

Current Perspectives and Recommendations for the Development of Mass Spectrometry Methods for the Determination of Allergens in Foods

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Allergen detection and quantification is an essential part of allergen management as practiced by food manufacturers. Recently, protein MS methods (in particular, multiple reaction monitoring experiments) have begun to be adopted by the allergen detection community to provide an alternative technique to ELISA and PCR methods. MS analysis of proteins in foods provides additional challenges to the analyst, both in terms of experimental design and methodology: (1) choice of analyte, including

multiplexing to simultaneously detect several biologically relevant molecules able to trigger allergic reactions; (2) choice of processing stable peptide markers for different target analytes that should be placed in publicly available databases; (3) markers allowing quantification (e.g., through standard addition or isotopically labeled peptide standards); (4) optimization of protease digestion protocols to ensure reproducible and robust method development; and (5) effective validation of methods and harmonization of results through the use of naturally incurred reference materials spanning several types of food matrix.

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Allergen Management

Providing food that is safe for consumers to eat is of paramount importance to the food industry and involves managing microbiological, chemical, and physical hazards. A relatively new hazard that has emerged over the last 20 years is food allergens. While innocuous to the majority of consumers, allergens (even in trace amounts) can represent a (sometimes) life-threatening hazard to allergic consumers, with allergic individuals even reacting to a kiss from someone who has been eating "their" problem food (1). Since there is currently no cure for food allergy, individuals with this condition have to practice food avoidance, which can be a challenge with regard to manufactured foods. In order to make food avoidance easier for allergic consumers, food labeling regulations have been developed around the world—such as the Food Allergen Labeling and Consumer Protection Act in the United States (2) and 2003/89/EC (3) and 2007/68/EC (4) in the European Union (EU)—which make it mandatory to label certain allergenic ingredients used in foods, irrespective of their level of inclusion. However, this legislation excludes the unintentional presence of allergenic ingredients at low levels that can result through the use of common equipment and processing lines. In the EU, this is covered by general food safety legislation that specifically mentions the requirement to address the needs of specific consumer groups, such as food allergic consumers (EU Regulation 178/2002/EC, EC 2002; The General Food Law). Currently, only two countries have regulations that specifically address cross-contact issues: Japan, where the legislation covers both deliberately added allergenic ingredients and cross-contact allergens (specifically cereals, eggs, peanuts, milk, and buckwheat); and Switzerland, where an ingredient on the Codex Alimentarius Commission allergen labeling list that is present in a food because of cross-contact at levels exceeding 1 g/kg must be listed as an ingredient.

In general, such cross-contact allergens are managed through effective allergenic risk assessment and application of Hazard Analysis Critical Control Points. In some countries, guidelines for managing allergens in such foods have been developed by regulators, such as the United Kingdom's Food Standards Agency (5) and industry-led initiatives such as Vital (6), the British Retail Consortium Guidelines (2005), and Confederation of the Food and Drink Industries of the EU (CIAA; 7). However, the application of such management strategies requires effective methods of analysis to allow the food industry to verify and validate cleaning protocols (8), and for control authorities to monitor for allergens in food, both deliberately added ingredients and those that carry precautionary statements. Lastly, in the event of an allergic consumer suffering from an unexpected severe, or even fatal, allergic reaction, there is a need for analytical tools to support identification of the allergenic ingredients responsible. This is especially important since many severe or fatal reactions occur as a result of consumption of manufactured foods and/or eating away from home (9).

During the last decade, immuno-based (primarily ELISA) and some PCR techniques became the methods of choice for

allergen analysis (10, 11). However, the rapid developments in protein MS driven by advances in hardware and software mean that, although still more expensive than antibody or DNA-based methods, such instrumental approaches are becoming more commonplace. While methods such as ELISA and PCR have been widely used for allergen analysis in a routine setting (12), they can lack the precision and rigor required, especially in circumstances where there are liability issues. In the wake of rapid advances in both instrumentation and informatics, protein MS offers an alternative to ELISA and PCR. It has much promise as an alternative method with high precision and accuracy. Additionally, MS offers the prospect of an alternative method that may ultimately lead to the development of a reference method for allergen analysis, allowing the screening of several allergen indicators in the same analysis. Through the platform provided by the Allergen Working Group of the EU-funded Network of Excellence MoniQA [Monitoring and Quality Assurance in the total food supply chain (12)], an assessment of the current state-of-the-art with regard to application of MS to allergen analysis has been undertaken in this paper, with a view to defining what would constitute best practice.

