

SPECIAL GUEST EDITOR SECTION

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

Issues related to food allergens has been a hot topic for the last 20 years, so it is a relatively new field. Food fraud has existed for centuries; however, it is in the last decade that it has gained the attention it deserves. Fraudulent activities affecting food take place when modifications of the properties of a food or ingredient are carried out with the purpose of economic gain. However, many of these modifications can introduce hazardous components, jeopardizing the product's safety. This is where food allergens and food fraud converge, as, for example, in the voluntary replacement of a premium food ingredient with a cheaper allergenic one that provides the food with a similar appearance. Analytical methods are very useful tools both to support compliance (e.g., food allergen labeling) and to help uncover fraudulent practices.

MS technology has seen tremendous developments from the early days of single quadrupoles and inefficient vacuum pumps to highly sensitive, high-resolution triple quadrupoles with very efficient vacuum pumps. When looking at routine testing laboratories, MS has always been used predominantly for relatively small molecules. Food contaminants such as pesticides, veterinary drug residues, and mycotoxins and foodborne contaminants such as acrylamide are the typical contenders when it comes to mass spectrometric analysis.

Food Allergens

MS has been used for the characterization of food allergens for some time. However, the technology and sample preparation methods have evolved to improve the sensitivity and resolution of these methods. It was around early 2000 (1) when the first mass spectrometric applications for food allergen analysis were developed.

Food allergens were, and still are, predominantly analyzed either by immunological methods such as ELISA or

by DNA-based assays such as PCR. These assays have been used in laboratories for decades, and technical staff tend to be very familiar with their execution.

Commercial allergen ELISA tests, especially for relevant allergens such as peanuts, egg, and milk, have been available for more than 20 years now, and the ease of use makes them suitable for routine tests. In addition, allergen ELISA targets proteins of the same class as food allergens. Therefore, a positive finding of ELISA is directly linked with the presence of the offending molecule in the tested product that poses a risk to the allergic individual. ELISA is the method of choice when it comes to allergen testing in most cases. However, these immunological tests come with a number of challenges. For one, the antibodies in different commercial ELISA kits have been generated against different target proteins. Antibodies are dependent on the protein structure with which they were produced, and the modification of such structures may affect their ability to bind the target. Moreover, ELISA kits are often calibrated with different materials, which contributes to a higher result variability among kits. Some proficiency test providers therefore assign values per ELISA kit rather than per proficiency sample, as is common for proficiency tests for pesticides and mycotoxins. In addition, the targets chosen by ELISA kit manufacturers to generate the antibodies may be more or less heat-stable, meaning that antibodies produced against a native protein may no longer be detected when the food has been heat-treated while the allergenicity may still be maintained. Also, one of the drawbacks of commercial ELISA is that it is not multiplexed, meaning that each kit detects only one allergenic ingredient (e.g., milk, egg, or peanut). This makes testing samples for different allergens expensive for food manufacturers, authorities, and laboratories. For this reason, deciding which allergens to test is usually based on risk assessment, for which often only insufficient data are available.



Bert Popping
Guest Editor



Carmen Diaz-Amigo
Guest Editor

PCR, a DNA-based method, allows the analysis of more than one allergen-containing species at a time and has here a clear cost advantage over ELISA methods. However, PCR also comes with a number of challenges. DNA is considered a marker for the presence of allergenic material because it is not the same compound class as proteins (i.e., food allergens). Both DNA and proteins can be separated from each other during food processing. An example is protein concentrate, in which the amount of DNA is usually significantly decreased while the amount of protein is significantly increased (e.g., soy protein concentrate). In a worst-case scenario, it means that a negative PCR

result does not equate to the absence of the allergen. Egg and milk are also good examples of ingredients in which, naturally, the protein content is high, but the DNA content is low. Another limitation for PCR testing is that it relies on the pH of the food. In low pH environments, the DNA degrades and may no longer be detectable, but the allergen is still present.

