil nuts, cashew nuts, hazelnuts, walnuts, peanuts, wheat, and soybeans) and evaluated it in thermally processed foods (bread, cookie, fried fish, and frozen pasta). To select significant peptides to monitor, we used a bioinformatics-based approach and experimental confirmatory analysis. It was demonstrated that the developed method could detect target food ingredients from thermally processed foods successfully.

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Development and In-House Validation of an LC–MS and LC–MS/MS Assay for the Determination of Food Fraud for Different Fish Species

Philipp Lasch, Steffen Uhlig, Carsten Uhlig, Christian Wilhelm, Nicola Bergmann, Stefan Wittke

Background: Fish and fish products are one of the most important food sources of high commercial interest. The global food trade and the associated risks are constantly presenting new challenges to consumer protection and public authorities, which, among other things, demand state-of-the-art analytical methods to ensure food authenticity. Objective: The establishment of MS-based strategies plays a decisive role alongside the (further) development of ELISA- or DNA-oriented methods. Methods: In the present work, therefore, the development and in-house validation of an LC–MS and LC–MS/MS-based assay for authenticity testing of certain fish species is described. Results: Based on the execution of a validated bottom-up LC–electrospray–MS and MS/MS assay and multivariate analysis, the commercially available species Lutjanus malabaricus (red snapper) and Sebastes spp. (redfish) are distinguished from each other, whereas an additional 68 samples [nine additional marine species such as pangasius (Pangasianodon hypophthalmus), salmon (Salmo salar), turbot (Scophthalmus maximus), plaice (Pleuronectes platessa), sole (Solea solea), lemon sole (Glyptocephalus cynoglossus), halibut (Reinhardtius hypoglossoides), red salmon (Oncorhynchus nerka), and great scallop (Pecten jacobaeus)] served as blinded negative controls to ensure the specificity of the assay. Conclusions and Highlights: A promising LC–MS and LC–MSMS based assay has been developed that could enable the detection of fish fraud at the protein level in the future.

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Mass Spectrometry-Based Untargeted Proteomics for the Assessment of Food Authenticity: The Case of Farmed Versus Wild-Type Salmon

Giuseppina Fiorino, Marion Fresch, Ina Brümmer, Ilario Losito, Marco Arlorio, Jens Brockmeyer, Linda Monaci

Background: Omics technologies have been widely applied in different fields, among which, proteomics is gaining increasing interest for its application to the authenticity of food products. MS, typically coupled with LC, represents a key technique for proteomics-related studies dedicated to fish and other seafood products by using a bottom-up approach. Objective and Methods: In this paper, the optimization of an untargeted proteomics-based method using LC separation and MS detection relying on a quadrupole time-of-flight mass spectrometer is described and applied to the analysis of Canadian farmed and wild-type salmon, followed by statistical analysis based on principal component (PC) analysis. Results and Conclusions: This untargeted approach, using a data-independent acquisition MS scheme, demonstrated the ability to effectively discriminate salmon belonging to the two classes. Furthermore, selected peptides showing high loadings on PC1 could represent potential candidate peptide markers able to discriminate farmed from wild-type salmon samples in the future.

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Detection and Quantification of Allergens in Foods and Minimum Eliciting Doses in Food-Allergic Individuals (ThRAll)

Clare Mills, Karine Adel-Patient, Hervé Bernard, Marc De Loose, Nathalie Gillard, Anne-Catherine Huet, Collette Larré, Chiara Nitride, Rosa Pilolli, Olivier Tranquet, Christof Van Pouke, Linda Monaci

Risk-based approaches to managing allergens in foods are being developed by the food industry and regulatory authorities to support food-allergic consumers to avoid ingestion of their problem food, especially in relation to the traces of unintended allergens. The application of such approaches requires access to good quality data from clinical studies to support identification of levels of allergens in foods that are generally safe for most food-allergic consumers as well as analytical tools that are able to quantify allergenic food protein. The ThRAll project aims to support the application of risk-based approaches to food-allergen management in two ways. First, a harmonized quantitative MS-based prototype reference method will be developed for the detection of multiple food allergens in standardized incurred food matrices. This will be undertaken for cow's milk, hen's egg, peanut, soybean, hazelnut, and almond incurred into two highly processed food matrices, chocolate and broth powder. This activity is complemented by a second objective to support the development and curation of data on oral food challenges, which are used to define thresholds and minimum eliciting doses. This will be achieved through the development of common protocols for collection and curation of data that will be applied to allergenic foods for which there are currently data gaps.

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Also Available

Guest Editor Special Section of the Journal of AOAC International on Food Allergens

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Upcoming Events

133rd AOAC Annual Meeting

September 6-12, 2019
Denver CO, USA

Food Allergen Scientific Sessions

September 11, 2019

This year, the AOAC Annual Meeting will have three scientific sessions dedicated to different aspects of food allergens and gluten.

8:15 am to 9:45 am

Community Perspective of Food Allergen Measurement
Co-chaired by Ashley Beasley-Green (NIST), David Bunk (NIST), Jupiter Yeung (Nestlé Nutrition R&D Center) & Carmen Diaz-Amigo (FOCOS)

10:15 am to 11:45 am

Latest Developments in Gluten Analysis
Co-chaired by Girdhari Sharma (FDA) & Carmen Diaz-Amigo (FOCOS)

1:00 pm to 2:30 pm

Validation and Implementation of Emerging Methods for Food Allergen and Gluten Measurement
Co-chaired by Girdhari Sharma (FDA) & Melanie Downs (University of Nebraska)

In addition to the scientific sessions, the Food Allergen Community will also hold its Annual Meeting (date and time to be determined). The co-chairs of the Community, Carmen Diaz-Amigo and Jupiter Yeung, would like to welcome all to join the group to participate and benefit from this information exchange platform.

More information

AOAC Food Allergen Community Newsletter

Contribute with articles, news items or suggestions.

Submission deadline for the 2nd issue of 2019: June 28

Send your articles to AOAC.Allergens@gmail.com

Topics for publication

- ✓ Regulatory Updates
- ✓ Food Industry Initiatives
- ✓ Regional developments
- ✓ Your research
- ✓ Upcoming events
- ✓ Questions for our Experts
- ✓ Interested in a topic?

Article requirements*

- ✓ Short title
- ✓ Length: 400 words max.
- ✓ 1 figure or table (optional)
- ✓ Author & Affiliation
- ✓ Related links
- ✓ No advertising

*** All articles are subject to review by the Editorial Board.**

AOAC Food Allergen Community

The AOAC Food Allergen Community is a forum serving the scientific community working on Food Allergens: The community aims to help AOAC INTERNATIONAL in its consensus-based scientific and advisory capacity on methods of analysis for allergens in foods and other commodities. It is also meant to serve the broader Stakeholder Community whose objectives it is to enhance the protection of food allergic consumers worldwide.

Contact us at
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