Allergen Detection in Foods—Common Issues

There are several issues regarding allergen detection that are common to any analytical method, including the choice of analyte and the effects of food processing and the food matrix on the extraction and subsequent analysis of the target analyte, which is particularly problematic when they are proteinaceous. Current legislation requires labeling of allergen-containing foodstuffs in manufactured foods but does not specify protein components or even the species from which the ingredients originate (Commission Directive 2007/68/EC). Because almost without exception, the molecules triggering allergic reactions to foods are proteins, in general risk assessors consider that "no protein means no problem." Consequently, methods have generally been developed that are able to determine the presence of protein components in allergenic ingredients either directly or by inference. In general, a variety of proteins in a food are responsible for the elicitation of an allergic response (13). This poses the question: is it really necessary to choose the allergenic molecule itself or is it sufficient to use stable marker proteins for the determination of the presence of the offending food? All methods suitable for food allergen detection have to deal with this critical point, namely the choice of target analyte for food allergen detection.

DNA-based methods detect DNA rather than the component—the protein—responsible for triggering an allergic reaction. Consequently, it is very difficult to conclude from the presence of DNA copies that are indicative of contamination of a product with an allergen what the corresponding protein or allergen content might actually be. Allergen immunoassays exploit the special binding properties of antibodies (high affinity and specificity) in combination with their corresponding proteinaceous antigens. Depending on the antibody source (polyclonal or monoclonal antibodies),

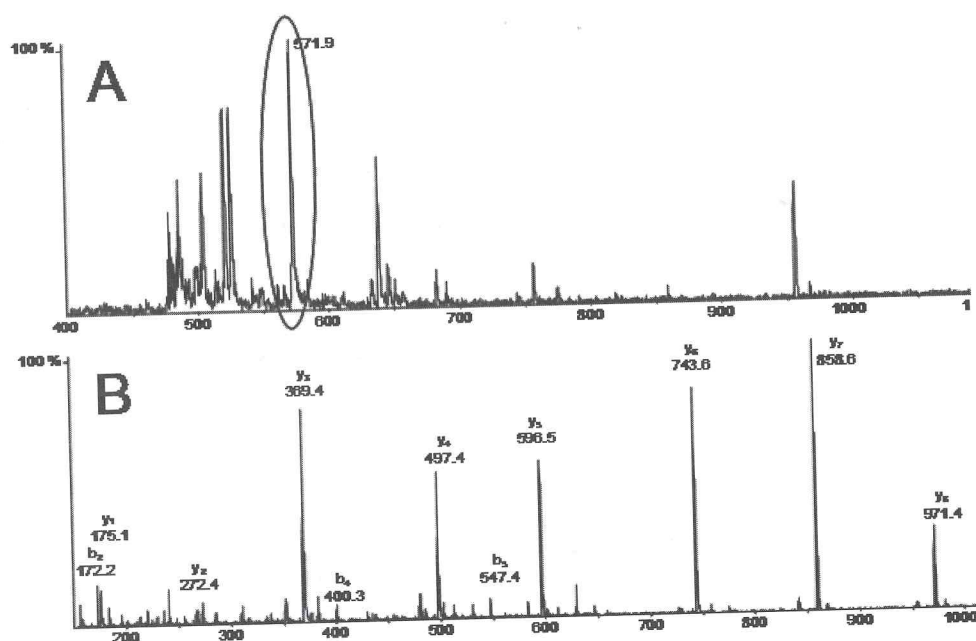


Figure 1. MS1 spectrum of a proteolytic digest of almond proteins and MS2 spectra of the 2+ charge state of peptide GNLDVQPPR (from prunin, an 11S storage globulin of almond). A: MS1 spectrum with the 2+ charge state of target peptide ($m/z = 571.9$) circled. B: MS2 (product ion) spectrum of GNLDVQPPR. The mass event at $m/z 571.9$ was selected for fragmentation. The b - and y -ion series (resulting from fragmentation of the peptide bonds from the N and C termini, respectively) are denoted. One or more of these y or b ions may be selected as a transition for quantification. Such transitions are usually denoted as (parent m/z)/(product m/z), e.g., 571.9/743.6 in the case of the y_6 ion.

the protein targets differ. Polyclonal antibody preparations raised to a whole food extract recognize many different protein components, or alternatively they can be developed to, and be specific for, individual proteins components or allergens. While monoclonal antibodies offer advantages of consistency and (in theory) unlimited supply of reagent, they generally recognize a single protein target, possibly the allergenic molecule itself. Antibody preparations to individual proteins can present problems when the target analyte is either poorly abundant or absent. For example, ELISAs for the whey protein β -lactoglobulin function effectively to detect whey isolates used as ingredients in foods, but not for the detection of caseinates. The decision to target allergenic molecules or simply protein markers of the allergenic foodstuff also has to be made for MS-based methods.