The most prominent problem that drove the development of MS was not as much cost as it was the fact that some allergens could not be detected with most ELISA methods after heat treatment as well as the need for confirmatory methods. The extraction of tightly matrix-bound proteins requires harsh conditions, such as the presence of reducing and chaotropic agents. This type of extraction may jeopardize the integrity of antibodies and the antibody–target binding. For MS, this is not as much of a problem because the proteins can be subject to such extraction conditions, which are followed by enzymatic digestion. Moreover, one of the desired advantages of MS is its multiplex capability, which could allow the detection of all regulated food allergens in a single analysis. The first method that describes the detection of several food allergens was published by Heick et al. (2). It demonstrated that seven allergens could be detected in a single run. MS methods are not free from challenges, some of which are shared with ELISA or PCR methods. Heick and coworkers' method came with challenges of its own; in order to achieve appropriate sensitivities, interfering substances had to be separated from the proteins, and the proteins had to be concentrated. This was done using a molecular filter that retained the proteins. This step was followed by an overnight digestion and alkylation. Although it could be demonstrated that the method worked, the sensitivity when testing raw materials for some allergens was not as low as ELISA. However, it could be shown that for processed egg, this method was significantly more sensitive than ELISA. In addition, it took almost 24 h to perform the method, which is not ideal for routine laboratories. This publication was followed by an increasing number of manuscripts showing method improvements toward time reduction, sensitivity, and the selection of more suitable peptides. The quantification of allergenic ingredients based on the quantities of peptides found remains a challenge for these MS-based methods, and while MS for food allergens is a very useful addition to existing methods such as ELISA and PCR, not all challenges are resolved. However, the first methods will be collaboratively trialed in 2019 and will provide additional data that will likely allow the further evaluation of the variability, precision, and accuracy of these methods.

Food Fraud

Mass spectrometric methods have not only been developed for food allergens but also to detect food fraud. In some cases, the adulterants were actually food allergens.

Food fraud hit the headlines in 2008 with the melamine crisis, leading to the death of several infants, and again in 2013 with the horse meat scandal, in which horsemeat not destined for human consumption was mixed with beef and not declared. Adulteration (i.e., substitution) with

different, lower-value species is a common type of fraud, and the challenge is to identify the adulterating species out of a large number of possibilities. As an example, Oceana (3) conducted a study and found that approximately 30% of fish were incorrectly labeled and often sold as higher-value species (Report February 2013). In this case, MS can help to identify different fish species in a single analysis. The same is true for nuts: Where peanuts have been used as adulterants, a multinut method has been developed (4).

MS analysis to detect food fraud is not limited to detecting targeted peptides. The United States Pharmacopeia has recently developed guidance for the development and validation of nontargeted methods to detect adulteration (5). Nontargeted methods generate a fingerprint for the ingredient or product (e.g., milk, cheese, olive oil), which may be based on many different parameters, and this is compared to an authentic profile. The advantage of such an approach is that many different parameters are evaluated at the same time, making it difficult for fraud to go unnoticed. On the other hand, such screening methods require robust profile databases and algorithms to avoid false-positive and false-negative results. For example, to assess whether Italian olive oil is authentic, oil from all the Italian regions needs to be collected and analyzed to generate the profile of an authentic Italian olive oil. Equally, the olive oils from other growing regions (e.g., Greece, Spain, Tunisia, the United States, etc.) need to be collected to build a nonauthentic profile, including blends of authentic and nonauthentic oils. This work is time-consuming and costly. While this approach is very comprehensive in its ability to detect food adulteration, time will tell if the benefits outweigh the economic challenges of generating the databases.

MS for food fraud and food allergens is still at a comparatively early stage and will see further development over the next years. The manuscripts included in this special section describe the status quo of these developments for both food fraud and food allergen detection.

—Bert Popping and Carmen Diaz-Amigo
FOCOS
Zum Kälterhaus 6b
63755 Alzenau, Germany
E-mail: bert.popping@focos-food.com

References

- (1) Shefcheck, K.J., & Musser, S.M. (2004) *J. Agric. Food Chem.* **52**, 2785–2790. doi:10.1021/jf035129h
- (2) Heick, J., Fischer, M., & Popping, B. (2011) *J. Chromatogr. A* **1218**, 938–943. doi:10.1016/j.chroma.2010.12.067
- (3) Warner, K., Timme, W., Lowell, B., & Hirshfield, M. (2013) http://oceana.org/sites/default/files/reports/National_Seafood_Fraud_Testing_Results_FINAL.pdf
- (4) Korte, R., & Brockmeyer, J. (2016) *Anal. Bioanal. Chem* **408**, 7845–7855. doi:10.1007/s00216-016-9888-y
- (5) *Food Chemicals Codex* (2018) 11th Ed., U.S. Pharmacopeial Convention, Rockville, MD, Appendix XVIII, 1510–1522