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CASE STUDY FARRP

Discovery-driven development of mass spectrometry methods for food allergen detection and quantification

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Food allergies, immune-mediated adverse reactions foods, can have detrimental impacts on the quality of life of food-allergic individuals and families.

Allergic reactions to foods can be life threatening, and in the United States, food allergies have been estimated to cost $24.8 billion annually.[1] Despite the impacts and potential severity of food allergies, a recent report from the National Academies of Sciences, Engineering, and Medicine has identified many knowledge gaps requiring additional research and resources.[2] The current lack of any approved treatments means that food-allergic individuals must follow strict avoidance diets. A critical part of being able to avoid a food of concern is getting clear and accurate information about what food allergens are present in foods. Accordingly, many regulatory authorities have implemented mandatory labeling requirements for major food allergens, or ingredients derived from thereof, when used as intentional ingredients. In the U.S., the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) mandates that the following eight allergenic foods, which are responsible for an estimated 90% of food allergies, be listed in plain English on the label of FDA-regulated packaged foods: peanuts, tree nuts, soy, wheat, milk, eggs, fish, and crustacean shellfish.

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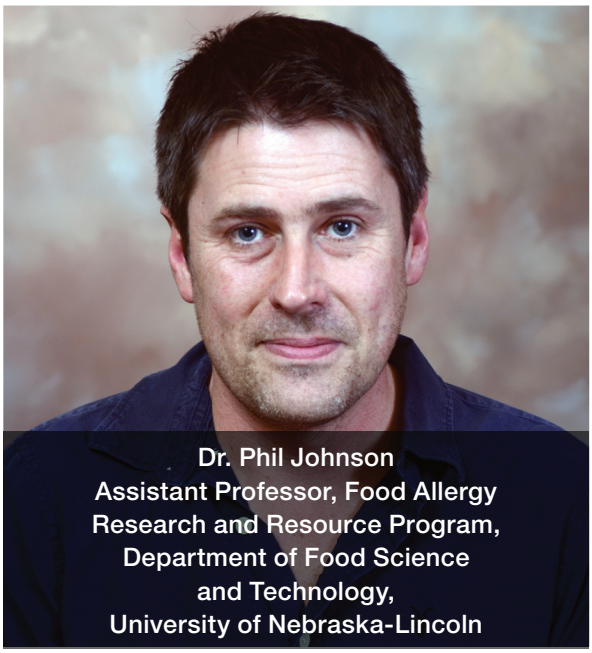

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"MS offers tremendous potential for changing the way we detect allergens..."

—Drs. Phil Johnson and Melanie Downs

The presence of undeclared food allergens in a product is of concern for the food industry, regulators, and allergic consumers, alike. The recently implemented rules for the Food Safety Modernization Act (FSMA) also recognize the potential hazard associated with undeclared food allergens, either from failure to label food allergen ingredients or due to cross-contact occurring during production. Under the Final Rule on Preventive Controls for Human Food, manufacturers must specifically implement cGMPs to prevent allergen cross-contact and implement preventive controls for food allergen hazards that are foreseeably likely to occur, as part of their written food safety plan.

The Food Allergy Research and Resource Program (FARRP) is an industry-funded research consortium in the Department of Food Science and Technology at the University of Nebraska-Lincoln. FARRP was formed in 1995 to provide research-based information, expert opinions, and tools to the food industry to assist in the effective management of food allergens. In order to achieve that mission, FARRP conducts research to answer critical clinical, regulatory, and analytical questions. FARRP's co-directors, Drs. Steve Taylor and Joseph Baumert, are joined by Drs. Melanie Downs and Phil Johnson, who are spearheading FARRP's protein mass spectrometry research.

Detection of food allergens

Accurate labeling of food allergens requires knowledge of which allergens are or may be present in finished foodstuffs. This allergen management is often facilitated by detection methods which allow sensitive, accurate and above all specific detection of small amounts of an allergen in products. Testing for the presence of allergens can be quantitative (how much) or non-quantitative (present or absent), and is currently performed using either immunological or polymerase chain reaction (PCR)-based techniques.