An additional point that must be addressed when selecting a target analyte for allergen detection is that most allergen testing is focused on manufactured foods. Hence, the effect of cooking procedures on target protein analytes needs to be considered together with the effects of the matrix, including other proteins, carbohydrates, fat, and other components. Food processing treatments have a tremendous impact on proteins, causing changes in conformation and introducing chemical modifications. Data about such processing-induced changes are not necessarily available in databases where researchers might source target protein sequences for allergen detection. Processing-induced modifications also affect analyte extraction. There are differences in the solubility of proteins from raw and processed food that can make a

difference to any analytical method for which protein is the target analyte. This also presents an added complication for immuno-based methods as processing-induced changes may affect antibody reactivity and specificity. For DNA-based methods, it is often the case that no DNA can be extracted from highly processed products or protein concentrates. Similarly for MS-based detection methods, extractability of the selected target analyte has to be guaranteed to a certain extent, otherwise detection is compromised.

MS Methods for Allergen Detection

MS technologies allow a broad range of different types of protein analysis to be undertaken for allergen analysis, including protein identification, characterization, and quantification. However, it is anticipated that in the majority of cases the presence of protein from an allergenic ingredient will be inferred by the detection of relevant derivative peptides. These peptides are deliberately produced by the action of specific endoproteases (such as trypsin or chymotrypsin) on the target protein and are detected initially by single MS (MS1) with selected verification by tandem MS (MS2) sequencing after separation by LC (typically RP-HPLC; Figure 1). The workflow for the design of methods for the detection of an analyte protein and derived peptides is shown in Figure 2.

Although MS1 instruments may be used for reliable detection of allergens in some cases, the additional verification of sequence fidelity provided by MS2 instruments

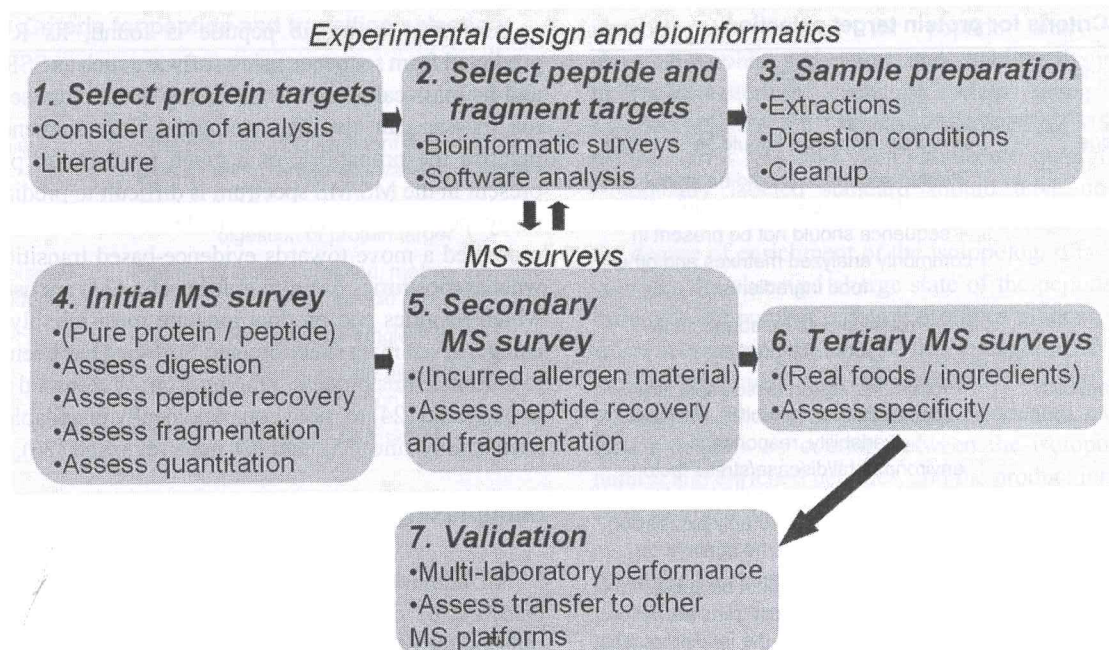


Figure 2. Overview of a workflow for design of an MS experiment to detect allergens in food. Initial experimental design and bioinformatics informs MS analysis. Experimental results from MS surveys of purified protein or peptides, incurred materials (allergens introduced into model matrixes during preparation), and real foods refine target selection.

gives additional specificity, especially when detecting allergens in the complex mixtures represented by most foodstuffs. Currently, most published MS allergen detection methods are single-reaction monitoring type using triple quadrupole (QQQ) instruments (14). However, other hybrid multistage instruments such as quadrupole-time of flight (Q-TOF), TOF-TOF, and linear ion trap-Fourier transform-ion cyclotron resonance have also been used in allergen research (15), including “top down” analyses without protease digestion (16).

The selection of an endoprotease to generate peptides from a parent protein that are suitable for MS depends on the pH of the extraction material, size, and amino acid sequence of the resultant peptides. It needs to generate consistent and predictable products. A commonly used protease in MS is trypsin, as it generally gives predictable cleavage at a pH close to neutral, and usually generates a mixture of peptide products of appropriate mass. However, lysine modification via the Maillard reaction is common in thermally processed food matrixes and may affect the completeness and reproducibility of tryptic digestion. Thus, the choice of protease needs to consider modifications of the target protein that might be adjacent to, or modify, cleavage sites and render digestion less effective. Many allergens contain disulfide bonds and are notoriously resistant to endoprotease digestion (17), and, hence, if they are selected as target analyte(s), tryptic digestion conditions should be assessed for allergens on an individual basis to ensure that the method can reproduce complete digestion.

Allergen MS: Target Analyte Selection

One advantage of MS detection over current methods for allergen detection is that it allows a large degree of freedom in selection of the analyte used to infer the presence of the allergenic foodstuff. This freedom does, however, require the analyst to consider many factors when selecting the optimal analyte to use. Published criteria for target selection exist for MS, including a points-based system for selection (18), but such criteria do not as yet exist for proteins. Given the importance of proteins in triggering allergic reactions and the fact that protein analysis by MS can be extremely specific to individual foodstuffs, we consider proteins to be the most suitable and likely target for inferring the presence of an allergenic foodstuff. Online resources are available that define the sequences of allergenic molecules in foods but they are focused on either genetically modified allergenic risk assessment, such as Allergen-online (19), or clinical diagnosis, such as the International Union of Immunological Services nomenclature database (20). Efforts are underway to update the InformAll database, originally developed to convey allergen information to the consumer (21), to provide such sequences in a readily downloadable format more tractable to bioinformatic analysis used to develop MS methods for allergens. However, such databases cannot, as yet, deliver clues about protein or peptide MS-characteristics like flight properties, sensitivity, or transition quality.

While in theory any protein target(s) unique to the foodstuff may be used to infer the presence of the foodstuff itself, in practice several considerations will guide the selection of individual proteins as targets for analysis

Table 1. Criteria for protein target selection

Selection criteria	Note
Prior knowledge	Full protein sequence should be available.
Uniqueness	Protein sequence should be unique to foodstuff to be detected. At a minimum, sequence should not be present in commonly analyzed matrixes and other food ingredients.
Abundance	Protein should be abundant in foodstuff/ingredient.
Expression variability	Protein expression (tissue specificity, species/cultivar variability, temporal variability, response to environmental/disease/stress factors, post-harvest treatment) should be characterized and preferably expression variability should be minimal.
Modification	Protein should ideally not be subject to modification (either post-translational or processing-induced) in the foods/matrixes commonly analyzed. If present, modifications must be considered when developing peptide targets and MS methodology.
Stability	Protein should not be subject to hydrolysis during storage or food processing.
Extractability	Protein should be released from the matrix into solution for detection.
Digestibility	Protein should be reproducibly digested by endoprotease used in the chosen sample preparation technique.