Immunological methods such as ELISA rely on the use of antibodies specific to proteins from the allergenic food. In an ELISA format, such antibodies are most often used to give quantitative data, and many commercial ELISA kits are available for the testing of allergens. FARRP primarily uses ELISA kits to detect food allergens. Antibodies can also be used in other formats, such as lateral flow devices (LFDs - sometimes referred to as 'dipsticks').

Such LFDs can provide rapid, on-site present or absent determinations and are a popular way of verifying if production equipment is free from particular allergens.

It is important to note that the above immunological techniques recognize proteins, and often specific proteins known to cause allergic reactions, within an allergenic food. However, DNA can also be used to detect food allergens, most often using PCR. Advantages of such PCR methods include extreme sensitivity and specificity, and again commercial kits are available for the testing of many allergens by PCR. The use of DNA to detect food allergens, rather than the protein molecules which cause reactions, can, however lead to issues, especially in foods which are highly processed.

More recently, mass spectrometry has emerged as a method showing great promise for allergen detection. Using proteomic workflows, we can detect molecules which cause reactions (proteins) while maintaining specificity by detecting one or more peptides which occur only in our allergenic food. We detect proteins in a food by detecting shorter peptides that result from protease digestion. Mass spectrometry requires a large initial investment and expertise to develop methods. It is likely that mass spectrometry will be initially used in order to confirm results from immunological or DNA-based methods rather than as a 'front-line' method. However, the rapid pace of development of MS makes it almost inevitable that this method will find more widespread use in the future. For this to occur, however, laboratories such as those here at FARRP must develop robust methods which can be employed elsewhere.

Challenges of allergen-derived ingredients

In order to enhance functionality and minimize waste, the food industry takes foods, including allergenic foods, and turns them into a variety of food ingredients. Soy and milk are two particularly relevant examples of this, with each being transformed into a myriad of isolates, concentrates, and other functional ingredients by a variety of processes (e.g. fractionation, filtration, heat treatment, chemical modification, and hydrolysis). These processes can affect the protein composition of the end product and properties of the proteins themselves, both of which can impact subsequent detection of an ingredient.

In order to develop widely-applicable food allergen detection methods, taking these different allergen-derived ingredients into consideration during the method development phase, rather than during the validation phase, may be more effective. In our lab, we have been using the discovery proteomics capabilities of the Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer to profile several different soy- and milk-derived ingredients before proceeding to targeted method development. The Q Exactive series offers us a unique ability to transition from high quality discovery proteomics to targeted PRM methods on the same platform. Our analysis of several different soy- and milk-derived ingredients has found that individual peptide abundances do, in fact, vary among different ingredients, and obtaining this type of information will be beneficial to selecting peptides for targeted methods capable of detecting a diverse range of food allergen-derived ingredients.

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Challenges of detecting allergens in matrices

Foods are complex environments for protein molecules. Not only are foods often mixtures of different plant and animal tissues, but they frequently undergo thermal processing during preparation. Such processing changes the food matrix itself as well as proteins within it, causing proteins to stick together (aggregate) and introducing chemical changes to the protein molecules themselves. Other food processing methods such as high-pressure treatment (HPT) and pulsed-electric field (PEF) are becoming more common, and often have different effects on food proteins.

Detecting specific proteins in these complicated mixtures can be very difficult, both for ELISA and MS-based methods. We often find that a method that works well in one food matrix, for example, a cookie, performs poorly in another (for example, chocolate). This is often because the peptides we choose to detect are not released efficiently from our food. Furthermore, it is nearly impossible to predict how well the method will perform in a given food matrix until the method has been finalized and it is at the validation stage. Here at FARRP we are using high-resolution mass spectrometry to identify peptide targets which work well in food matrices before we develop our MS method by performing indiscriminate (untargeted) analysis of our food allergen with a food matrix. We can then see which peptides release well from different types of food and base our methods on these. We have the example of peanut in spices to develop this technology. The ability to use one instrument for both our target selection and final method is greatly beneficial, as instrument to instrument variability is not an issue.

Conclusions and outlook

Mass spectrometry offers tremendous potential for changing the way we detect allergens in foods and therefore manage allergens in our food supply chain. We can tailor methods to suit particular foods, particularly those which are highly-processed, which are nearly impossible to effectively detect using other methods.

References

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