(Table 1). It is important to note that the food matrix in which the protein is present plays a crucial role in many aspects of protein target selection. In general, the community is currently favoring targeted determination of a particular protein using selected reaction monitoring, which requires the design of one or more protein-specific transitions (Table 2). A transition comprises a parent ion mass and product ion mass that uniquely identifies a peptide specific to the protein of interest. Transitions are also often associated with an expected HPLC retention time (RT). To design a transition it is first necessary to identify a peptide specific to the protein of interest. For a protein taken from a single species, this is achieved simply by extracting the proteolytic peptide sequences from the protein and BLASTing (Basic Local Alignment Search Tool) these against the proteome of that species to determine uniqueness. If the food sample to be analyzed contains material from multiple species—as is common in foods—peptides must be BLASTed against the proteomes of all species present. This is difficult to achieve for many allergenic foods and ingredients since their proteomes have either not yet been fully characterized or are absent from proteome databases, such as Proteomics Identifications and PeptideAtlas, and at present requires literature-based searching.

Assuming a unique peptide is found, its RT can be predicted from sequence using software such as SSRCalc (22) and its mass calculated by summing residue masses. Product ion masses can also be determined from sequence, but in practice the probability of a given product ion peak being present in the MS/MS spectrum is difficult to predict. Indeed, many peptides are not detectable at all in MS. This has prompted a move towards evidence-based transition design, where repositories of proteomic spectra (23) are used to infer which peptides and product ions are most reliably observed using a given mass spectrometer. Software has been produced to automate this process, ranging from Web-based tools such as MRMAid (24) to more complex locally installable software such as SkyLine (25), and has been reviewed (26).

Multiplexing

An additional potential benefit of MS is that it allows detection of multiple analytes within the same experiment, offering the opportunity to track multiple allergenic molecules from one allergenic ingredient or to target multiple analytes representative of one or more foodstuffs to provide a screening tool for ingredients such as tree nuts (27). The simultaneous quantification of multiple analytes [e.g., (28)] offers great potential in terms of analytical throughput and cost-saving. Typically, transitions of more than one peptide will be examined in any case for experimental rigor. One important issue that should be addressed in analysis is the nature of the reporting units used. In an MS analysis of a foodstuff for allergen content there will typically be multiple determinations, one for each transition used. We recommend that the analyte giving the highest determination of allergen content be used as the primary reporting unit; any discrepancy between recoveries of transitions in a sample is likely to be a result of a decrease in the efficacy of extraction, digestion, or detection.

Standards and Calibrants

The quantification of allergenic proteins via MS can be achieved in a number of ways. Many of the most sensitive methods require predigestion of the target protein to specific peptides. The quantification of the peptides could be achieved using proteolytic peptides derived from a known quantity of the protein or via synthetic peptide standards. Ideally, the use of both protein and peptide standards would add extra confidence in any result obtained, but neither comes without some nontrivial practicalities that need to be addressed.

The use of a specific intact protein standard would seem the logical choice for the quantification of a specific allergenic protein. However, an assessment of any "purified" protein for use as a calibrator for quantification requires consideration of a number of issues. In most commercially supplied proteins, where the primary intention of supply is not for use as a standard, the levels of other proteins, water, and buffer salts remaining after the cleanup process need to be determined as these will alter the amount of the actual protein in any standard produced. Therefore, characterization of any solution

Table 2. Criteria for peptide and transition selection

Criteria	Note
Reproducible	Peptide and MS2 fragment reproducibly produced from analysis of various matrixes
Unique	Peptide/MS2 fragment produced only from digestion of protein target
Minimize potential modifications	Preferably lacking cysteine, methionine, and glutamic acid
RT (LC)	Reproducible single RT
Charge	2+ or 3+ ions generally give the best fragmentation
Size	Typically 6–12 residues

standards produced would be sensible. This can be done via a dry mass approach, quantification using amino acid analysis, (if no other proteins are present), or enzyme hydrolysis and quantification of the evolved peptides. However, even if such practices are followed, consideration needs to be given to any process related modifications that may occur during isolation and preparation of the standards. These modifications may be amino acid-specific, which may alter the choice of peptides selected for quantification. Also, solubility issues and other normal protein handling practices need to be addressed. However, the major advantage in using a protein standard is that if brought through the whole extraction procedure any issues of recovery, incomplete digestion, and extraction derived modifications can be nullified, assuming the process is identical for the sample and standard alike.

Alternatively, peptide standards may be considered as they have the major advantage of being synthesized in a relatively pure form and in quantities that can be gravimetrically prepared to make solution standards with low uncertainties. However, current practice among many peptide manufacturers is to express the purity of the product in terms of the presence (or absence) of other peptides. For use as a standard for quantification, the amount of peptide (mg of chosen peptide/g synthetic product, i.e., chemical purity) is required. Therefore, a thorough assessment of the peptide purity is vital. Where gravimetric preparation is not possible, the peptide purity can be easily checked and the amount present in a solution determined via quantification of the amino acids. Once selected and prepared, equimolar solutions of the peptides can be used as standards to quantify the amount of the peptides present in a digested sample extract.

As with all LC/MS quantification, the best accuracy and precision is normally observed by the incorporation of appropriate internal standards (29). For the most part, the incorporation of enriched stable isotopes of constituent elements into the molecule being quantified forms the ideal internal standard for MS. The choice and position of the enriched isotope can influence the enriched molecule's ability to act as an internal standard. The incorporation of ^{13}C , ^{15}N , and ^2H is now common, but the carbon and nitrogen heavy

isotopes are favored for peptide analysis due to chromatographic separation and stability issues such as hydrogen/deuterium exchange when using deuterium. Labeled peptides must be easily resolved by MS from their natural forms. As with small molecule quantification, the isotopically labeled standard should have no detectable amounts of the naturally occurring isotopolog. When deciding the degree of enrichment of the isotopolog, it is important to consider the observed charge state of the peptide. This may influence the number of heavy isotopes to incorporate, since when increasing the charge, the separation of natural and labeled molecules will decrease. Ideally, the degree and position of labeling will result in protonated molecules for which there is no overlap between the isotopologs of the natural and enriched peptides, and the product ions generated from each are resolved.

Incorporation of an isotopically enriched protein would form the ideal internal standard for the entire measurement procedure. If the samples were spiked at the outset, any difference in digestion, extraction, and stability would be cancelled, especially if standards were put through the same procedures. To ensure the traceability and, hence, comparability of any results generated, the incorporation of properly equilibrated labeled protein (i.e., bound to the same matrix constituents, modified in the same way as the target protein, homogeneously spread throughout the sample, etc.) and the use of a traceable protein standard offer the best solution. Ideally, the labeled peptides resulting from the digestion of the isotopically enriched protein would have the same attributes as those described for the synthetic labeled peptides discussed previously. The major disadvantage is that, although reduced in price, the cost of producing labeled proteins in the quality and quantity required is very expensive and difficult to achieve for certain allergenic molecules because of their highly disulfide-bonded nature, and plant origin still presents a challenge for heterologous expression.

The advantage of using labeled peptides is that they can be easily synthesized. The incorporation of readily available labeled amino acids into the synthetic process makes them by far a cheaper alternative to labeled proteins. The incorporation of internal standards helps by reducing the impact of any difference in ionization in the MS source caused by differences between the standard and sample matrixes. However, as with the use of nonlabeled peptide standards, they do not reduce any issues relating to losses at the protein level or incomplete digestion of the target proteins for release of the peptides. Also, when using labeled peptides, one needs to assess the ideal point to add the internal standards. If protein cleanup methods are incorporated, spiking at the start of the analytical process is pointless as the peptides are likely to have very different characteristics compared to those of the protein of interest. If spiking predigestion, the stability of the peptides and the rate at which they are released from the intact protein need consideration. The degree of resistance of a peptide to degradation is often very different if the peptide is free in solution or incorporated in a protein. While it is ideal to have isotopically labeled standards, it may be cost-prohibitive to

have these for all relevant peptides for all allergens listed in Annex IIIa of 68/2007/EC in routine analysis.

Reference Materials

The incorporation and use of any of the above standards, natural and/or labeled, does not remove the requirement for a full validation of the method. One of the best tools for use in the validation is the analysis of a matrix certified reference material. However, there are currently few matrix reference materials available for allergens in foods (12). Also, for any of the proteins mentioned, the preprocessing of the food may modify the protein in a manner that makes the use of any unmodified protein or peptides as a standard in these instances unwise. Consideration of the extent and type of modification to the protein and its likely impact on the choice of standard prior to quantification would be required. This being the case, any MS method must be rigorously evaluated on a range of relevant target matrixes.

Conclusions and Recommendations

Choice of Analyte

MS analysis presents the opportunity to directly analyze for the presence of the molecules that cause allergic reactions. While the legislation governing labeling of allergenic food ingredients and safety of foods does not specify what is to be managed, the best hazard management and risk assessment processes require methodology that measures the hazard itself. Thus, the ultimate goal for food allergen management is to have methods that can detect biologically relevant molecules in foods able to trigger an allergic reaction, with an appropriate level of sensitivity, in relevant food matrixes. However, the nature of the allergenic hazard remains a matter of research and debate, making the choice of target molecules difficult. The ability of MS methods to multiplex provides an unprecedented opportunity to determine the levels of several biologically relevant molecules simultaneously, thus facilitating improved allergen hazard management. This may be especially useful when analyzing certain fractionated ingredients, as illustrated by the need for different tools to quantify both whey and casein food ingredients in addition to total milk. We recommend that methods target proteins, and that multiplexing MS methods that can simultaneously detect several biologically relevant molecules able to trigger allergic reactions should be an objective of future research and development.

Choice of MS Marker Peptides

One crucial issue for allergen analysis is the selection of peptide markers. As previous research has shown (30), not all proteins and peptides are stable after common processing steps in the food industry like heating, baking, roasting, or pressure treatment. In order to ensure that the most appropriate peptides are chosen, these need to be thoroughly evaluated for their behavioral characteristics in various matrixes under different processing conditions. To avoid variability of results, we recommend that methods utilizing

such marker peptides should be placed in publicly accessible databases. Such an approach avoids looking for explanations of different results for the same sample when different peptides/proteins markers have been chosen, as is often the case with commercially available ELISA kits containing different antibodies for the same target allergen.

Choice of Standard

The optimal choice of standard is the isotopically labeled target protein analyte, but, given the likely nature of many of these molecules linked with the difficulty and expense of obtaining labeled protein standards, isotopically labeled peptides provide a realistic alternative. This is especially true for the development of reference methods.

Optimization of Protease Digestion

Quantitative protein MS requires reproducible and effective protease digestion steps to be developed. It is likely these will need to be optimized for each target analyte and each common food matrix to ensure reproducible and robust method development.

Harmonization of Methods and Results

With the development of MS techniques and the movement towards routine applications, it is essential that the data obtained are comparable among laboratories. Because a number of factors, including interoperator variability, extraction method, and equipment used, all contribute to interlaboratory comparability, some essential factors can be readily standardized, thereby contributing to a harmonization of results. As with other methods for allergen determination, harmonization will be greatly facilitated through the development of naturally incurred reference materials (31, 32) spanning several types of food matrix and through method validation and interlaboratory trials. Validation studies according to AOAC/International Union of Pure and Applied Chemistry harmonized guidelines may initially prove difficult due to the currently small number of laboratories. Bearing in mind that so far only very few ELISA methods have undergone full validation despite a large number of laboratories, different approaches have to be found for validation of methods, possibly a performance parameter based approach allowing validation of individual methods by single laboratories. Such activities will also contribute to the development of standardized units allowing better comparability of MS methods with more conventional immuno- and DNA-based methods.

Future Perspectives

In the future there could be recommendations regarding appropriate markers for qualitative and quantitative analysis of food allergens at the European Commission level (33). However, protein MS is a rapidly developing field; at present it is more practical to use flexible approaches, such as industry Codes of Practice. This would facilitate rapid adoption of markers with improved characteristics as they are found and new approaches, such as MS^E (34), as they become available.

Such activities would similarly lead to a significant improvement in harmonization of methods and, in turn, to more coherent data, ultimately benefitting consumers, laboratories, and enforcement agencies alike.

To facilitate effective analysis of allergens in foods by MS there is a need to improve the quality and quantity of food proteomic data available in the proteomics community databases. The rapid expansion in proteomics methodologies and platforms in the food chemistry community is enabling this, and there is a need to adapt databases originally developed for model organisms and human proteomics to the needs of the food community. This paper deals with issues common to all analytical methods currently used for allergen detection, together with new issues specifically linked to MS analysis. The latter are common to many applications of proteomic techniques in the food arena, including food authenticity and supporting the increasing need for traceability and monitoring in the global food market place. In addition, online resources with collated, authenticated allergen sequences suitable for protein determination would also facilitate allergen analysis.

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